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**Gordan S. KARAMAN**<sup>1</sup>

**TWO NEW GENERA OF THE FAMILY NIPHARGIDAE FROM GREECE  
(CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 287)**

**ABSTRACT**

From the subterranean waters of Tzani spilios Cave, Lefka Ori Massif, W. part of Crete Island, Greece, a new genus and species of the family Niphargidae (Crustacea, Amphipoda) is described and figured, *Exniphargus tzanisi*, gen. nov., sp. n., and its relationship to other genera of the family Niphargidae is discussed.

The species *Niphargobates lefkodemonaki* Sket, 1990, known from Crete Island, Greece, is removed to the new genus *Niphargobatoides*, gen. nov. as *typus generis* (type species); differences and relationship of this genus regarding other genera are presented. New diagnosis of the genus *Niphargobates* Sket, 1981 is given and the key to the genera of the family Niphargidae is composed.

**Keywords:** Amphipoda, Niphargidae, new genera, new species, key to the genera, Greece.

**INTRODUCTION**

The dominant subterranean amphipods (Crustacea, Amphipoda) in the subterranean waters of Europe and Near East are the members of the family Niphargidae, replaced in North America by the family Crangonyctidae, especially genus *Stygobromus* Cope, 1872: 422 (T. sp.: *Stygobromus vitreus* Cope, 1872) (G. Karaman, 1974). The family Niphargidae is presented in Europe by 12 known genera:

*Carinurella* Sket, 1971 [Type sp.: *Carinurella paradoxa* (Sket, 1964)];

*Exniphargus*, gen. nov. [Type sp.: *Exniphargus tzanisi* sp. n.];

*Foroniphargus* G. Karaman, 1985 [Type sp.: *Foroniphargus pori* G. Karaman, 1985];

*Haploginglymus* Mateus & Mateus, 1958 [Type sp.: *Haploginglymus bragai* Mateus & Mateus, 1958];

*Karamaniella* Sket, 1962 [Type sp.: *Karamaniella pupetta* Sket, 1962];

*Microniphargus* Schellenberg, 1934 [Type sp.: *Microniphargus leruthi*, Schellenberg, 1934];

*Niphargellus* Schellenberg, 1938 [Type sp.: *Niphargus arndti*, Schellenberg, 1933];

*Niphargobates* Sket, 1981 [Type sp.: *Niphargobates orophobata* Sket, 1981];

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*Niphargobatoides* gen. nov. [Type sp.: *Niphargobates lefkodemonaki* (Sket, 1990)];

*Niphargopsis* Chevreux, 1922 [Type sp.: *Niphargopsis casparyi* (Pratz, 1866)];

*Niphargus* Schiödte, 1849 [Type sp.: *Niphargus stygius* (Schiödte, 1847)];

*Pontoniphargus* Dancau, 1970 [Type sp.: *Pontoniphargus racovitzaei*, Dancau, 1970].

Among them, genus *Niphargus* is the most numerous, with over 200 known taxa divided into various subgenera often not clearly divided to each other.

During our recent study of the family Niphargidae from Greece, we discovered a new species belonging to the new genus *Exniphargus*, gen. nov., based on some specific characters of its mouthparts (maxilla 1, maxilliped, etc.) and combination of other morphological characters. On the other hands, based on the morphological analysis of genera of the family Niphargidae, the species *Niphargobates lefkodemonaki* Sket, 1990 is removed to the new genus *Niphargobatoides*, gen. nov. as a typus generis.

## MATERIAL AND METHODS

The collected material was preserved in the 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All appendages were temporarily submersed in the mixture of glycerine and water for study and drawing. Later, all appendages have been transferred to Liquid of Faure on permanent slides. The body-length of examined specimens were measured by tracing individual's mid-trunk lengths (from tip of head to end of telson) using camera lucida. All illustrations were inked manually.

Some morphological terminology and setae formulae follow G. Karaman's terminology (Karaman, G., 1969; 1993; 2012a) regarding the last mandibular palpus article [A= setae on outer face; B= setae on inner face; C= additional setae on outer face; D= lateral marginal setae; E= distal long setae] and propodus of gnathopods 1 and 2 [S= corner spine; L= lateral slender serrate spines; M= facial setae; R= subcorner spine on inner face]. Terms "setae" and "spines" are used based on its shape, not origin.

All studies in this work are based on the classic morphological, ecological and zoogeographical studies.

## TAXONOMICAL PART

### Family NIPHARGIDAE

Genus *EXNIPHARGUS* gen. nov.

DIAGNOSIS of the genus:

Eyes absent; body and telson *Niphargus*-like. Antenna 1 with 2-articulated accessory flagellum; antennal gland cone short.

Mouthparts: labrum entire; labium with small inner lobes; mandibles with triturative molar; right mandible: molar with long lateral seta, incisor with 5



teeth, and lacinia mobilis with 4 teeth, accompanied by several rakers. Left mandible: incisor with 4 teeth, lacinia mobilis with several teeth, accompanied by several rakers; mandible palpus 3-articulated, last article subfalciform, with A, B, D and E-setae. Maxilla 1: inner plate well developed, with distal setae, outer plate broad, with numerous pectinate spines, palpus 2-articulated, well developed. Maxilla 2 outer plate slightly larger than inner one, both plates with marginal setae only. Maxilliped: inner and outer plate well developed but relatively short, palpus article 4 with 3 distal nails (spines) and single short setae.

Coxae relatively short, coxa 4 without distinct posterior lobe, coxa 5 much shorter than 4; gnathopods 1-2 *Niphargus*-like. Pereopods 5-7 with dilated article 2. Pleopods and uropods 1-2 biramous, *Niphargus*-like. Uropod 3: inner ramus short, scale-like, outer ramus elongated, 2-articulated, *Niphargus*-like. Telson *Niphargus*-like. Sexual dimorphic characters scarce. Coxal gills occur on the corresponding legs 2-6, oostegites occur on the corresponding legs 2-5.

TYPUS GENERIS: *Exniphargus tzanisi*, sp. n.

TAXA: Only type species is known.

DISTRIBUTION: Crete Island, Greece.

DERIVATIO NOMINIS: The name *Exniphargus* alluding to Latin words “former *Niphargus*”.

*EXNIPHARGUS TZANISI*, sp. n.

Figs. 1-8

MATERIAL EXAMINED:

**Greece:** Tzanis spilios Cave, Lefka Ori Massif, W. part of Crete Island, September, 1988, 5 exp. (leg. B. Sket).

DIAGNOSIS

Body *Niphargus*-like, eyes absent, accessory flagellum of antenna 1 consisting of 2 articles. Labium with small inner lobes. Mandible *Niphargus*-like, palpus well developed, 3-articulate. Maxilla 2 inner plate slightly smaller than outer plate, both plates with distal setae. Maxilla 1 inner plate narrowed, outer plate broad, with 14 slender pectinate spines, palpus 2-articulated, well developed. Maxilliped inner and outer plate well developed, palpus article 4 with 3 distal nails (spines). Coxa 5 shorter than coxa 4. Gnathopods 1-2 and pereopods 3-7 *Niphargus*-like. Uropods 1-2 biramous. Uropod 3 biramous, inner ramus consisting of one article, scale-like, outer ramus 2-articulate, second article short. Telson incised.

DESCRIPTION. **Male 8.5 mm.**

Body moderately slender, *Niphargus*-like, mesosomal segments naked; metasomal segments 1-3 with 3-4 dorsoposterior marginal setae (fig. 2G). Urosomal segment 1 on each dorsolateral side with 1 seta (fig. 4H); urosomal segment 2 on each dorsolateral side with one spine and one seta; urosomal segment 3 naked. Urosomal segment 1 in both ventroposterior corners with one spine near basis of the uropod 1 peduncle (fig. 4H).

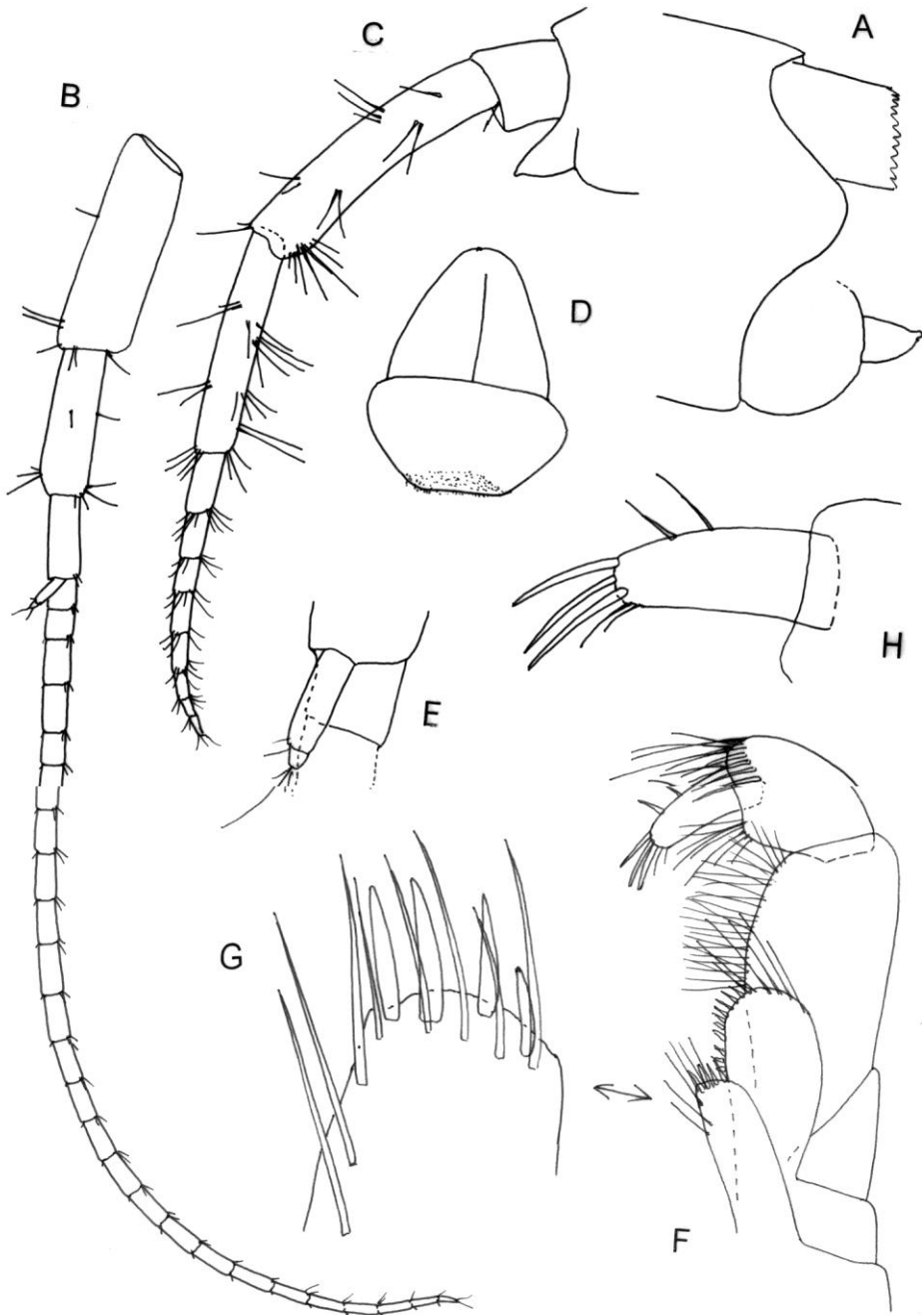


Figure 1. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, male 8.5 mm (holotype): A= head; B= antenna 1; C= antenna 2; D= labrum; E= accessory flagellum; F= maxilliped; G= inner plate of maxilliped; H= palpus article 4 of maxilliped.

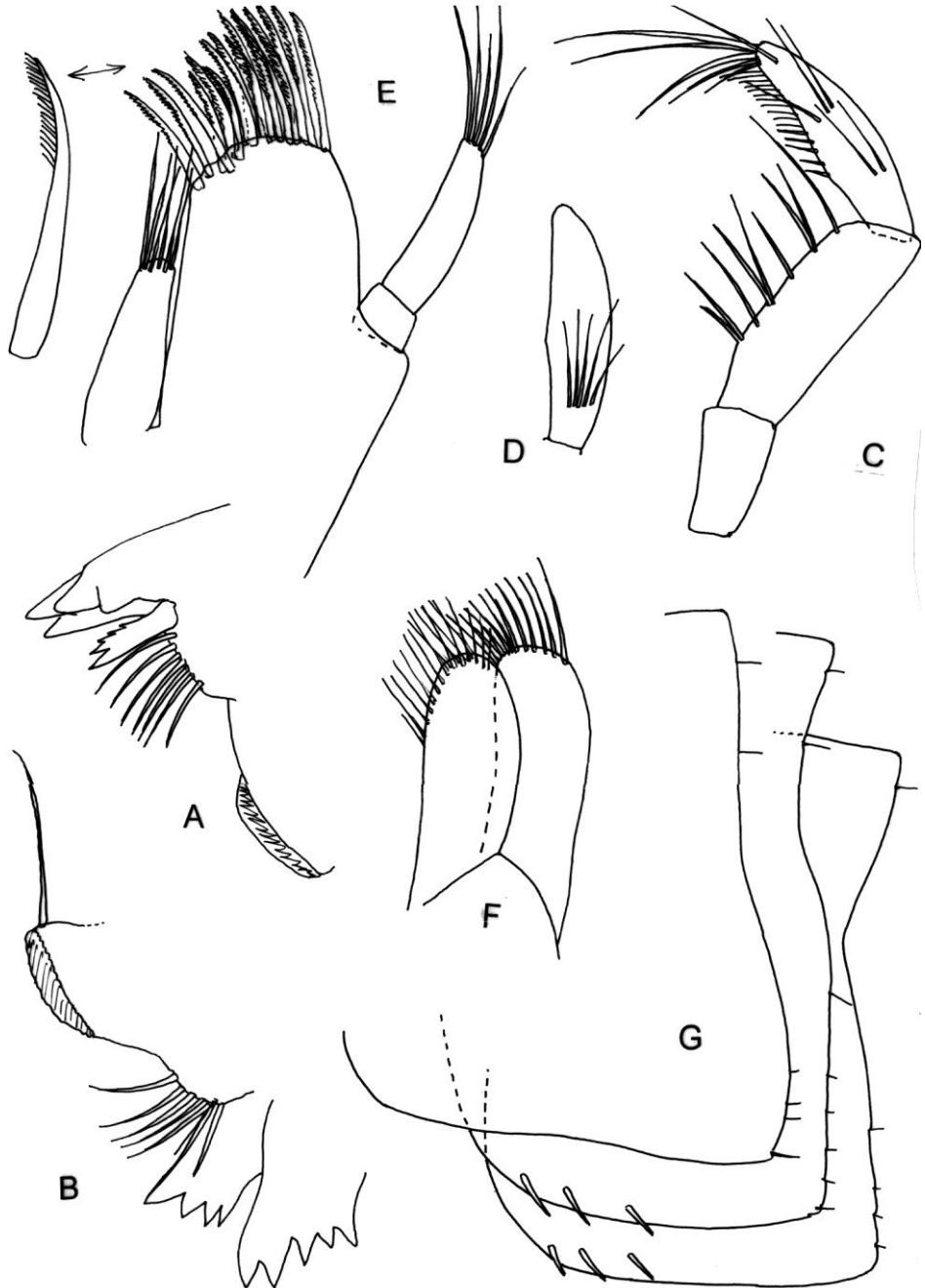


Figure 2. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, male 8.5 mm (holotype): A= left incisor and lacinia mobilis; B= right incisor and lacinia mobilis; C= mandible palpus, inner face; D= distal mandible palpus article, outer face; E= maxilla 1; F= maxilla 2; G= epimeral plates 1-3.

Epimeral plates strongly angular. Epimeral plate 1 with distinctly marked ventroposterior corner by 1 slender spine, along posterior moderately convex margin with 3 short setae, ventral margin poorly concave in the middle. Epimeral plate 2 with marked ventroposterior corner by 1 spine-like seta, posterior distal margin straight, bearing 5-6 short setae. Epimeral plate 3 with angular ventroposterior corner, along posterior straight and poorly inclined margin appear 4 short setae; epimeral plates 2 and 3 with 3 subventral spines each (fig. 2G). Sexual papillae are attached at ventral margin of metasomal segment 3 (fig. 6B).

Head with short rostrum and short subrounded lateral cephalic lobes and concave ventroanterior excavation (fig. 1A), eyes absent.

Antenna 1 almost reaching the half of body-length (ratio: 85:40). Peduncle moderately slender, scarcely setose, peduncular articles progressively shorter (ratio: 58:39:22) (fig. 1B); main flagellum slender, consisting of 25 scarcely setose articles (most of them with one short aesthetasc). Accessory flagellum much shorter than last peduncular article and consisting of 2 unequal articles bearing distal setae (fig. 1E).

Antenna 2 shorter than antenna 1, peduncle moderately slender, peduncular article 3 short, nearly as long as broad (fig. 1C); peduncular article 4 slightly longer than article 5 (ratio: 70:59), along ventral margin with 3 bunches of short setae (fig. 1C), along dorsal margin with 3 groups of 2-3 setae each; peduncular article 5 at ventral margin with 4 bunches of setae (the longest setae exceeding the diameter of article itself), along dorsal margin with 3 groups of several setae each. Flagellum longer than last peduncular article (ratio: 74:59), consisting of 8 articles; antennal gland cone short (fig. 1C).

**MOUTHPARTS.** Labrum twice broader than long (fig. 1D). Labium broader than long, inner lobes short, outer lobes broad, entire, convex distally (fig. 3A).

Mandible well developed. Right mandible with triturative molar bearing long lateral seta (fig. 2B), incisor with 5 teeth, lacinia mobilis with 4 teeth accompanied by 7 rakers (fig. 2B). Left mandible: triturative molar without long lateral seta, incisor with 4 teeth, lacinia mobilis with several teeth accompanied by 7 rakers (fig. 2A). Palpus of left and right mandible symmetric: first article short, naked (fig. 2C); second article with 9 lateral setae; third article poorly shorter than second article (ratio: 60:64), subfalciform, bearing nearly 16 D-setae and 5 E-setae (fig. 2C), on outer face appear one row of 5 A-setae (fig. 2D), on inner face are attached 3 groups of B-setae (2-3-1) (fig. 2C).

Maxilla 1: inner plate narrowed, with 6 distal setae (fig. 2E), outer plate with 14 densely pectinate slender spines (fig. 2E); palpus 2-articulated, poorly exceeding basis of outer plate spines and provided with 5 distal setae (fig. 2E).

Maxilla 2: outer plate slightly larger than inner one, bearing distal setae only (fig. 2F); inner plate with distolateral setae (fig. 2F).

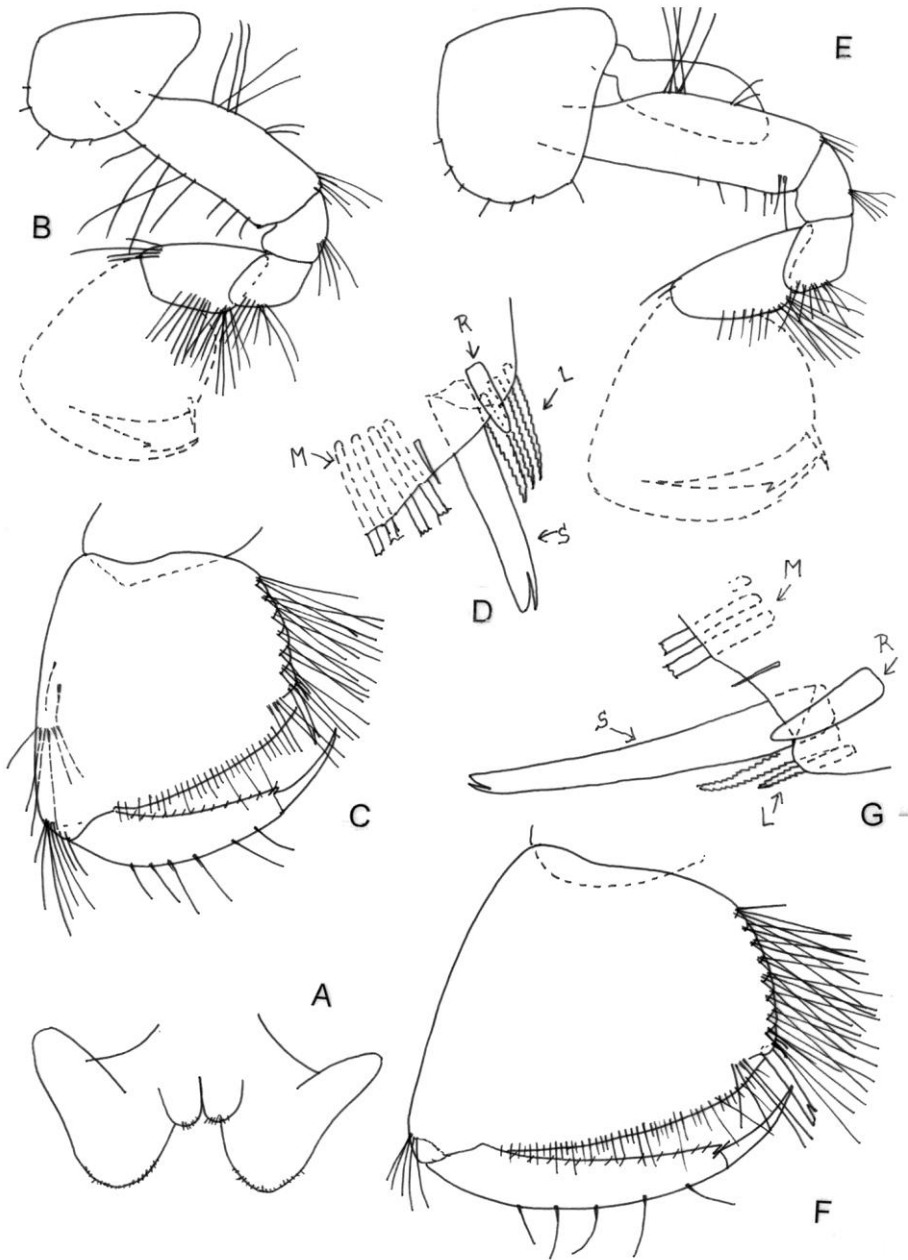


Figure 3. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, male 8.5 mm (holotype): A= labium; B-C= gnathopod 1, outer face; D= distal corner of gnathopod 1 propodus, inner face [S=corner spine; L= lateral spines; R= subcorner spine; M= facial M-setae]; E-F= gnathopod 2, outer face; G= distal corner of gnathopod 2 propodus, inner face [S= corner spine; L= lateral spines; R= subcorner spine; M= facial M-setae].

Maxilliped: inner plate reaching outer tip of palpus article 1, bearing 3-4 distal pointed spines intermixed with single setae longer than spines, as well as with 3 lateral setae at inner margin (fig. 1G); outer plate short, not exceeding half of palpus article 2, with row of pointed distoexternal marginal spines and several distal long setae; palpus article 2 at inner margin with numerous setae (fig. 1F); palpus article 3 at inner margin with bunch of setae, at distoexternal margin with row of setae and spine-like setae (fig. 1F). Palpus article 4 with 3 distal strong nails (spines) (equivalent to nail in *Niphargus* taxa) accompanied by 2 slender setae (fig. 1H), at outer margin with 1-2 strong median setae.

Coxae relatively short. Coxa 1 broader than long (ratio: 45:33), with subrounded ventroanterior corner and provided with 5 marginal setae (fig. 3B). Coxa 2 as long as broad, with convex ventral margin bearing 7 marginal setae (fig. 3E). Coxa 3 slightly longer than broad (ratio: 58:46), at ventral convex margin with 6 setae (fig. 4A). Coxa 4 slightly longer than broad (ratio: 62:50), with convex ventral margin and without distinct ventroposterior lobe, bearing 7 marginal setae (fig. 4C).

Coxa 5 much shorter than coxa 4, broader than long (ratio: 60:35), anterior subrounded lobe only poorly longer than posterior lobe (fig. 5A).

Coxa 6 similar to coxa 5 but smaller, broader than long (ratio: 51:30), with convex anterior lobe poorly longer than posterior one (fig. 5C).

Coxa 7 much broader than long (ratio: 56:22), with convex ventral margin (fig. 5F).

Propodus of gnathopod 1 and gnathopod 2 are remarkably larger than corresponding coxa. Gnathopod 1: article 2 along anterior margin with row of long setae (setae in proximal part are longer than these in distal part), along posterior margin with median and distal bunch of long setae; article 3 at posterior margin with one bunch of setae (fig. 3B); article 5 shorter than article 6 (propodus) (ratio: 32:47), along anterior margin with distal bunch of setae (fig. 3B). Propodus trapezoid, hardly longer than broad (ratio: 72:69), along posterior margin with 6 transverse rows of setae (fig. 3C). Palm poorly convex, inclined over half of propodus length, defined on outer face by one corner S-spine accompanied laterally by 3 slender serrate L-spines and facial 4 long M-setae (fig. 3D), on inner face by one short subcorner R-spine (fig. 3D). Dactylus reaching posterior margin of propodus, along outer margin with 6 single median setae, along inner margin with row of numerous short setae (fig. 3C).

Gnathopod 2 is slightly larger than gnathopod 1; article 2 along anterior margin with row of short setae in distal part, no setae in proximal part of article; along posterior margin of article 2 appears one proximal bunch of long setae and one median pair of short setae, as well as distal bunch of shorter setae. Article 3 at posterior margin with one bunch of setae (fig. 3E); article 5 shorter than article 6 (propodus) (ratio: 40:50), at anterior margin with distal bunch of setae. Propodus nearly as long as broad, along posterior margin with 8 transverse rows of setae (fig. 3F); palm slightly convex and inclined almost 2/3 of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 2 L-spines and 4 long M-setae, on inner face by one subcorner R-spine (fig. 3G). Dactylus reaching posterior margin of propodus, bearing 5 median single setae along outer margin and numerous short setae at inner margin (fig. 3F).

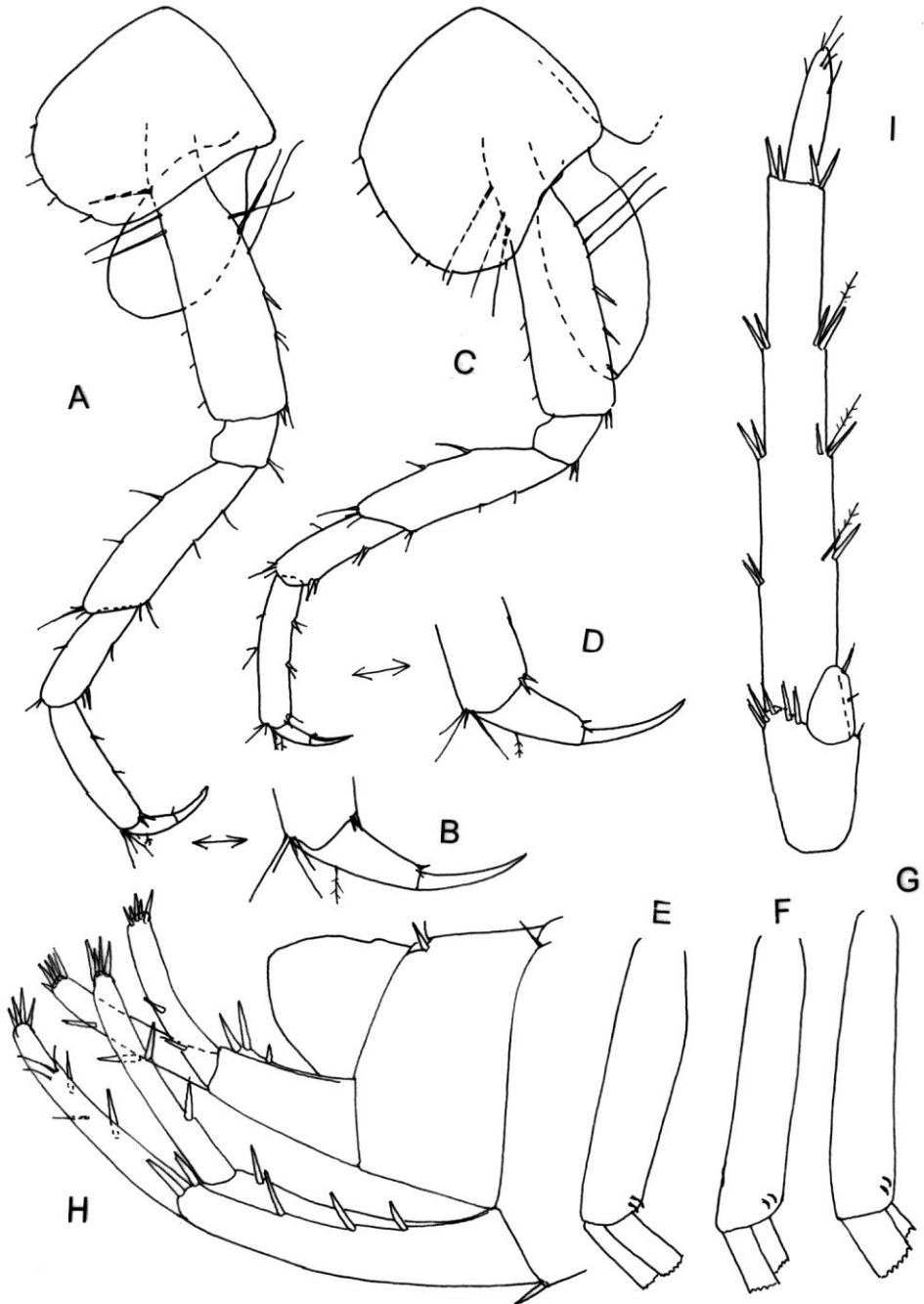


Figure 4. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, male 8.5 mm (holotype): A-B= pereopod 3; C-D= pereopod 4; E-F-G= peduncle of pereopods 1-3; H= urosome with uropods 1-2; I= uropod 3.

Pereopods 3 and 4 similar to each other. Pereopod 3: article 2 with several long proximal setae at anterior and posterior margin, and 3-4 short setae in distal part of anterior margin; one median spine and pair of short setae are attached at posterior margin and tip. Articles 4-6 are of different length (ratio: 50:29:37), scarcely setose (fig. 4A), single setae and spines are shorter than diameter of articles themselves. Dactylus moderately strong and elongated, shorter than article 6 (ratio: 24:37), along inner margin with one spine-like seta near basis of the nail, along outer margin with one median plumose seta (fig. 4B), nail rather shorter than pedestal (ratio: 28:35).

Pereopod 4: posterior margin of article 2 with 3 proximal long setae and 4-5 short spines, along anterior margin provided with 3 long proximal setae and 3 short setae in distal part (fig. 4C); articles 4-6 of different length (ratio: 47:32:39); articles 5 and 6 at posterior margin with bunches of short spines and single setae; dactylus rather elongated, shorter than article 6 (ratio: 23:38), at inner margin with one spine-like seta or slender spine near basis of the nail, along outer margin with one median seta (fig. 4D); nail shorter than pedestal (ratio: 28:30).

Pereopod 5 is remarkably shorter than pereopods 6 and 7 (fig. 5A, C, F). Pereopod 5: article 2 longer than broad (ratio: 73:46), along anterior convex margin with row of 6 slender spines, along posterior slightly convex margin with 13 short setae, ventroposterior lobe not developed (fig. 5A). Articles 4-6 of unequal length (ratio: 48:60:65), along both margins with short spines and setae not exceeding the diameter of articles themselves (fig. 5A); article 2 is longer than article 6 (ratio: 73:65). Dactylus shorter than article 6 (ratio: 28:65), at inner margin with one short spine-like seta and one short seta near basis of the nail, along outer margin with one median plumose seta (fig. 5B); nail shorter than pedestal (ratio: 29:37).

Pereopod 6: article 2 longer than broad (ratio: 85:51), along anterior margin with row of short spines, along posterior margin with 12 short setae, ventroposterior lobe not developed (fig. 5C). Articles 4-6 of different length (ratio: 56:80:100), along both margins with bunches of short spines and single setae not exceeding the width of articles themselves (fig. 5D). Article 2 is shorter than article 6 (ratio: 85:100). Dactylus shorter than article 6 (ratio: 34:100), at inner margin with short spine-like seta and short seta near basis of the nail (fig. 5E), along outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 30:50).

Pereopod 7: article 2 longer than broad (ratio: 87:51), along anterior margin with row of 6 pairs of single short slender spines, along posterior margin with 12 short setae, ventroposterior lobe not fully developed (fig. 5F); articles 4-6 of unequal length (ratio: 53:77:100), along both margins with bunches or single short spines not exceeding diameter of articles themselves (fig. 5G). Article 2 is shorter than article 6 (ratio: 87:100). Dactylus is much shorter than article 6 (ratio: 33:100), at inner margin with short small spine and seta near basis of the nail (fig. 5H), along outer margin with one median plumose seta; nail shorter than pedestal (ratio: 31:50).



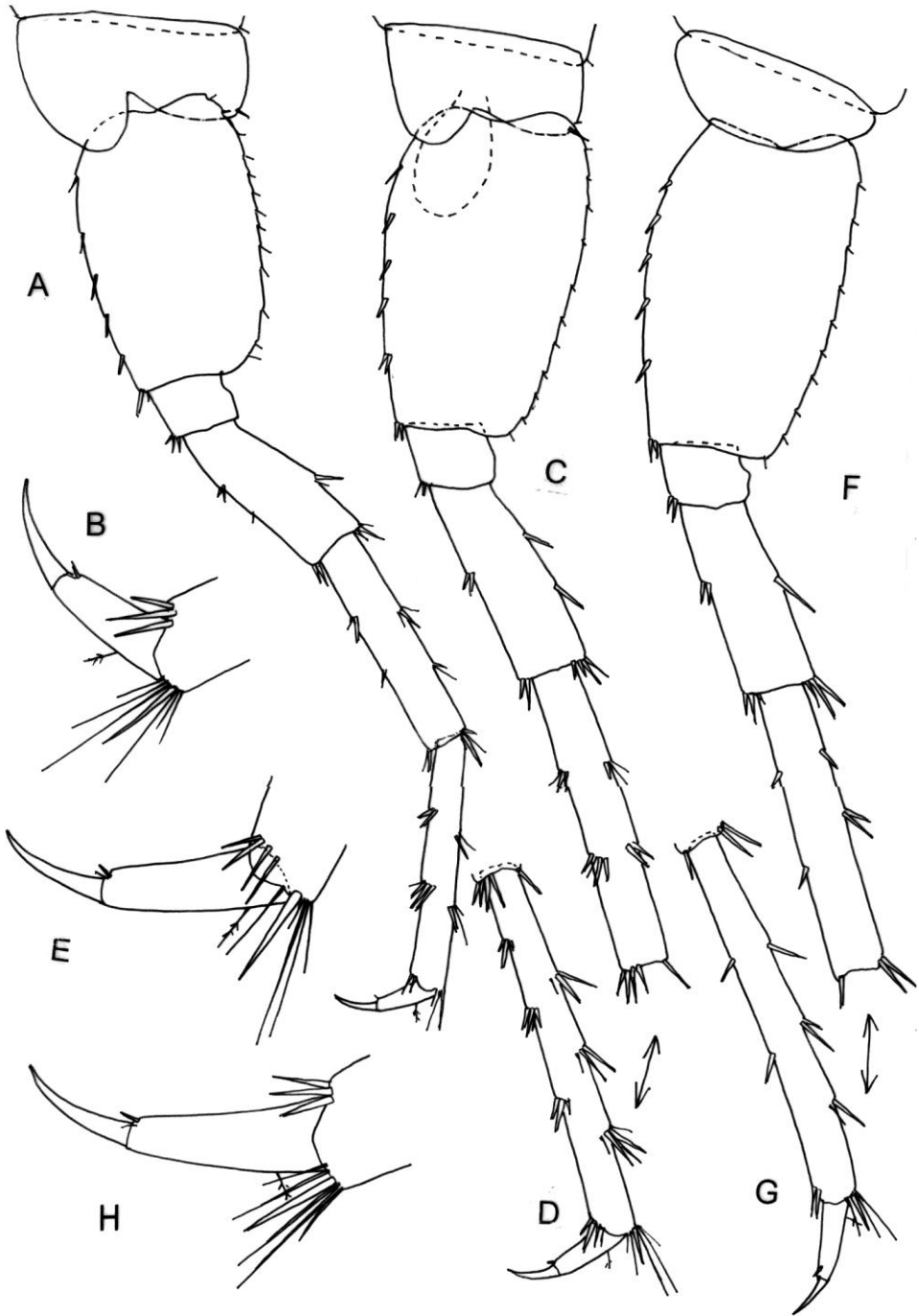


Figure 5. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, male 8.5 mm (holotype): A-B= pereopod 5; C-D-E= pereopod 6; F-G-H= pereopod 7.

Pleopods 1-3 with 2 retinacula each, peduncle of all pleopods naked (fig. 4E, F, G).

Uropod 1: peduncle longer than rami, with dorsoexternal row of strong spines, on dorsointernal margin appears only distal spine (fig. 4H); inner ramus scarcely longer than outer one, bearing 2 median strong spines at lateral margin and 4 distal short spines (fig. 4H); outer margin with 2 lateral median single strong spines and 2 bunches of 1-3 short setae, as well as with 4 distal short spines (fig. 4H).

Uropod 2: outer ramus scarcely twisted (in lateral projection), bearing 2 lateral single spines and 5 distal short spines; inner ramus scarcely longer than outer one, or almost equally long, bearing 2 lateral single strong spines and 5 distal short spines (fig. 4H).

Uropod 3 moderately long: peduncle less than twice as long as broad, with several distal spines; inner ramus consists of one article, short, scale-like, shorter than peduncle and bearing one lateral seta and one distal spine (fig. 4 I); outer ramus 2-articulated: first article along outer margin with 4 bunches of short strong spines, along inner margin with 4 bunches of spines accompanied by single plumose setae longer than spines themselves (fig. 4 I); second article much shorter than first article (ratio: 35:145), bearing several distolateral simple setae (fig. 4 I).

Telson slightly longer than broad (ratio: 81:70), incised over 2/3 of telson-length, slightly gapping, lobes slightly tapering distally and bearing 3 short distal spines each (fig. 6A); a pair of unequal plumose setae appears on each lobe in upper half of its outer margin.

Coxal gills ovoid, relatively short, never reaching ventral margin of corresponding article 2 of the legs 2-6 (figs. 3E, 4A, C; 5C).

Left and right genital palpus on ventral median surface on metasomal segment 3 is well developed.

#### **FEMALE 8.1 mm** with setose oostegites:

Body is similar to male. Metasomal segments 1-3 with 4 dorsoposterior marginal short setae each. Urosomal segment 1 on each dorsolateral side with 1 seta; urosomal segment 2 on each dorsolateral side with 2 spines or 1 spine and 2 setae; urosomal segment 3 naked.

Epimeral plate 1 poorly concave ventrally and with obtusely angular ventroposterior corner defined by 1 corner slender spine, posterior margin almost sinusoid, with several short marginal setae (fig. 8A); epimeral plate 2 poorly acute with obtuse ventroposterior corner defined by one corner spine-like seta, posterior margin with 3-4 short marginal setae; epimeral plate 3 poorly acute, with obtuse ventroposterior corner defined by 1 spine-like seta, posterior margin poorly inclined and provided with 5-6 short setae. Epimeral plates 2 and 3 with 3 subventral spines each (fig. 8A).

Antenna 1 almost reaching half of body-length (ratio: 40:81), main flagellum consisting of 21 articles.

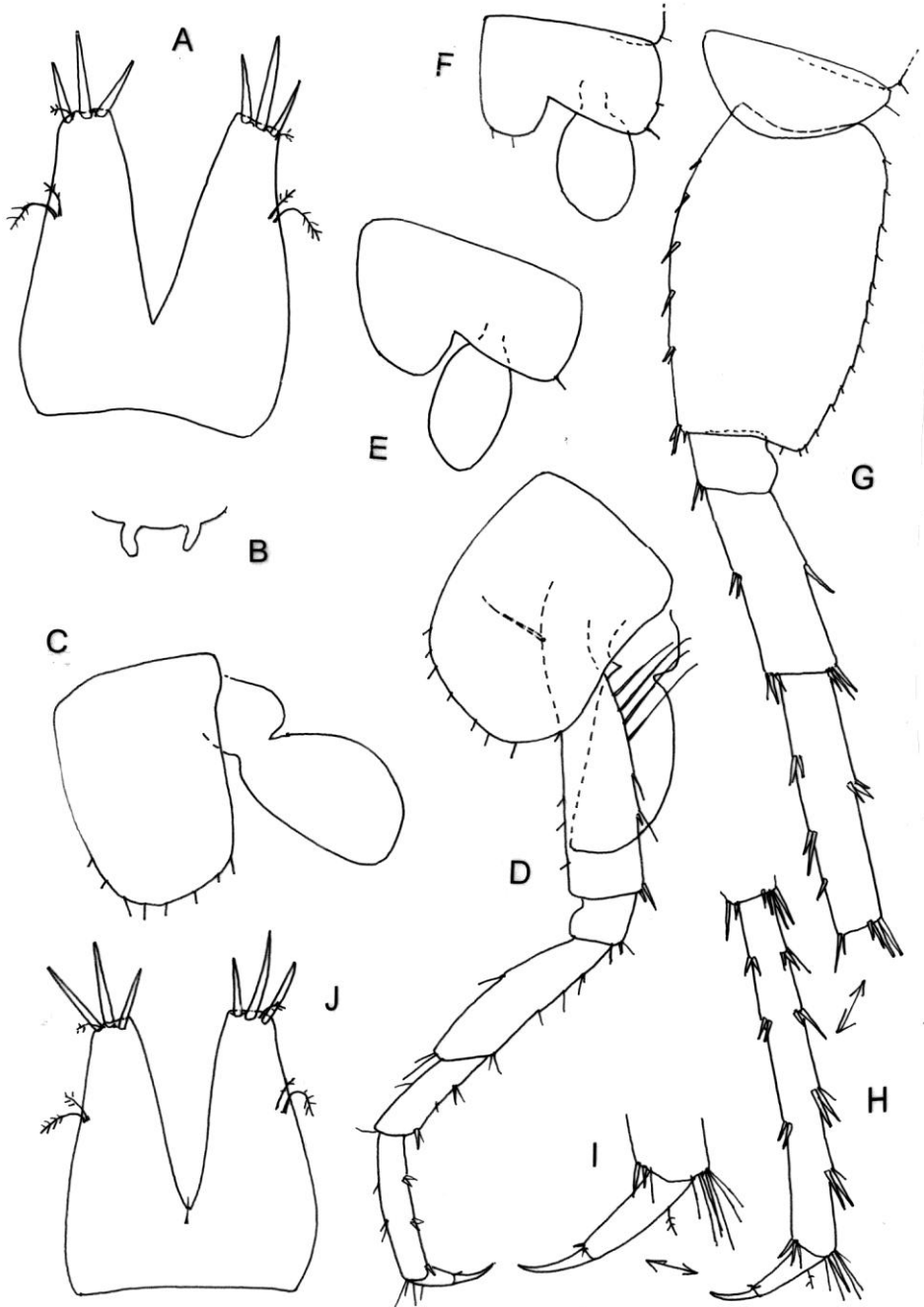


Figure 6. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, male 8.5 mm (holotype): A= telson; B= ventral sexual papillae.

**Female** 8.2 mm (paratype): C= coxa 3; D= pereopod 4; E= coxa 5; F= coxa 6; G-H-I= pereopod 7; J= telson.

Antenna 2 like that in male, flagellum consisting of 8 articles. Mouthparts like these in male. Maxilla 1 like that in male.

Maxilliped: inner plate with 3 distal spines accompanied by several strong distal and distolateral spine-like setae (fig. 7A); right palpus article 4 with 3 distal nails accompanied by 4 short setae sitting below the nails, at outer margin with one median seta (fig. 7B). Left palpus article 4 with 3 distal nails accompanied by 3 distoventral short setae, at outer margin with 1 median strong seta (fig. 7C).

Coxa 1 broader than long (ratio: 43:33), with subrounded ventroanterior corner and bearing 4 marginal setae (fig. 7D). Coxa 2 as long as broad, having on subrounded ventral margin 9 short setae (fig. 7F). Coxa 3 longer than broad (ratio: 62:44), at ventral margin with 8 short setae (fig. 6C); coxa 4 slightly longer than broad (ratio: 65:50), with 8 short marginal setae (fig. 6D). Coxa 5 broader than long (ratio: 62:38), with subrounded anterior margin (fig. 6E). Coxa 6 smaller than 5, broader than long (ratio: 47:30) (fig. 6F). Coxa 7 entire, broader than long (ratio: 51:24), with convex ventral margin (fig. 6G).

Gnathopods 1-2 rather similar to these in male. Gnathopod 1: article 2 along anterior and posterior margin with numerous long setae (fig. 7D); article 3 at posterior margin with one bunch of setae; article 5 shorter than propodus (ratio: 32:43), at anterior margin with distal bunch of setae (fig. 7D). Propodus trapezoid, slightly longer than broad (ratio: 65:60), along posterior margin with 5 transverse rows of setae (fig. 7E). Palm convex, inclined hardly over half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 slender L-spines and facial 5 M-setae (fig. 7E), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with 6 strong median setae, along inner margin with over 12 short setae (fig. 7E).

Gnathopod 2: article 2 with naked proximoanterior margin, and with 6 shorter setae in distal margin, along posterior margin with long setae in proximal part and short setae in distal part; article 3 at posterior margin with one bunch of setae (fig. 7F); article 5 shorter than 6 (ratio: 38:52), at anterior margin with one distal seta. Propodus trapezoid, poorly longer than broad (ratio: 80:75), along posterior margin with 8 transverse groups of setae (fig. 7G); palm convex, inclined poorly over half of propodus-length, defined on outer face by one corner S-spine accompanied by 2 L-spines and 4 long facial M-setae (fig. 7F), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with 5 median setae, along inner margin with nearly 11 short setae (fig. 7G).

Pereopods 3 and 4 similar to each other, scarcely setose. Pereopod 4: article 2 along anterior margin with 1 long proximal seta and several short setae in distal part, along posterior margin with several short setae and spines (fig. 6D). Articles 4-6 of different length (ratio: 47:30:39), bearing very short setae and single spines; dactylus shorter than article 6 (ratio: 22:39), at inner margin with one short spine-like seta, on outer margin with one median plumose seta (fig. 6D), nail slightly shorter than peduncle (ratio: 10:13).

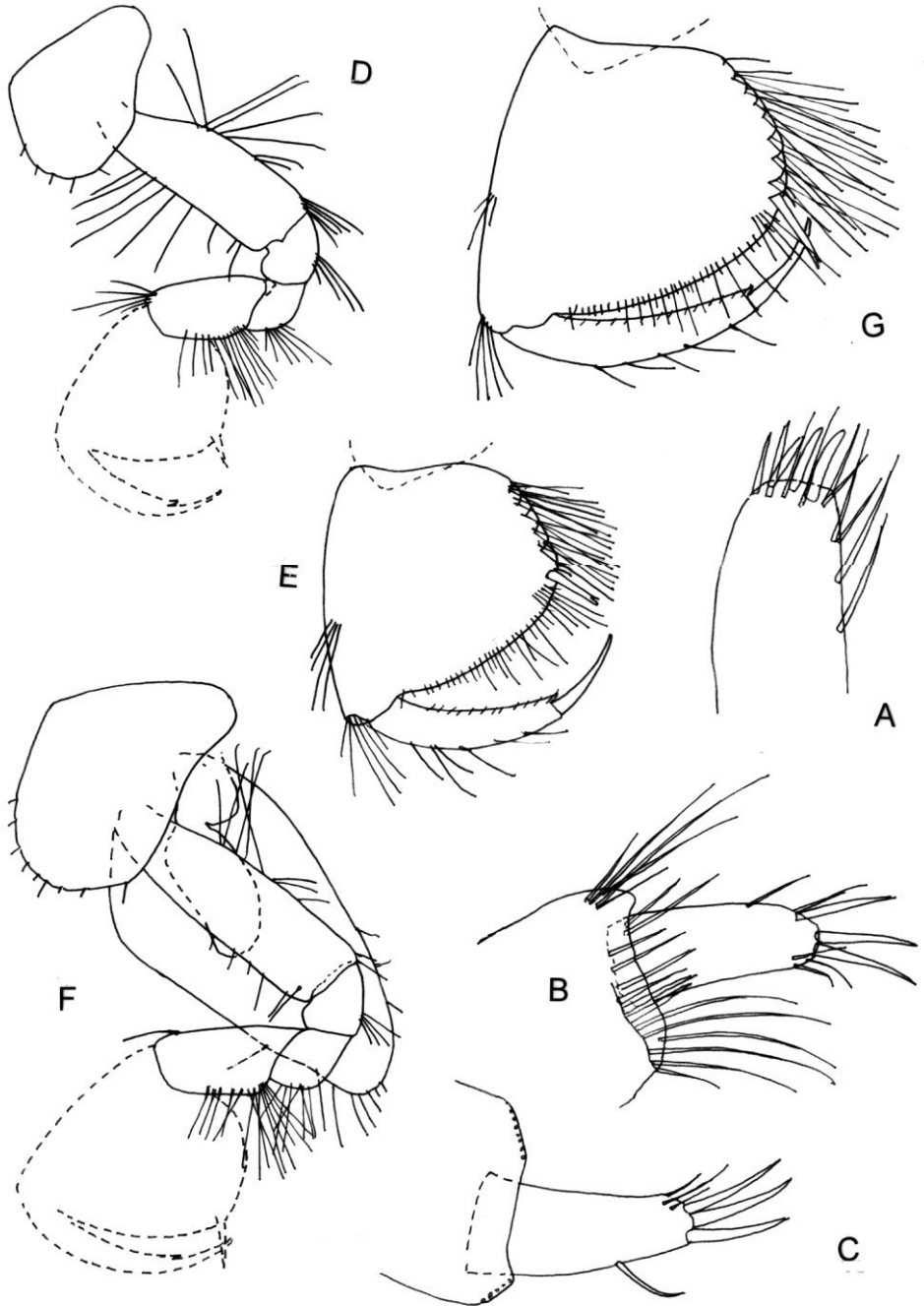


Figure 7. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, female 8.2 mm (paratype): A= inner plate of maxilliped; B= right article 4 of maxilliped palpus; C= left article 4 of maxilliped palpus; D-E= gnathopod 1, outer face; F-G= gnathopod 2, outer face.

Pereopods 5-7 similar to these in male. Pereopod 7: article 2 longer than broad (ratio: 87:54), along anterior convex margin with 6 groups of spine-like setae, along posterior convex margin with 15 short setae, ventroposterior lobe not fully developed (fig. 6G). Articles 4-6 of different length (ratio: 52:70:96), along both margins with bunches of short strong spines; article 2 slightly shorter than article 6 (ratio: 87:96) (fig. 6H). Dactylus shorter than article 6 (ratio: 30:96), at inner margin with one short slender spine and one short seta near basis of the nail, at outer margin with one median plumose seta (fig. 6 I); nail shorter than pedestal (ratio: 20:33).

Pleopods 1-3 with 2 retinacula each; peduncle of all pleopods naked.

Uropod 1: peduncle slightly longer than rami, with dorsoexternal row of strong spines, dorsointernal margin with distal spine only (fig. 8B). Rami equally long, outer ramus with several lateral and 4 distal short strong spines; 2 groups of 1-2 setae are attached at lateral margin; inner ramus with 2 lateral spines and 4 distal short spines (fig. 8B).

Uropod 2: peduncle with dorsodistal spines; rami of equal length: outer ramus hardly recurved, with one lateral and 5 distal short strong spines (fig. 8C); inner ramus with one lateral and 5 distal short spines.

Uropod 3: peduncle rather longer than broad (ratio: 38:22), with several distal spines (fig. 8D). Inner ramus short, consisting of one article, scale-like, shorter than peduncle and provided with one distal spine and lateral short seta (fig. 8D); outer ramus 2-articulated: first article at outer ramus with 3 bunches of spines not exceeding diameter of article itself; along inner margin are attached 5 bunches of short strong spines mixed with single plumose setae longer than spines. Second article much shorter than first one (ratio: 26:125), bearing one lateral and one distal bunch of short simple setae (fig. 8D).

Telson slightly longer than broad (ratio: 73:66), incised over 2/3 of telson-length, rather gaping; lobes tapering distally and provided with 3 short spines (the longest spines not reaching 1/3 of telson-length; a pair of short plumose setae is attached rather over middle of outer margin in each lobe (fig. 6J).

Coxal gills like these in male, not reaching ventral margin of corresponding article 2 of legs (figs. 6D, 7F). Oostegites appear on corresponding legs 2-5, very large, with marginal setae (fig. 7F).

**VARIABILITY.** Rather variable position of distal nails on palpus article 4 in maxilliped was observed; rami of uropod 1 and 2 are of equal length or almost unequal (inner ramus hardly longer).

The stable characters are the presence of 3 nails on maxilliped palpus, presence of numerous slender pectinate spines on outer plate of maxilla 1.

**HOLOTYPE:** male, 8.5 mm. Holotype and paratypes are deposited in KARAMAN's Collection in Podgorica, Montenegro.

**DERIVATIO NOMINIS.** The name of the species "tzanisi" was nominated according to the name of the locality Tzanis on Crete Island.

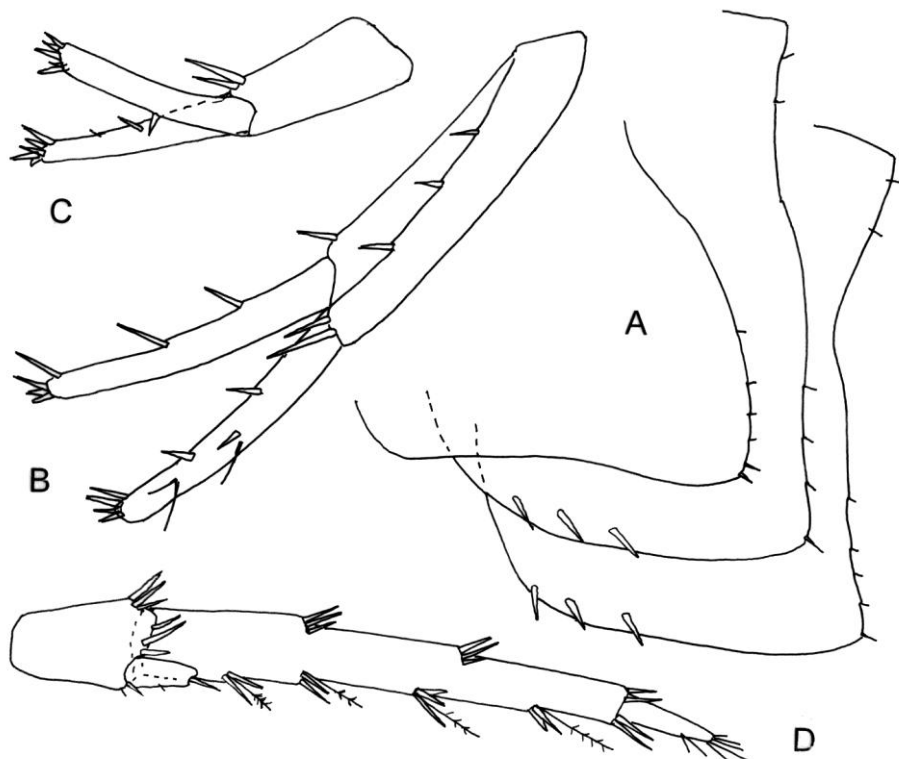


Figure 8. *Exniphargus tzanisi*, gen. n. sp. n., Tzanis spilios, Crete Island, female 8.2 mm (paratype): A= epimeral plates 1-3; B= uropod 1; C= uropod 2; D= uropod 3.

#### REMARKS AND AFFINITIES.

The new species, *Exniphargus tzanisi* differs from all known taxa of the family Niphargidae by presence of 3 distal nails (spines) of maxilliped palpus article 4.

On the other side, within genus *Niphargus* the most of taxa have one nail on maxilliped palpus article 4 (*Niphargus cvijici* S. Karaman 1950, etc.). Some species of this genus are provided with 2 nails of maxilliped palpus article 4 [*Niphargus krameri* Schellenberg, 1935 (loc. typ.: Pazin, Istra, Croatia)], but all other taxonomic characters of *N. krameri* agree with diagnostic characters of genus *Niphargus*.

Genus *Pontoniphargus* has outer plate of maxilla 1 with 7 pectinate spines, and maxilliped palpus article 4 with distal setae (*Pontoniphargus racovitzae* Dancau, 1970).

The presence of elevated number of distal spines on maxilla 1 outer plate was observed in various species of genus *Niphargus* [8-9 spines with one lateral tooth each in *Niphargus remyi* S. Karaman, 1934) (G. Karaman, 2012b), but always with other taxonomic characters of genus *Niphargus*.

There are several other genera within family Niphargidae with numerous distal pectinate spines on maxilla 1 outer plate (genus *Foroniphargus* with 9-10

pectinate spines and genus *Niphargopsis* with numerous (over 20) pectinate spines, genus *Niphargobates* with numerous multiserrate spines), but with different other taxonomical characters.

Among them, genus *Niphargopsis* seems to be more close to *Exniphargus*, but differs from *Exniphargus*, except maxilliped palpus, also by long lobes of maxilliped, very small inner plate of maxilla 1 bearing only one distal seta, *kochianus*-type gnathopods, much higher number of pectinate spines on maxilla 1 outer plate, etc., what eliminate the possibility to identify of taxon *tzanius* to the genus *Niphargopsis*.

By this way, the species *tzanius* differs from all other known genera by combination of taxonomic characters (maxilla 1, maxilliped, accessory flagellum, uropod 3, maxilla 2, etc.) and we created a new genus *Exniphargus*, gen. nov. (diagnosis see above).

The species *Niphargobates orophobata* Sket, 1981 (Type species of genus *Niphargobates* Sket, 1981), described from the subterranean waters of Slovenia, is with following diagnosis:

#### DIAGNOSIS OF GENUS *Niphargobates* Sket, 1981

Accessory flagellum is consisting of one article. Mandible *Niphargus*-like but with high number of rakers, palpus 3-articulate but shortened, palpus article 3 is not subfalciform, bearing distal E-setae only. Maxilla 1 outer plate dilated, with numerous pectinate spines and reduced very small palpus consisting of one article bearing one small distal seta. Inner plate of maxilla 2 is much larger than outer plate. Maxilliped: outer plate is remarkably longer than inner plate, palpus article 4 short, with distal nail. Uropods 1-2 slender, biramous,

uropod 3 very short and stout, inner ramus consisting of one article, scale-like; outer ramus consisting of one article, short, spinose distally and with some lateral strong spines, no plumose setae. Telson very short and broad, broadly excavated distally less than half of telson-length.

TYPUS GENERIS (Type species): *Niphargobates orophobata* Sket, 1981

TAXA: Type-species known only.

DISTRIBUTION: Planinska Jama Cave near Postojna, Slovenia.

The species *Niphargobates lefkodemonaki* Sket, 1990, differs remarkably from the type species of this genus (*Niphargobates orophobata* Sket, 1981}, by elongated uropod 3 having 2-articulated outer ramus, *Niphargus*-like; accessory flagellum 2-articulated; telson normal, *Niphargus*-like; normal distal subfalciform palpus article 3 of mandible, bearing at least D and E-setae. Based on these differences we removed the taxon *N. lefkodemonaki* to the new genus *Niphargobatoides*, gen. nov. as a type species.

#### DIAGNOSIS OF THE GENUS *Niphargobatoides*:

Eyes absent, head with short rostrum, accessory flagellum 2-articulated; labium without inner lobes; mandible *Niphargus*-like, with triturative molar and well developed 3-articulate palpus having A, B, D and E-setae. Maxilla 1 inner plate well developed, with distal setae; outer plate dilated, bearing numerous



recurved serrate spines, palpus shortened, consisting of one article, with distal setae. Maxilla 2 inner plate much larger than outer plate, both lobes with marginal setae. Maxilliped inner plate remarkably shorter than outer plate, palpus article 4 (dactylus) short, with distal nail. Coxae moderately large, coxa 5 almost as long as coxa 4. Gnathopods and pereopods 3-7 like these in *Niphargus*. Pleopods 1-3 well developed. Uropods 1 and 2 biramous, rami well developed, with spines, *Niphargus*-like. Uropod 3 *Niphargus*-like, with inner ramus short, consisting of one article, scale-like, outer ramus 2-articulate, second article short. Telson deeply incised, bilobed, with spines.

TYPUS GENERIS (Type species): *Niphargobatoides lefkodemonaki*  
(Sket, 1990)

TAXA: Type species known only.

DISTRIBUTION: Crete Island, Greece.

### Key to the genera of the family Niphargidae

1. Accessory flagellum 1-articulate.....2
- Accessory flagellum 2-articulate.....5
2. Maxilla 1 palpus consisting of one article.....*NIPHARGOBATES* Sket, 1981
- Maxilla 1 palpus consisting of 2 articles.....3
3. Uropod 3 outer ramus consisting of one short article.....*CARINURELLA* Sket, 1971
- Uropod 3 outer ramus consisting of 2 articles.....4
4. Mandible palpus partially reduced, bearing distal E-setae only. Body slender, elongated.....*MICRONIPHARGUS* Schellenberg, 1934
- Mandible palpus well developed, subfalciform, with at least D and E-setae. Body adapted to the rolling in the ball.....*KARAMANIELLA* Sket, 1962
5. Uropod 3 outer ramus consisting of 1 article.....6
- Uropod 3 outer ramus consisting of 2 articles.....7
6. Maxilla 1 palpus consisting of 1 shortened article.....*NIPHARGOBATOIDES* gen. nov.
- Maxilla 1 palpus well developed, consisting of 2 articles.....*HAPLOGINGLYMUS* Mateus & Mateus 1958
7. Uropod 3 inner ramus well developed, long, exceeding half of outer ramus.....*PONTONIPHARGUS* Dancau, 1970
- Uropod 3 inner ramus short, more or less scale-like, much shorter than first article of outer ramus.....8
8. Maxilliped palpus article 4 with 3 distal nails (maxilla 1 outer plate with numerous pectinate slender spines).....*EXNIPHARGUS* gen. nov.
- Maxilliped palpus article 4 with one, exceptionally 2 distal nails.....9
9. Maxilla 1 outer plate with over 20 pectinate spines..... *NIPHARGOPSIS* Chevreux, 1922
- Maxilla 1 outer plate with 7-8, rarely up to 10 simple or pectinate spines.....10
10. Mandible palpus article 3 partially reduced, bearing distal E-setae only.....*NIPHARGELLUS* Schellenberg, 1938
- Mandible palpus article 3 well developed, subfalciform, with at least D and E setae..... 11
11. Maxilliped inner and outer plate short and broad; maxilla 2 both plates are short and broad; maxilla 1 inner plate very strong, broader than palpus, outer plate with 9-10 pectinate spines, palpus short, not exceeding basis of outer plate spines.....*FORONIPHARGUS* G. Karaman, 1985
- Maxilliped inner and outer plate slender; maxilla 2 both plates are slender; maxilla 1 inner plate is slender, narrowed distally, outer plate with 7-9 simple to pectinate spines, palpus longer, exceeding basis of outer plate spines..... *NIPHARGUS* Schiödte, 1849

## CONCLUSION

Based on the morphological characteristics of the members of the family Niphargidae, we tried to recognize the distinct genera within the family Niphargidae, taking in consideration the most stable taxonomic characters, which are not variable within members of each genus. By this way each genus can be recognize based on certain stable morphological characteristics. This is not the phylogenetic approach, because we tried to facilitate the recognition of single species and genera based on external morphological data. We can suppose that the further genetic and molecular investigations of these taxa will have maybe some different ideas about the division of taxa within the Niphargidae family, but we hope that our work will facilitate further investigations of this problem.

The genus *Niphargus* (sensu auctorum), having polyphyletic origin, probably is composed of more different groups known recently as different subgenera or Complex of species, needs further detailed morphological, genetic and other studies to understand the taxonomy of this genus.

## ACKNOWLEDGEMENTS

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## **N<sub>2</sub>-FIXING TREES FOR PROFITABLE FARM FORESTRY**

### **SUMMARY**

N<sub>2</sub>-fixing casuarinas and acacias are indigenous components of the Australian flora, both families making significant contributions to the nitrogen economy of natural ecosystems. For the brigalow-belah dominated areas in northern Australia, their inputs of nitrogen allowed production of wheat using the high levels of NH<sub>4</sub><sup>+</sup>-saturated clay for many years. However, despite the centres of origin for both families being in Australasia, their potential in Australian farm-forestry using rotations of less than five years has been little explored. Active applied research into the Casuarinaceae (*Allocasuarina*, *Casuarina*, *Gymnostoma*, *Ceuthostoma*) and its N<sub>2</sub>-fixing microsymbiont *Frankia* is largely restricted to India, China and France. Farmers in India are pioneering profitable farming of casuarinas for paper manufacture in 3-year rotational cycles with rice. We propose major investment in a new rural scheme to promote the application of casuarinas on Australian farms and to develop local rural secondary industries with strong cash flow, responsive to market forces. Large scale carbon sequestration (up to 20 tonnes dm/ha/annum) can theoretically be achieved, reducing N-fertiliser use and expanding farmers' product and cash flow options, using vertically integrated production to maximise their value. These forest products include wood and fuel pellets, paper pulp, charcoal for hydrogen production, N-fertiliser and volatile chemicals, even including ammonia and hydrogen. One million ha of casuarinas could produce \$50 million of C-neutral N-fertiliser per annum as by-product, or adequate ammonia as stock for electricity generation or H<sub>2</sub> production using the N<sub>2</sub> fixation reaction for heat storage. This paper will examine the prospect for integrated uses of these under-utilised genetic resources, planning the required applied research in profitable fire-resistant landscape design and community economic development, also confirming feasibility in terms of underlying principles of energetics and action thermodynamics.

**Keywords:** *Casuarinaceae*, *Acacias*, *Frankia*, nitrogen fixation, hydrogen, ammonia

### **INTRODUCTION**

Casuarinas are fast-growing, N<sub>2</sub> -fixing species indigenous to Australia (Ganguli and Kennedy, 2013). This review proposes and examines the feasibility

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of a major innovative research program to make better use of this genetic resource. Firstly, short term (3-5 year) farm-forest rotations are identified as a significant gap in farming research; their increased use in these rotations offers prospects for large scale carbon sequestration (10- 20 tonnes dm/ha/annum) with reduced fertiliser-N use, while improving farmers' product options. Diversity in farm design is important; the more viable choices farmers have, the better they respond to changes in climate, commodity prices and market conditions defining profitability.

Secondly, farm-forestry is increasingly regarded as having potential for providing new options for local secondary industry, maximising value. These activities will be on a local scale and include wood products, paper pulp (Parthiban et al., 2012), charcoal for hydrogen production, N-fertiliser and volatile chemicals, potentially including ammonia and hydrogen gas. Thirdly, farm-forestry with frequent rotation minimising waste materials acting as fuel can be a timely response to national emergencies such as bushfire. Australia's recurrent cycle of flood and excessive vegetation and fuel generation followed by devastating fires, promoted by drought conditions of extreme low humidity has now become a chronic condition. Invariably, there is a lack of preparedness for extreme conditions and huge insurance losses are suffered on an annual basis, quite apart from loss of human life and livestock. By contrast, intelligent and strategic landscape design using all the benefits of GPS mapping could provide a new kind of water and fire management, using all the technological resources available. This future would avoid high risk monoculture stands of forest trees encouraging both disease and fire.

### **Project outputs**

Casuarinas species are chosen as highly water-efficient nitrogen-fixers and an under-exploited natural resource. A comprehensive research project would include the following objectives:

- A significant review of previous research outputs on casuarinas, with reassessment for farm-forest rotations. This should incorporate close examination of successful research elsewhere, particularly in India (Kumaravelu et al., 2012) and in China.
- Experimental field data on farm productivity of casuarinas for carbon and nitrogen fixation in cropland and rangeland landscapes.
- Data on the suitability of farm-forest rotations in areas with steeper terrain or on land potentially needing rehabilitation, such as highly acidified areas (Kennedy, 1992). Other species such as nitrogen-fixing acacias may also be suitable providing biodiversity.
- Data on the potential for farm-forest rotations with deeper roots to provide better water management, remediating acidification.

Managing water tables, often saline, can also be achieved by deep-rooted species - of casuarinas like *C. obesa* (Hollingworth, 2007). An eventual

requirement will be implementation of new carbon sequestering technology on farms, but focus on the preliminary research is needed showing its feasibility. Full implementation will require a new participatory action phase where the technology will be adapted regionally and climatically. The program will also require the support of new government policy, allowing implementation of farm forestry on the large scale. Widespread extension of necessary tools to farming and rural communities will be essential, no doubt with new legislation. Major success in the technology will require social readjustments and reintegration on a large scale, involving more jobs in factories close to farm supplies to minimise transport costs. Only high value timber and other elite forest products can be transported economically to long distances. Power generation, itself a product of the new developments would be localised. A

position paper produced in stage 1 of the program will be a quantitative technical and economic assessment, analysing the potential for farm-forestry to enhance the productive capacity of farms normally growing other produce. The feasibility to establish an ethical supply of renewable energy able to completely replace coal will also be assessed (e.g. 7.5 million ha of casuarinas on favoured farmland, 25% of current Australian cropland, could produce 150 million tonnes of dry wood or pellets for electricity per annum, based on recent Indian data for ethical paper pulp and paper manufacture (Jain, 2010; Parthiban et al., 2012).

## MATERIAL AND METHODS

### Farm forestry

This activity will aim to establish a new farming practice and demonstrate benefits as well as expose the challenges. The outputs from the field trials and data analysis sought will include: (i) information on the nutrients most susceptible to depletion on any site; (ii) the age of greatest nutrient stress; (iii) the quantities of nutrients removed with harvesting strategies; (iv) the potential cost of nutrient replacement; (v) optimum planting density; (vi) best genetics for casuarinas (Suresh et al., 2012), needed for cloned production of highest productivity lines; (vii) optimal ages for harvesting for different purposes (viii) ability to rehabilitate acidified or salinised soil (ix) choice of species and lines to adapt to climate change.

### Biological technologies – microbiology and forest genetics

At the University of Umea, a set of *Frankia* isolates have been maintained by Anita Sellstedt (Sellstedt, 1995), from a prior CSIRO collection of the 1980s. In collaborative research, these and other cultures now cultured at the University of Sydney will be characterised using molecular analysis of ribosomal DNA so that strain typing can be achieved. These techniques will then allow correlations between root-nodulating *Frankia* strains and casuarina productivity to be made, a necessary part of allowing the symbiosis to be optimised for effectiveness and to achieve maximal productivity. *Frankia* are difficult organisms to isolate from soil or nodules, partly because of their slow growth. Molecular techniques will

allow identification of strains and also provide some measure or numbers, using real time PCR, collaborating with overseas experts in this area at the University of Umea (Anita Sellestedt) and in CIRAD in Montpellier, France (Claudine Franche).

### **Plant improvement by breeding and cloning**

In India, large scale breeding of better clones of casuarinas selected for homogeneous and rapid growth has been achieved (Nicodemus et al., 2010; Kannan et al., 2012; Karthikeyan et al., 2013). This work will need to be repeated in Australia, with new selections to examine the range of diversity. In all this research, quantitative information will be sought, including the relative value of products such as timber, paper pulp, charcoal, biomass pellets, organic volatiles, N-fertilisers, ammonia, hydrogen and soil enrichment or depletion. The feasibility to establish an ethical supply of renewable energy able to complement the use of coal or even replace it eventually will be assessed. For example, 7.5 million ha of casuarinas on favoured farmlands could produce 150 million tonnes of dry wood or cogeneration pellets for electricity per annum, of the same order as the current consumption of coal in power stations.

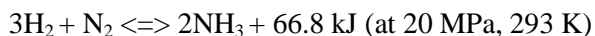
## **RESULTS AND DISCUSSION**

### **Secondary industry**

Forest products are well recognised as supplying valuable feedstock for industry. In Western Australia some progress has been made in mallee farming in harvestable alleys (Sudmeyer et al. 2012), helping with ongoing landscape restoration. This followed a period of excessive land clearing for wheat cropping that seriously failed, leading to widespread salinisation in the southwest of Western Australia.

### **Ammonia as feedstock for H<sub>2</sub> generation**

The root nodules of N<sub>2</sub>-fixing casuarinas are clearly a major source of ammonia, normally used for plant growth. H<sub>2</sub> is often proposed as a clean fuel that would overcome many environmental problems related to pollution. But its main sources such as steam and gas are considered to be too expensive. Electrolysis of water to 2H<sub>2</sub> and O<sub>2</sub> uses large quantities of electricity and has only been used where cheap hydroelectric sources are available. However, ammonia itself can also be used as a viable fuel to directly power motor vehicles. Less known is the fact that the Haber reaction normally used to prepare N-fertilisers can be reversed to produce hydrogen gas, consuming a considerable amount of heat (see Figure 1).



This reaction, usually catalysed using Fe-based catalysts and other metals and high pressures, is unusual in that it is poised and able to be conducted in either direction, depending on the conditions of reaction. As an exothermic



reaction as written, it releases heat raising the entropy of its environment. Technology to allow solar energy to be stored in a system operating in sunlight and then to be reversed at night has already been well developed at a pilot scale (Lovegrove et al., 2004) and is awaiting investment. This approach would be very appropriate for decentralised power generation, using portable factory units able to be installed from pre-fabricated units.

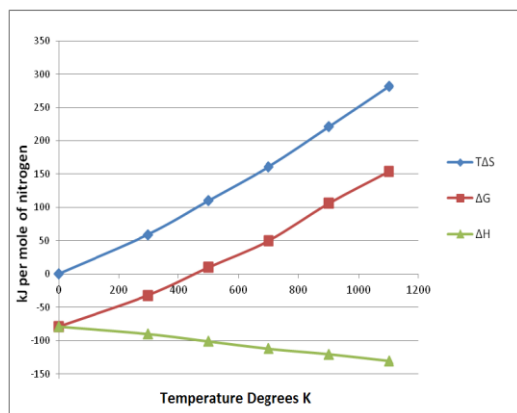


Figure 1: Thermodynamics of the nitrogen fixation reaction  $3\text{H}_2 + \text{N}_2 \rightleftharpoons 2\text{NH}_3$  under standard conditions of 1 atmosphere pressure of reactants and product at temperatures shown.  $K_p$  is equal to 1.0 at 450 K ( $\Delta G=0$ ), where  $\Delta H$  is equal to  $-\text{T}\Delta\text{S}$  and  $\Delta G$  is equal to zero.

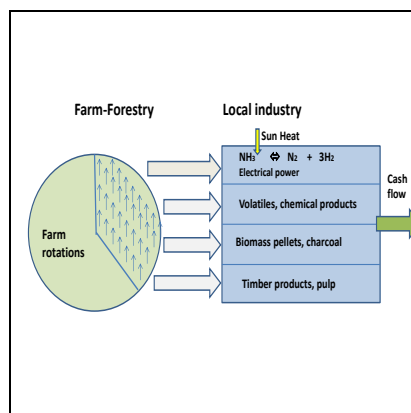


Figure 2: Integrated farm forestry-local industry would be based on an action-entropy model sustained by optimised support resources (Rose et al. 2008) allowing diversification and decentralised populations.

In principle, casuarinas could offer a relatively inexpensive source of ammonia from their foliage, harvested on a continuous basis if farms operate on a 3-4 year rotation cycle. It is not unreasonable to assume that 100 kg of ammonia could easily be harvested per ha on an annual cycle without loss of the forest resource or even more using rotations 1000 ha would yield a minimum of 100 tonnes of ammonia of value \$50,000 at \$US50 per ha. While there is a prospect for making  $\text{H}_2$  which is a highly desired clean fuel, ammonia itself is an excellent fuel, releasing 317 kJ per mol of ammonia burnt ( $\Delta H = -317$  kJ/mol).



By comparison, the reaction of  $\text{H}_2$  with oxygen releases 285 kJ per mole of  $\text{H}_2$  burnt ( $\Delta H = -285$  kJ/mol). The specific energy cost in 2004 was \$US13.3 per GJ (Lovegrove et al., 2004). Thus, one tonne of ammonia would yield 19.2 GJ of energy, worth \$US255, or \$US25.5 yield per ha as electricity.

Hence, ammonia from farm-forests has the potential to be used in small scale electricity stations, forming  $\text{H}_2$  by day while generating solar power and

releasing heat by night allowing continuing power generation from steam (Lovegrove et al., 2004). Such flexibility can be contrasted with the very large economic inertia when chemical products are manufactured in super-large installations by the Haber process or power is generated in facilities able to supply whole states with millions of consumers..

## **CONCLUSIONS**

### **Project outputs**

Ultimately, the outputs of this project would be the basis for new rural industries, on the scale of millions of hectares of products, able to satisfy a significant portion of our needs for fuel (high density casuarina matches coal in calorific content, burning to a pure white ash), paper pulp, chemical products and nitrogen fertiliser. Such production, responsibly integrated for genetic diversity, site specificity and productivity, and product selection governed by current commodity markets, would be certifiable to high standards of environmental stewardship. The project would need to strongly adhere to the ethical guidelines of the Montreal Process (see [www.montrealprocess/](http://www.montrealprocess/)).

### **A new rural vision based on partnerships**

This research and development project will quantify the feasibility of new technologies related to achieving carbon- effective farming using indigenous nitrogen-fixing casuarina species for short-term agroforestry on a 3-5-year production cycle. It will generate detailed information on the potential for soil carbon sequestration from foliage-fall and nitrogen-addition to fertility of the soil in farming-forestry in a range of habitats, using versatile species of casuarina that are collectively capable of growing in a range of habitats and adverse climatic conditions, often too harsh for most other plants. The potential agro-industrial impact of using these trees as an intercrop forestry system in farming landscapes, for land rehabilitation but with particular emphasis on generating cash products such as charcoal, volatile chemical by-products and nitrogen fertilisers will be investigated.

An integrated program (see Figure 2) must be linked to completed research in industrial chemistry such as for the N<sub>2</sub> fixation reaction as a source of electrical power using its heat-storing properties. The data thus obtained could be utilized to generate more sustainable plantations of casuarinas, for increasing soil carbon and fertility in a given habitat in the context of local industries as by-products, similar to those being sought in mallee farming. It will target the greening of vast marginal lands of Australia by significantly increasing the population of N<sub>2</sub>-fixing native casuarina trees. It will aim to reverse the trend of depopulation of the countryside and even to substantially reverse it, making rural communities much more attractive places to live. The overall result would be a more resilient agriculture, united in a diversity of production and rewarding activities

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## **EFFECTIVENESS OF CLINKER ASH IN REMOVING NITRATE NITROGEN FROM AGRICULTURAL WASTE WATER**

### **SUMMARY**

Clinker ash is a type of coal ash generated by thermal power plants. At present, reuse of coal ash has been required, because large amounts of coal ash are generated by coal-fired power plants every year. In this study, the ability of clinker ash to purify nitrogen pollution water was evaluated by using two kind of water, which was contaminated with nitrate nitrogen and ammonium nitrogen.

Four kind of clinker ash, which differ particle size distribution were used as specimens for the column experiment. Each type of clinker ash was placed in a cylindrical column. Then, we prepared two kind of test water contaminated with nitrate nitrogen (10 mg/L) and ammonium nitrogen (10 mg/L). Two-test water was filtered through each column at 200 mL/day for 66 days. The nitrogen concentration of the water that flowed out of the column was measured.

The column experiment confirmed that nitrogen is removed by nitrogen adsorption of clinker ash. Namely, it was clarified that clinker ash has a great capacity to adsorb NO<sub>3</sub>-N and NH<sub>4</sub>-N. It was also found that fine-particle clinker ash is able to adsorb large amount of nitrogen. As the results, we concluded that clinker ash could be useful for nitrogen adsorbent of agricultural wastewater treatment. However, it is necessary to pay close attention to the particle size distribution of clinker ash when developing a water purification system using clinker ash.

**Keywords:** Clinker ash, Nitrate-nitrogen, Ammonium-nitrogen, Adsorption, Wastewater

### **INTRODUCTION**

Nitrogen is an essential component for food and feed production. On the other hand, excessive nitrogen application on farmland is used around the world. Excessive use of nitrogen can lead to nitrogen discharge from farmland to river (Okazawa, et al. 2010 and Yoshinaga, et al. 2007) and to serious problems related to human health and ecosystem vulnerability such as acidification of soil

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and eutrophication of coastal system (Mosier et al, 2004). Therefore, if it were possible to construct a nitrogen mitigation system in which the nitrogen running off of farmland is captured, then agriculture with environmental burdens would be possible.

Coal is a vital role for global energy security. The coal that is used for thermal power plants is a solid, which makes it safer to transport than LNG (Liquefied Natural Gas), and it is more economical than LNG because of its abundant reserves. At present, about 40% of the world's energy is generated by coal-fired power plants (World coal association, 2015). In Japan, 20% of electricity is provided by coal-fired thermal generation. 220 million kg clinker ash, which is a kind of coal ash, emits from coal-fired power plant every year. Clinker ash was specified as an item that should be recycled, since the Revised Recycling Law of Japan established in 2001. However, most of it ends up in landfills without recycle; thus recycling of clinker ash is an important environmental issue (JETA, 2005).

Clinker ash has been reported to be able to purify water contaminated with ammonium nitrogen. Because of clinker ash is a numerous pore material; it has good potential as a water purification material (Yamamoto et al., 2011). Okazawa and Fujikawa (2014) reported that clinker ash has a great capacity to adsorb  $\text{NH}_4\text{-N}$ . It was also found that fine-grained clinker ash is able to adsorb large amount of nitrogen, which can be used by plants as compost. However, nothing is the experimental result relating to nitrate nitrogen contamination. In this study, the ability of clinker ash to purify water was evaluated in laboratory experiments using the cylindrical columns with high concentration nitrate nitrogen solution water and ammonium nitrogen solution water.

## MATERIAL AND METHODS

Clinker ash of four different particle size distributions (No. 1 to No. 4) were used as specimens for the experiment. The particle size distribution curves of No. 1 to No. 4 are shown in Fig.1. The No.1 is the smallest particle size distribution and the No.4 is the largest particle size distribution.

Table 1 shows the physical and chemical properties of four specimens of clinker ash. The coefficient of permeability and the dry density were similar between four specimens and those ranges were  $2.33 \times 10^{-2}$  cm/s to  $1.45 \times 10^{-1}$  cm/s and  $0.81$  g/cm<sup>3</sup> to  $0.93$  g/cm<sup>3</sup>, respectively. The specific gravity of all specimens was  $2.19$  g/cm<sup>3</sup>. The values of pH and EC varied from 8.5 to 8.9 and from 0.1 dS/m to 0.9 dS/m, respectively. Total-nitrogen (T-N) included in the clinker ash before the experiment ranges from  $2.3 \times 10^{-5}$  kg/kg to  $2.9 \times 10^{-5}$  kg/kg and those values were very low.

Column experiment had been conducted in the laboratory (Temperature is 24°C and illumination is 550 lux) to examine the nitrogen purification (adsorption) capacity of clinker ash as shown in Fig. 2. Each specimen was poured into the PVC cylindrical column that has 44 mm in diameter and 350 mm in length. Two kinds of solution water, which contaminated by nitrate nitrogen

with 10 mg/L and ammonium nitrogen with 10 mg/L supplied to each column at a flow rate of 100 to 200 mL/d for 66 days. The drainage tube was lifted in the column upper part to make it a saturation state. The Total Nitrogen (Total-N) concentration of the water that flowed out of the column was measured every second or third day.

The nitrogen balance in each column during the experimental period was calculated by Eq. (1).

$$L_{in} = L_{out} + L_{ads} \tag{Eq. (1)}$$

where  $L_{out}$  is nitrogen discharge from each column (mg),  $L_{in}$  is nitrogen supply to each column (mg),  $L_{ads}$  is nitrogen removed in each column (mg). In this study, we assumed that  $L_{ads}$  is equal to nitrogen adsorption on clinker ash.

### RESULTS AND DISCUSSION

Figs.3 and 4 shows the time series data of nitrogen concentration in drainage water in each column. In the column experiment with NH<sub>4</sub>-N solution water, all specimens recorded the lowest Total-N concentrations at 2.99 mg/L (No.1), 3.01 mg/L (No.2), 2.87 mg/L (No.3) and 2.98 mg/L (No.4) at 1st or 2nd day, respectively. From then on the concentration rose gradually, stabilizing in the range between 6 mg/L to 10 mg/L. All specimens also recorded lower concentrations.

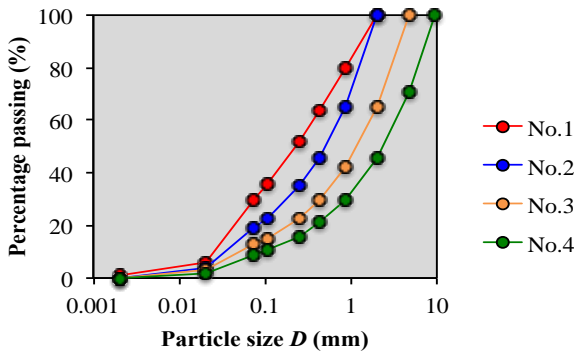


Fig.1 Particle size accumulation curve

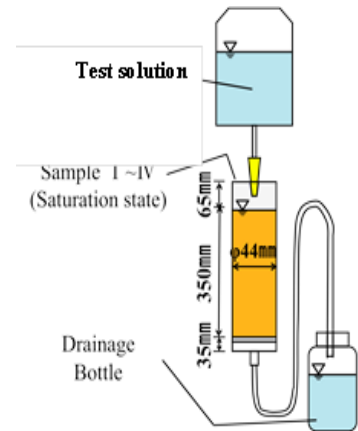


Fig.2 Apparatus of column experiment

Total-N than that in the incoming solution (10 mg/L) throughout the experiment period, consistently demonstrating the presence of a water purification mechanism. It was clarified that clinker ash contained many fine particles seems to be effective in mitigating nitrogen pollution of water, because the Total-N concentration of No.1 and No.2 were lower than those of No.3 and No.4.

Table 1 Physical and chemical properties of four specimen(s) of clinker ash

S	Physical properties*				Chemical properties**		
	PS	CP k (cm/s)	SG $\rho_s$ (g/cm <sup>3</sup> )	DD $\rho_d$ (g/cm <sup>3</sup> )	pH <sub>1:2.5</sub>	EC <sub>1:5</sub> (dS/m)	Total-N ( $\times 10^{-5}$ kg/kg)
No 1	Finest	1.09E-02	2.19	0.93	8.5	0.3	2.4
No 2	Fine	2.33E-02	2.19	0.89	8.6	0.2	2.6
No 3	Coarse	1.45E-01	2.19	0.88	8.9	0.2	2.9
No 4	Coarsest	5.43E-01	2.19	0.81	8.9	0.1	2.3

\* PS; Particle size, CP; Coefficient of permeability, SG; Specific gravity, DD; Dry density\*\*EC; Electric conductivity

In the column experiment with NO<sub>3</sub>-N solution water (Fig.4), on the other hand, recorded the highest Total-N concentrations, at 5.0 mg/L (No.1), 9.28 mg/L (No.2), 6.26 mg/L (No.3) and 6.67 mg/L (No.4) at 1st day, respectively. Then, the Total-N concentration of water that ran through the column decreased to the smallest values by the 12th day for all specimens.

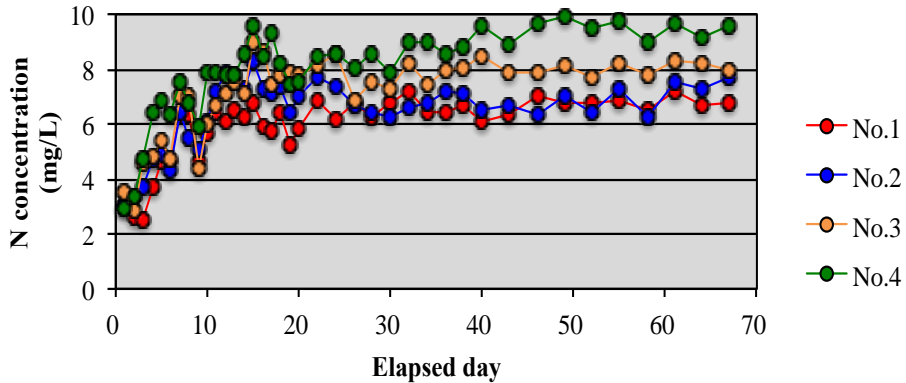


Fig.3 Time series data of outflow nitrogen concentration  
(The result of the column experiment with 10mg/L of NH<sub>4</sub>-N solution water)

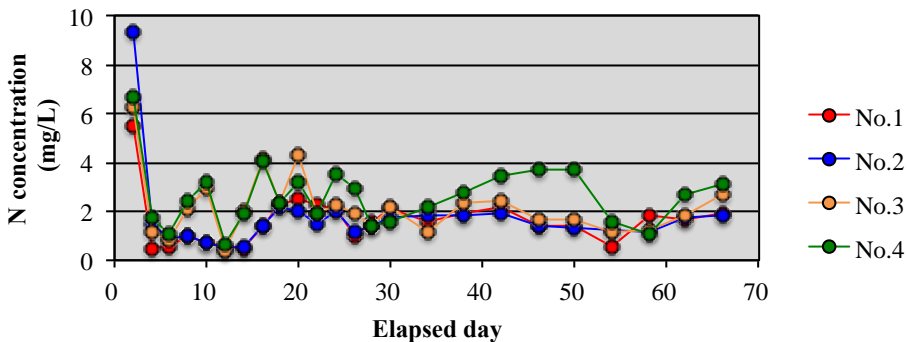


Fig.4 Time series data of outflow nitrogen concentration  
(The result of the column experiment with 10mg/L of NO<sub>3</sub>-N solution water)



Table 2. Nitrogen balance of the column experiment for 66 days by using 10mg/L of NH<sub>4</sub>-N and NO<sub>3</sub>-N solutions water as a test water

NB	NH <sub>4</sub> -N solution water as a test water				NO <sub>3</sub> -N solution water as a test water			
	No 1	No 2	No 3	No 4	No 1	No 2	No 3	No 4
<i>L<sub>in</sub></i> (mg)	139.2 100%	139.8 100%	143.0 100%	139.3 100%	134.4 100%	130.3 100%	137.7 100%	133.6 100%
<i>L<sub>out</sub></i> (mg)	87.1 63%	93.1 67%	107.1 75%	118.7 85%	21.7 16%	22.6 17%	34.9 25%	37.0 28%
<i>L<sub>ads</sub></i> (mg)	52.1 37%	46.6 33%	35.8 25%	20.6 15%	112.7 84%	107.8 83%	102.8 75%	96.6 72%
Calculation method of nitrogen balance: Nitrogen balance defines as the equation; $L_{in} = L_{out} + L_{ads}$ <i>L<sub>in</sub></i> ; nitrogen supply to a column (mg), <i>L<sub>out</sub></i> ; nitrogen discharge from a column (mg), <i>L<sub>ads</sub></i> ; nitrogen removed in each column (mg).								

The Total-N concentration showed great variations. The changes in Total-N concentration in the water of Nos. 1, 2 and 3 were great throughout the experiment period. The Total-N concentration values of the water after filtration for all specimens were much smaller than those before filtration (10mg/L) throughout the 66 days of the experiment period. The above results demonstrate that the ratio of fine particle size of clinker ash is related to the performance of nitrogen mitigation. Furthermore, for all the specimens, the concentration of Total-N showed a steady level, even with increases in the elapse day. Based on the above finding, it was clarified that nitrate nitrogen was constantly removed for the long term.

Table 2 shows the nitrogen balance in each column for 66 days by using NH<sub>4</sub>-N solution water and NO<sub>3</sub>-N solution water. In the case of NH<sub>4</sub>-N solution water, the ratios of effluence (*L<sub>out</sub>* /*L<sub>in</sub>*) of each specimen were respectively 63% (No.1), 67% (No.2), 75% (No.3) and 85% (No.4). This illustrates that when the nitrogen solution passes through a column containing clinker ash, the nitrogen can remove from wastewater.

In the case of NO<sub>3</sub>-N solution water, the ratios of effluence (*L<sub>out</sub>* /*L<sub>in</sub>*) of each specimen were respectively 16% (No.1), 17% (No.2), 25% (No.3) and 28% (No.4). Those values were extremely lower than the result of NH<sub>4</sub>-N solution water. It was clarified that clinker ash was more effective in purifying NO<sub>3</sub>-N wastewater than NH<sub>4</sub>-N wastewater as an adsorption material.

## CONCLUSIONS

The column experiment confirmed that nitrogen is removed by nitrogen adsorption of clinker ash. Namely, it was clarified that clinker ash has a great capacity to adsorb NO<sub>3</sub>-N and NH<sub>4</sub>-N. It was also found that fine-grained clinker ash is able to adsorb large amount of nitrogen. As the results, we concluded that clinker ash could be useful for nitrogen adsorbent of agricultural

wastewater treatment. However, it is necessary to pay close attention to the particle size distribution of clinker ash when developing a water purification system using clinker ash. In addition, we have to clear the mechanism of nitrogen removal by clinker ash in high concentration nitrogen solution water, since the mechanism of nitrogen removal by clinker ash could not be clarified in this study.

### ACKNOWLEDGEMENTS

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## **TEMPORAL EVOLUTION OF THE WATER CHEMICAL QUALITY FOR AGRICULTURAL IRRIGATION IN PEGO (ALICANTE, SPAIN)**

### **SUMMARY**

The purpose of this communication is to present the evolution of the chemical quality of surface water and groundwater used for irrigation of citrus and vegetables in Pego village (Southeast of Spain), during the years 2004-2014, considering the annual rainfall and the vulnerability of the area to the real risk of seawater intrusion and contamination by the application of organic fertilizers and synthetic origin. This is because irrigation is an area near the Mediterranean coast from a distance of between 2 and 11 km.

It is a very interesting study area because it brings together two Cretaceous aquifers formed by dolomites and limestones cracked very karst landforms formed by geological materials of different chemical and solubility composition with other detrital aquifer quaternary occupying the flat and depressed area and terrain marl and clay that forms a swampy marsh (dedicated to rice and natural park) that separates it from the sandy shoal and the line of dunes along the beach of the Mediterranean Sea immediate sand.

**Keywords:** groundwater, seawater intrusion, electrical conductivity, chlorides, nitrates, salinity.

### **INTRODUCTION**

The coastal plains are physiographic units of great importance for society, especially, when we study demographic and economic indicators of the municipalities. One can say that the center of gravity in many regions, as they are in Valencia, is located in the coastal plains.

These physiographic units bordering the foothills of the mountainous terrain, it is a result of the deposition of porous sediments from erosion, which extensively filled sunken tectonic structure that forms the coastline. Below, aquifers, technically called multilayer aquifers, have historically enabled the development of irrigated agriculture that has characterized the coastal regions worldwide with favourable climates, such as in the Mediterranean.

The undercurrents flowing through aquifers between descaling mixture of clays and alluvial gravel pebbles. These are the continental water recharge and discharge head to the coast this aquifer forming a characteristic circulatory system.

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These aquifers in the Spanish Mediterranean coast, such as Pego Village, enter into and bind to the valleys occupied by low course of the main surface drainage systems, which are traditional municipal orchards that have needed intensive aquifer throughout the twentieth century and so far this century.



Figure 1: Pego Valley view

Pego Valley has a horseshoe shape, with the people and the sea beyond the east. To the left of the image of Figure 1 shows the output of the aquifer Benicadell-Almirall-Mostalla, north of the valley; the right end, the aquifer Alfaro-Migdia-Segària is observed on south valley; Limit centre at sea, the quaternary aquifer Gandia-Denia. The flat area is the agricultural area irrigated by wells both Cretaceous aquifers and the marsh seated on the Quaternary aquifer.

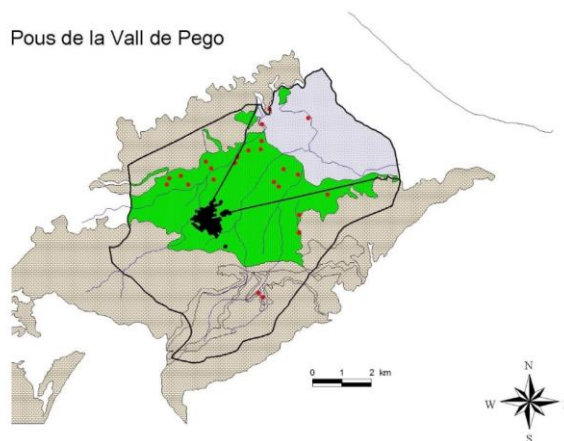


Figure 2: Pego area map

The map (figure 2) shows schematically the indications of figure 1 photograph. We can see the position of the irrigation wells, separated quaternary aquifer sea (dark line), (green) farmland and both Cretaceous aquifer at the foot of the mountains bordering the valley.

The work is based on the chemical characterization of the evolution of these aquifers, with the analytical water wells and some surface water irrigation in the municipality of Pego. We want to show the annual evolution of the quality of water and rainfall, the distance to the waterfront and the geological substrate of irrigated land, with regard to endogenous ions and mainly exogenous.

Since 2004, we analyzed nine wells continuously supplying virtually the entire municipality and three surface water between the two Bullent (Benirrama) and Racons (Marsh) rivers delimiting the marsh in the Quaternary aquifer and an artesian well in the centre there of (Ullal Bullentó). These rivers and surface outcrops favour the recharge of coastal aquifers and seawater intrusion dimmed. This is a serious problem in all semi-arid coastal areas of the world, not just the shores of the Mediterranean.

### **MATERIAL AND METHODS**

The study is conducted by collecting the water sample from each well and the surface in summer (July), when the water needs of crops are maximum and minimum rainfall, characteristic of the Mediterranean climate, during the intensive irrigation season.

In short, this is a field where the first activity is proceeding with the collection of samples from different waters, then make the necessary laboratory analysis of water.

For this, the protocol analysis pH, electrical conductivity and different ions, both anions and cations, follows international standards quantization thereof. The electrical conductivity is measured with a conductivity meter, a pH meter for pH, volumetry for  $\text{Cl}^-$  and  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$  for colorimetry, photometry issue for  $\text{Na}^+$  and  $\text{K}^+$  and atomic absorption photometry for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .

### **RESULTS AND DISCUSSION**

Although fieldwork has many variables not controlled, it shows that despite the quantitative variations of the different parameters studied, usually we can observe a gradual increased electrical conductivity of all the waters of the aquifers.

Even more troubling is a slow, steady increase in the concentration of chloride ion indicator of progressive marine intrusion affecting this area, and in general the entire Mediterranean coast.

The advancement of marine intrusion is confirmed also by higher content of sodium and potassium ions. This can be seen in the following graphics (Fig. 4 and Fig. 5).

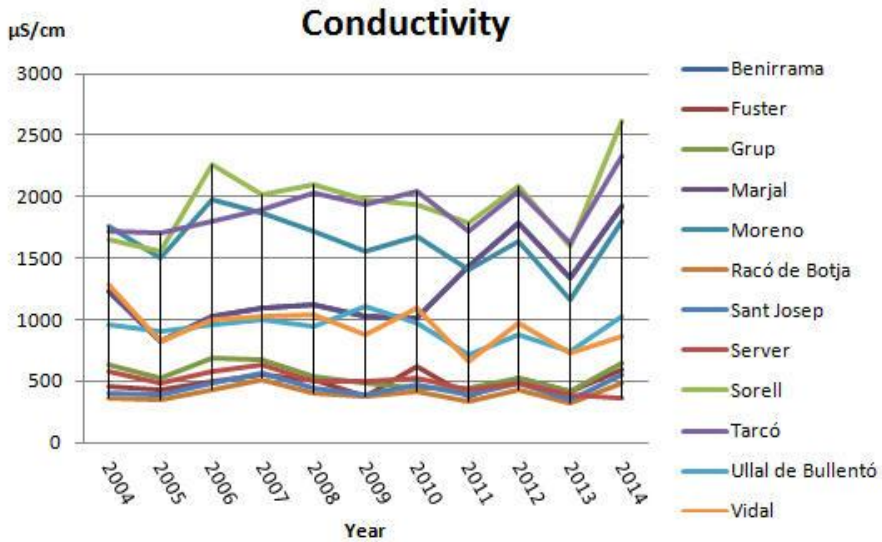


Figure 3: Conductivity evolution

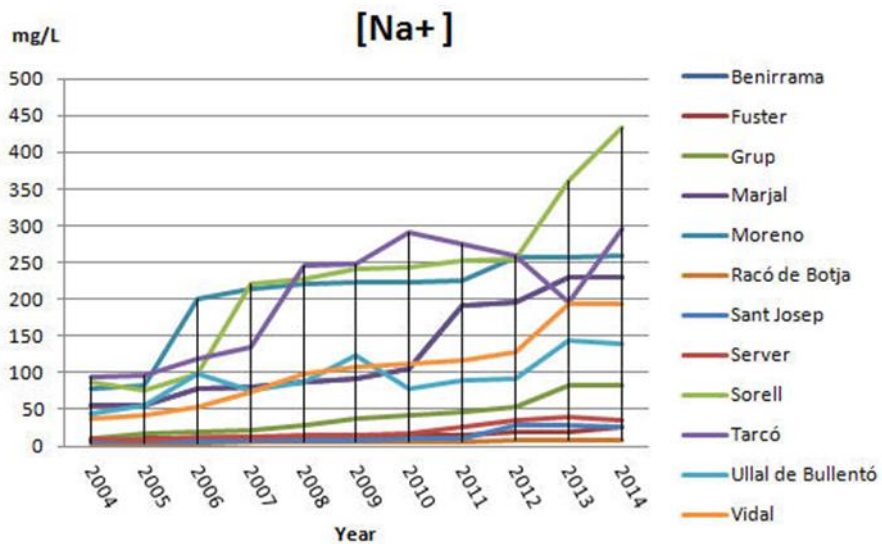


Figure 4: Sodium ion evolution

Although there may be a scenario under discussion regarding the effects of climate change, it should be noted that, according to data from the Observatory of Pego, the annual rainfall in recent years has decreased considerably and otherwise average temperature has had regular and persistent heat waves due to warm winds from the south and west. So 738.1 L / m<sup>2</sup> were recorded in 2013, with an average annual temperature of 19.8 °C, and only 350.5 L / m<sup>2</sup> was

amounted to in 2014 and the average annual temperature was 21.1 °C without so far in 2015 present a more optimistic outlook.

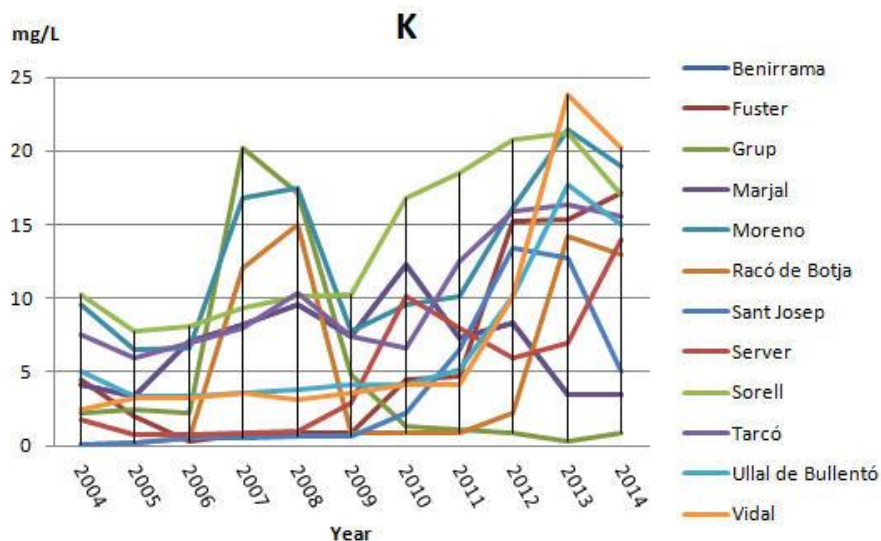


Figure 4: Potassium ion evolution

## CONCLUSIONS

Improper use causes very important qualitative and quantitative changes in water quality of these aquifers, which may be unsuitable for human use and agriculture.

We have found a gradual increase in the electrical conductivity of the water with the annual monitoring carried out. While soft containment of seawater intrusion in some coastal areas is observed. Possibly, this is due to lower water pumping for irrigation to be abandoned in recent years many citrus orchards. This fact is confirmed by the diminution of the concentration of nitrates, which is directly related to the amount of nitrogen fertilizer applied.

Other observations may be due to the effect of increased drip irrigation, which implies a more smooth and continuous extraction of water by pumping and reduced surface infiltration in some areas for development of the agricultural and natural space.

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## **RELATIONS BETWEEN FOOD AND NUTRITION SECURITY, DIETS AND FOOD SYSTEMS**

### **SUMMARY**

The food arena, which is at the interface of different economic sectors, is characterised by the presence of numerous actors with often different and even competing agendas. This is exacerbated by the lack of a deep and common understanding of the issues at play and their interdependencies. The review paper aims at highlighting the multifaceted and multidimensional relations and linkages between food and nutrition security, diets and food systems in the context of sustainability. The paper addresses also other issues, which are relevant in sustainable food systems context, such as sustainable consumption and production, typical and traditional foods, and food losses and waste. Food security is built on four pillars: availability, access, use, and stability. It is strongly linked to nutrition security. Sustainable diets are environmentally-friendly and contribute to food security and healthy life. The Mediterranean diet, which encompasses a plethora of traditional and typical foods, is considered by many scholars as an example of sustainable diet. Food systems should deliver food security and nutrition for present and future generations that is why all components of food systems should be sustainable, resilient and efficient. Moving towards more sustainable food systems implies also reducing food losses and waste along the food chain. Sustainability should be considered as a dimension of long-term food security. From such a perspective the concept of sustainable diets can play a key role. To address food and nutrition challenges, food systems have to be considered in their entirety, acknowledging the interdependency of consumption and production. These linkages are shown in the global Zero Hunger Challenge initiative and should be highlighted and further operationalized in global food-related strategies (e.g. 10 Year Framework of Programs on Sustainable Food Systems) as well as regional strategies such as the Sustainable Consumption and Production Action Plan for the Mediterranean.

**Keywords:** Food and nutrition security, Sustainable diets, Mediterranean diet, Food systems

### **INTRODUCTION**

The interactions between the diverse actors and processes in food systems are more complex than meets the eye (IPES-Food, 2015). Food system is at the

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interface of different economic sectors. The food arena is characterised by the presence of numerous actors with often different and even competing and contradictory agendas (Hopkins et al., 1982). This is exacerbated by the lack of a common understanding of the interdependencies of food-related issues at play. From persistent undernutrition to burgeoning obesity rates, from land evictions to agriculture's soaring environmental footprint, from dwindling fish stocks to mounting food waste, there has rarely been so much attention on the problems within food systems. However, there has been a tendency among scientists and policymakers to address the problems as individual pieces of the puzzle, and to over-look their interrelations (IPES-Food, 2015).

Realizing development and sustainability goals such as the reduction of hunger and poverty and the improvement of rural livelihoods and human health requires acknowledging the multifunctionality of agriculture and food sector. The challenge is to simultaneously meet development and sustainability while increasing agricultural production (IAASTD, 2009).

Unsustainable food consumption patterns are putting increasing stress on ecosystems, the supply of resources, goods and services, and human social systems and well-being. Food consumption and production patterns are among the most important drivers of environmental pressures (e.g. land degradation, declining soil fertility, unsustainable water use). The social and economic costs of diet-related illnesses are straining individuals, families and national healthcare budgets.

FAO (2012) pointed out that ending hunger requires that food consumption and production systems achieve more with less resources which encompasses fostering sustainable intensification of food production, encouraging sustainable food consumption and reducing food losses and waste. Drivers for consumption patterns and lifestyles are economic, technological, cultural, social and political. Sustainable food consumption is a powerful driver to reduce ecological scarcities and improve social equity. It involves harnessing global demand by promoting more sustainable diets.

The present review paper aims at highlighting relations and linkages between food and nutrition security, diets and food systems. The paper addresses also other issues such as sustainable consumption and production, Mediterranean diet, typical and traditional agro-food products and food losses and waste, which are relevant in the context of sustainable food systems. The ultimate aim is to see how sustainable diets can help developing sustainable food systems and achieving food and nutrition security.

## **RESULTS AND DISCUSSION**

Food security exists when all people at all times have physical, social and economic access to sufficient, safe and nutritious food. Food and nutrition security embraces meeting energy, protein and nutrient needs for healthy life (CFS, 2012). The Rome Declaration on World Food Security in 1996 defined its three basic dimensions as: availability, accessibility and utilization. In 2009, at

the World Summit on Food Security, the dimension of stability/vulnerability was added (Berry et al., 2014). Therefore, food security is built on four pillars (CFS, 2012; UN-HLTF, 2011; Ericksen, 2011): food availability: sufficient quantities of food available on a consistent basis; food access: having sufficient resources to obtain appropriate foods for a nutritious diet; food use: appropriate use based on knowledge of basic nutrition and care; and stability in food availability, access and utilization.

Food security is a problem from the individual to the global level: it is an individual issue; yet policies deal with it mostly at the national level, and it is measured at best at the household level. The existence of food security in a nation necessitates that all four interrelated and interdependent dimensions are present (Berry et al., 2014). Food security is a complex sustainable development issue. Ensuring that sufficient nutritious foods are available to all people and that they can access these foods at all times are critical elements of economic and social development.

A comprehensive approach for tackling the issue of food and nutrition security requires: (i) taking into account the interconnectedness and interactions between the food and nutrition security dimensions (availability, access, utilization and stability); (ii) addressing the full spectrum of food and nutrition security, including food production, sourcing and distribution; and (iii) ensuring multi-sectoral engagement and coordination of sectoral policies (e.g. agriculture, trade, health, education, nutrition) (UN-HLTF, 2011).

Food production and its physical availability are certainly essential to addressing hunger. However, producing more food alone will not be sufficient to achieve food and nutritional security. In fact, food and nutrition security is about more than just producing sufficient food as it encompasses the need to ensure access to food at all times. Achieving sustainable food security requires transition towards more sustainable food consumption patterns and diets and working on both sides of the food chain i.e. food production and food consumption. The focus on sustainable diets integrated in a wider food system (Fig. 1) is original in this sense and allows grasping the different facets and dimensions of food and nutrition insecurity (Capone et al., 2014).

Modern agro-food systems failed to resolve the problem of food insecurity. While the world currently produces enough food for its citizens, about 795 million people are undernourished (FAO, IFAD and WFP; 2015) while 1.4 billion are overweight or obese. Micronutrient malnutrition, often referred to as “hidden hunger”, affects approximately 2 billion people worldwide, more than one third of the global population. Food insecurity and malnutrition (undernutrition, overnutrition, and micronutrient malnutrition) problems are widespread. These as well as the social, economic and environmental impacts of the current food consumption patterns and diets highlight the inadequacy of the global food system (Capone et al., 2014).

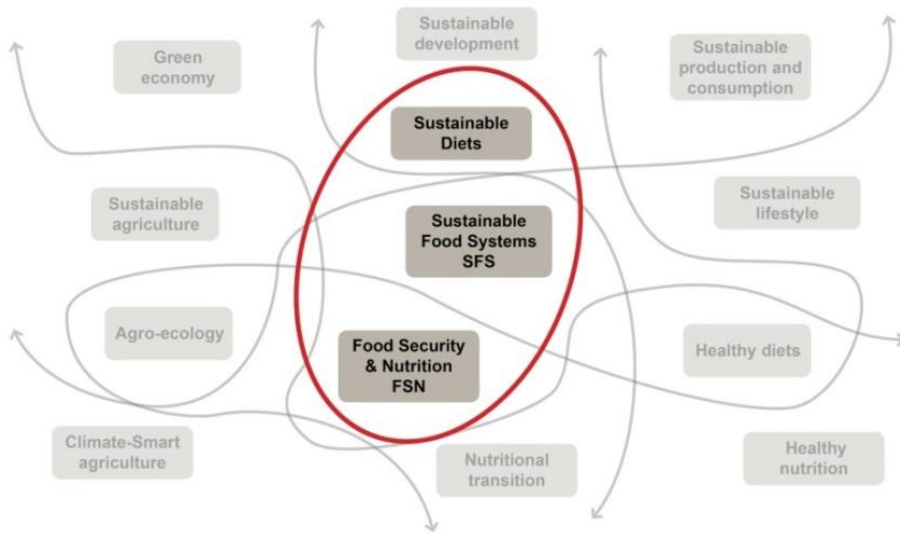


Figure 1. Sustainable diets in the contexts of sustainable food systems and food security and nutrition (Source: Gitz, 2014).

Food systems have to be considered in their entirety, acknowledging the interdependency of sustainable consumption and production. An analytical lens is needed in order to understand the various problems in food systems as the component parts of wider systemic problems (IPES-Food, 2015). In July 2014 the High Level Panel of Experts on Food Security and Nutrition (HLPE) provided the following definition for a food system: “A food system gathers all the elements (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities that relate to the production, processing, distribution, preparation and consumption of food and the outputs of these activities, including socio-economic and environmental outcomes”. Food systems overlap with agricultural systems in the area of food production, but also comprise the diverse set of institutions, technologies and practices that govern the way food is marketed, processed, transported, accessed and consumed (Ericksen, 2011).

Food systems also refer to the vast web of sectoral policies and regulatory frameworks (e.g. agriculture, environment, health and safety, trade, energy, etc.) that shape food systems as they interact with one another (IPES-Food, 2015). Previous policies and actions fell short from addressing the problem of food and nutrition security. In fact, most of the previous strategies focused on food availability - adopting a quantitative approach aiming at increasing agricultural production - while little attention has been devoted to the other components of food and nutrition security mainly food accessibility and food utilisation. The challenge of feeding the growing world population requires new strategies and approaches (Capone et al., 2014).

To address food and nutrition challenges, food systems have to undergo radical transformation for improving resource efficiency, improving equity and transitioning towards sustainable diets. According to FAO & Bioversity (2012) “Sustainable diets are those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources”. Transformation of food systems towards sustainable diets is an essential part of sustainable development. It offers vast economic and social opportunities, while preserving natural resources.

Changes in patterns of food production and consumption force to rethink agricultural systems and diets. Simplification of diets led to diets low in variety but high in energy that do not solve the problems of undernutrition and micronutrient deficiencies but also promote obesity and chronic diseases. A sustainable diets approach aims to address nutrition requirements, both in terms of energy and nutrients and resources used for food production, including local biodiversity, used to produce traditional and local foods. Traditional and typical foods represent the opposite of mass food production. They date back to before the large-scale alteration of traditional food production processes. Traditional foods can be at the epicentre of the effort to preserve diversity and collective identity. Communities and cultures that maintain their own traditional food systems are better able to conserve local food specialties with a corresponding crop and animal diversity (Trichopoulou, 2012). The Mediterranean diet offers a clear example.

The Mediterranean diet (MD), which encompasses a plethora of traditional and typical foods, is currently studied by CIHEAM and FAO as a case study for the assessment of the sustainability of dietary patterns in the Mediterranean area (Lacirignola et al., 2012). The Mediterranean diet, recognized by the World Health Organisation (WHO) as a healthy eating pattern and included in 2010 in the UNESCO’s List of Intangible Cultural Heritage of Humanity, represents a valid tool for promoting more sustainable consumption and production patterns.

The MD has been reported as a model of healthy eating. A greater adherence to it has been associated with significant health, nutrition and environmental benefits. People adhering to the Mediterranean dietary patterns comply better with recommended nutrient and micronutrient intakes. Different studies have confirmed the beneficial role of the MD with regard to mortality from all causes including cardiovascular diseases (CVD) and cancer, as well as obesity, type 2 diabetes and degenerative diseases. The Mediterranean dietary pattern (MDP) has also low environmental footprints and contributes to reducing biodiversity loss. The MDP can also effectively contribute to the worldwide efforts for climate change mitigation as it encompasses lower carbon footprints i.e. greenhouse gas emissions. Therefore, it should be promoted as a cornerstone in public health and sustainable consumption strategies. Unfortunately, there is

an ongoing decrease of adherence to the Mediterranean dietary pattern (CIHEAM & FAO, 2015).

Agriculture, food security, nutrition and sustainability are increasingly discussed in the same context (Lang, 2009). More recently, intergovernmental processes have emphasized the importance of food security as part of sustainability and vice-versa (Berry et al., 2014). There are very strong linkages between food and nutrition security, and food sustainability. They intersect in agricultural and food systems at the global, national and local levels. A sustainable food system supports food security. Food and nutrition security is a cornerstone of sustainable diets and food consumption patterns (Capone et al., 2014). According to Garnett (2013), broadly three perspectives are emerging on how to achieve sustainable food security and food system sustainability: efficiency oriented, demand restraint and food system transformation.

According to the HLPE (2014) “A sustainable food system (SFS) is a food system that delivers food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition for future generations are not compromised”. The unsustainability of food systems is the main reason for the existence of food insecurity and malnutrition; if food systems do not perform adequately in their environmental, economic and social dimensions, food security and nutrition are threatened. For ensuring food security and nutrition all components of food systems should be sustainable, resilient and efficient.

The unique opportunity to make agriculture and food systems more sustainable should not be missed. The primary objective of agriculture is to produce enough food to feed 9 or 10 billion people by 2050. This largely needs to be accomplished by crop and animal productivity increases as well as reducing food losses and waste and changing diets, always keeping in mind that the Earth’s natural resource base is finite. Aspirations of maximum consumption should be replaced by patterns of optimized consumption (Capone et al., 2014).

About a third of the global food production is lost or wasted with severe impacts on the world’s economy and environment (Gustavsson et al., 2011). Food losses and waste prevention and reduction would allow meeting the food needs of about one billion undernourished people. Reducing post-harvest losses and food waste – by using the available knowledge and technologies, education campaigns and adopting innovative legal frameworks – would reduce waste and ease the pressure on agro-ecological systems to meet the growing food demand, thus, contributing to improving food systems sustainability and ensuring food security and nutrition.

Any future initiative on food and nutrition security should embrace the sustainability paradigm and involve all the relevant actors along the food chain, create linkages with the existing global and regional multi-stakeholder processes and develop a multi-faceted research agenda. Research activities are needed to analyse the sustainability of the current dietary patterns in order to design

appropriate policy actions and measures (Capone et al., 2014; CIHEAM & FAO, 2015).

Sustainability can be considered as a precondition for long-term food security. The environment, climate and the obtainability of natural resources, are a precondition for the availability of food. Economic and social sustainability are necessary for the accessibility of all to food. Social sustainability is also a determinant for the utilization of food. Together, the three dimensions of sustainability – social and economic and environmental – also ensure the stability of the systems on which depends the constancy of the other dimensions of food security. On the other hand, food security is considered increasingly as a condition for sustainability (Fig. 2) (Berry et al., 2014).

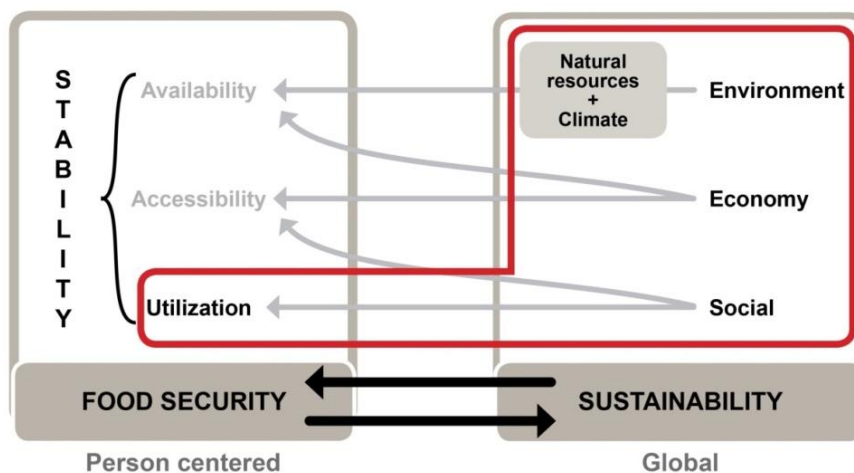


Figure 2. Linkages and interrelationships between food security and food systems sustainability (Source: Gitz, 2014; Berry et al., 2014).

Sustainability is a general concept applicable across all food security dimensions. Inclusion of sustainability recognizes that continued healthy well-being is the goal of sustainable food security (fig. 3) (berry et al., 2014). Sustainability should be regarded as an integral part of food security planning, monitoring and evaluation in determining the long-term viability of food system chains (searchinger et al., 2013). However, getting the concept of sustainability on the political agenda is a challenge as, in fact, the integration of food security as an explicit part of the sustainability agenda would go a long way towards such a goal. The task ahead is to build food security on sustainability and vice-versa (tilman & clark, 2014). The final common pathway of all these efforts is towards sustainable food security and nutrition (berry et al., 2014).

Sustainability should be considered as part of the long-term time dimension in the assessment of food security. From such a perspective, the concept of sustainable diets can play a key role as a goal and a way of maintaining nutritional well-being and health while ensuring the sustainability

for future food security. Without integrating sustainability as an explicit dimension of food security, today's policies and programmes could become the very cause of increased food insecurity in the future (Berry et al., 2014). Sustainability must serve as the benchmark for food systems reform, and to do so, it must be defined at the appropriate scales. Sustainability must also be defined in all of its dimensions, in line with the emerging definition of sustainable diets (IPES-FOOD, 2015).

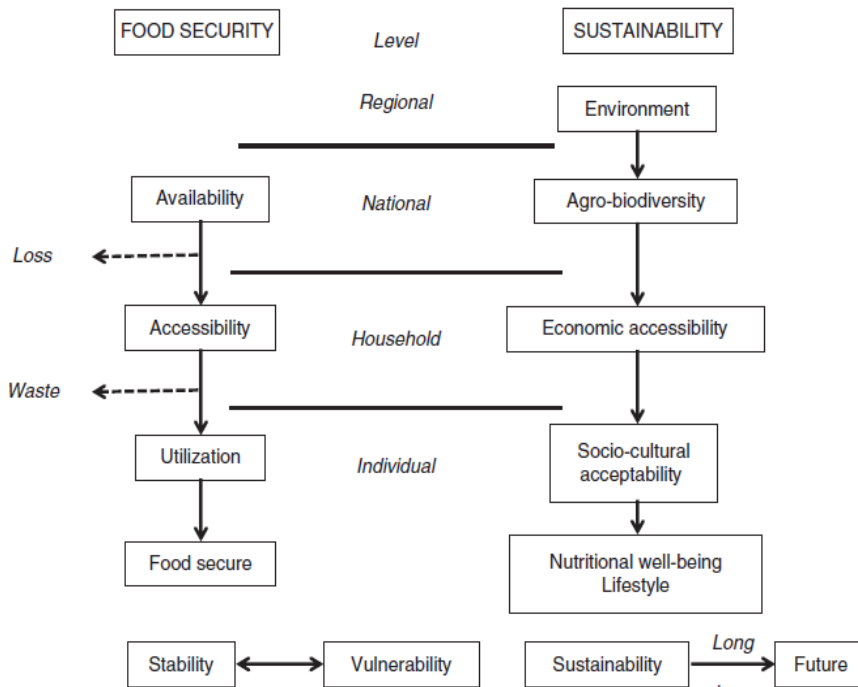


Fig. 3. The time dimension to food security: short-term stability (left) and long-term sustainability (right) (Source: Berry *et al.*, 2014)

## CONCLUSIONS

The Mediterranean region is passing through a “nutrition transition” in which problems of under-nutrition coexist with overweight, obesity and food-related chronic diseases. This nutrition transition is alarming as it has negative impacts on health systems. Therefore, changes in both food consumption and food production are necessary to ensure more sustainable food systems and to achieve food and nutrition security in the Mediterranean region. Since diets sustainability is crucial for achieving food and nutrition security, there is an urgent need to design and implement appropriate policies to improve the sustainability of the current food consumption patterns. Transition towards sustainable food systems in the Mediterranean area requires developing a set of comprehensive, coherent, integrated and holistic policies that deal with the



different food-related spheres and arenas. Coordinated actions are needed at local, national and regional level with the support of the private sector and the civil society, for fostering sustainable food systems by improving the sustainability of diets and food consumption patterns. The Mediterranean diet can be regarded as a model for validating methods, metrics and indicators for sustainable diets.

Relations and linkages between food and nutrition security, diets and food systems are shown in the Millennium Development Goals (MDGs) as well as the Zero Hunger Challenge initiative of the United Nations' Secretary General. They should be highlighted and further operationalized in global food-related strategies (e.g. 10 Year Framework of Programs on Sustainable Food Systems, Sustainable Development Goals) as well as regional strategies such as the Mediterranean Strategy for Sustainable Development and the Sustainable Consumption and Production Action Plan for the Mediterranean.

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## **THE INFLUENCE OF POSTAGROGENIC TRANSFORMATION ON BIOLOGICAL PROPERTIES OF SODDY SHALLOW CLAY LOAM PODZOLIC SOIL IN THE PREDURALIE**

### **SUMMARY**

The aim of this study was to assess changes in biological properties of a postagrogenic soddy shallow clay loam podzolic soil of a 45 years old stationary field experiment of the Perm State Agricultural Academy (Russia) in a grain-grass-tilled crop rotation. The design of the experiment facilitated an evaluation of the ecological recovery of fertilized soils as well as of discontinued tillage, where particular emphasis was put on the soil biota. Discontinuation of tillage and high availability of phosphorus and potassium were found to significantly enhance carbon dioxide production and urease activity, and to reduce phosphatase activity. We conclude that elevated amounts of available nutrients enhanced soil biological activity beyond its natural levels, and that preferential recultivation of such areas would minimize the risks of nutrient losses to neighboring environments.

**Keywords:** fallow land, tillage, carbon dioxide emissions, urease activity, alkaline phosphatase activity

### **INTRODUCTION**

Fallow land is a potentially valuable resource of the Russian Federation. In Russia, there are located more than 70 million hectares of unused farmland, mainly in areas with low bioclimatic potential: in the northern and eastern regions, as well as in the non-chernozem zone. These lands mainly belong to small farms or to private owners not involved in agriculture [2]. Discontinuation of cultivation resulted, for example, in an increase of pastures in Russia. The Ministry of Agriculture of the Russian Federation is interested in expanding arable areas through the development of fallow land to improve the food and food base of the country. According to the state program of agricultural development for 2013-2020, the funding of Russian farms is provided per hectare, which will entail an intensive process of return to agricultural use of areas under fallow [3]. Conservation and soil improvement can be achieved by science-based land management.

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Paper presented at the 6<sup>th</sup> International Scientific Agricultural Symposium "AGROSYM 2015".  
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

## MATERIAL AND METHODS

The study site was located on an experimental field of a 45 years old stationary field experiment of the Agrochemistry Department of the Perm State Agricultural Academy (Perm, Russia) on a very gentle slope with south-eastern exposure. The soil was a soddy shallow clay loam podzolic soil which has developed on red calcareous clayey loams of the Perm period.

The climate of this region is continental, with cold and long snowy winters and short warm summers. The typical vegetation in 2014 is presented in table 1 and figure 1.

Since 1969, in this experiment was conducted research on the effectiveness of various fertilizer systems, and on the forms and doses of fertilizers in a grain-grass-tilled crop rotation. The unique duration of the field experiment under controlled conditions of agronomic management has lead to the establishment of specific environmental effects.

Table 1 Botanical description of study site, 2014

Variant	Projective coverage, %		Species	
	live	dead	grass	trees
I	54	42	<i>Taraxacum officinale</i> , <i>Sonchus arvensis</i> , <i>Cirsium arvense</i> <i>Trifolium repens</i> , <i>Matricaria recutita</i>	<i>Alnus incána</i> ,
II	61	51	<i>Taraxacum officinale</i> , <i>Sonchus arvensis</i> , <i>Cirsium arvense</i> , <i>Matricaria recutita</i> , <i>Plantágo májor</i>	<i>Alnus incána</i> , <i>Acer negúndo</i>
III	5	2	<i>Matricaria recutita</i>	-

The postagrogenic phytocenosis of fallow variants (I and II) was presented by ruderal vegetation with dominating *Taraxacum officinale* and *Sonchus arvensis*. Plant residues consisted of leaves and straw. The projective coverages of live and dead vegetation were 12-20% higher in variant II compared to variant I. Mosses or other cryptogams did not form layers or biocrusts.



Figure 1. View of study site (a - I variant; b – II variant)

Levels of available phosphorus and exchangeable potassium established during the experiment (Table 2) further influenced the productivity of the phytocenoses.

Total biomass was higher by 15-23% in the variant with high phosphorus-potassium background compared to the control. Thus, agricultural crops and the level of mineral nutrition had a significant impact on the development of successions, which further influenced pedogenesis.

To study changes of edaphic properties, soil samples were taken in triplicate from the arable horizon of the following variants: (I) no tillage, no fertilization (fallow control), (II) no tillage with high levels of available forms of phosphorus and potassium (set by the experiment to 150 mg kg<sup>-1</sup>), and (III) tillage only, no fertilization (severely disturbed). Variants (I) and (II) were discontinued in 2011, variant (III) was continued to be cultivated to sampling. Thus, the following effects on biological activity could be studied:

- Variant (i) vs. Variant (ii): ecological recovery of fertilized soil
- Variant (i) vs. Variant (iii): influence of tillage
- Variant (ii) vs. Variant (iii): mutual exclusion of tillage and fertilization

Available phosphorus and exchangeable potassium were determined in extracts of 0,2 m kcl solution with a ratio of 1:5. The soddy shallow clay loam podzolic soil was characterized by a slightly acidic reaction and by high degrees of base saturation (v)

Table 2. Agrochemical properties of soddy shallow clay loam podzolic soil, 2014

Variant	pH <sub>H2O</sub>	pH <sub>KCl</sub>	mg-equiv/100 g of soil		V, %	Mg/kg of soil	
			Sum	CEC		P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
I	6.4	5.0	17.3	19.6	89	101	142
II	6.4	5.3	18.5	20.8	89	161	190
III	6.4	5.3	14.4	16.8	86	150	168

Respiratory activity was determined every two weeks in summer 2014 by CO<sub>2</sub> adsorption using the method of shtatnov in modification of makarov (1975). Enzyme activities were determined using spectrophotometric methods: urease according to hoffmann and teicher (1961); alkaline phosphatase according to tabatabai and bremner (1969).

## RESULTS AND DISCUSSION

Surface carbon dioxide emission differed significantly between all variants, and further depended on other factors, like temperature and humidity (sampling date comprised 37% of total variance) (Figure 1). For the tillage only

variant (III) the soil respiration rate was minimal in all periods of measurement and averaged to  $2.2 \pm 1.2$  kg CO<sub>2</sub>×ha<sup>-1</sup>×h<sup>-1</sup> (59±10% of control, sampling date effects excluded in relative data), expressing the negative effect of severe disturbance on soil biota.

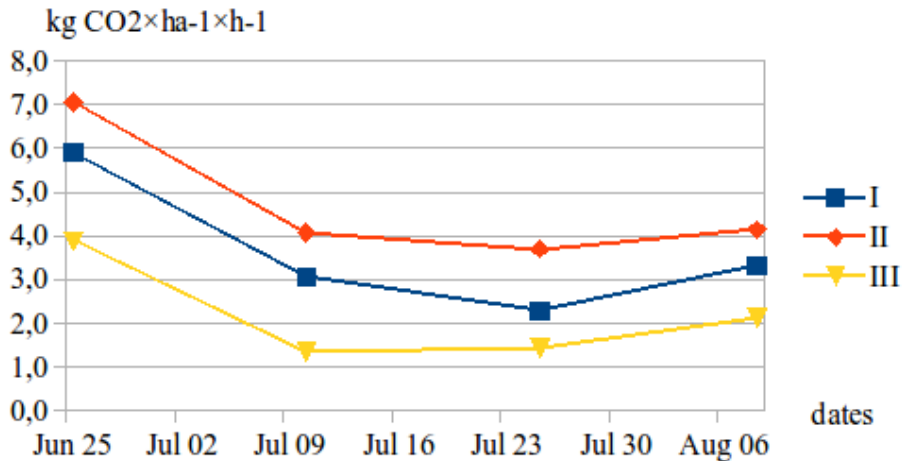


Figure 2: Carbon dioxide emissions of soddy shallow clay loam podzolic soil, 2014.

On the control plots under fallow, CO<sub>2</sub> discharge averaged to  $3.6 \pm 1.6$  CO<sub>2</sub>×ha<sup>-1</sup>×h<sup>-1</sup>, and in variant (II) with high phosphorus-potassium background it was highest ( $4.7 \pm 1.6$  kg CO<sub>2</sub>×ha<sup>-1</sup>×h<sup>-1</sup>, 135±19% of control). The results of urease activity confirm this finding (Table 1). On the fallow control it was 13-40% higher compared to the tillage only variant.

Urease activity averaged to 19.5 mg N-NH<sub>3</sub>×24h<sup>-1</sup>×10g<sup>-1</sup> soil in the tillage only variant (III), to 22.3 mg N-NH<sub>3</sub> ×24h<sup>-1</sup>×10g<sup>-1</sup> soil in the control, and to 31.4 mg N-NH<sub>3</sub>×24h<sup>-1</sup>×10g<sup>-1</sup>soil in variant (II) with high phosphorus-potassium background. Thus, humus turnover and urease activity remained on higher levels in variant (II) after discontinuation of the field experiment, presumably due to persisting favorable conditions for vegetation development and soil biota.

Table 3 Enzyme activities of soddy shallow clay loam podzolic soil, 2014

Variant	Urease, mg N-NH <sub>3</sub> × ×24h <sup>-1</sup> ×10g <sup>-1</sup> soil	Phosphatase, mg P <sub>2</sub> O <sub>5</sub> × ×h <sup>-1</sup> ×g <sup>-1</sup> soil
I (fallow control)	22.3	12.0
II (high PK background)	31.4	8.7
III (tillage only)	19.5	16.7
LSD <sub>01</sub>	4,0	4,4

The phosphatase activity was inversely related to concentrations of mobile phosphates, indicating that phosphatase activity decreases with phosphate availability. For the control, the activity was  $12.0 \pm 1.1 \text{ mg P}_2\text{O}_5 \times \text{h}^{-1} \times \text{g}^{-1}$  soil; for variant (II) with high phosphorus-potassium background  $8.7 \pm 2.8 \text{ mg P}_2\text{O}_5 \times \text{h}^{-1} \times \text{g}^{-1}$  soil, and significantly higher on the tillage only variant (III) –  $16.7 \pm 0.7 \text{ mg P}_2\text{O}_5 \times \text{h}^{-1} \times \text{g}^{-1}$ .

### CONCLUSIONS

It was concluded that elevated amounts of available nutrients enhanced soil biological activity beyond its natural levels. Preferential recultivation of such areas would promote food production and minimize the risks of nutrient output to their neighboring environment. It is further proposed that fallow facilitates the restoration of soil functionality in case of severe agrogenic disturbance.

### ACKNOWLEDGEMENTS

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**EFFECTS OF LIGHT AND MOISTURE ON GROWTH AND MORPHOLOGICAL CHARACTERISTICS OF HORSE CHESTNUT (*Aesculus hippocastanum* L.) SEEDLINGS IN THE WESTERN BLACKSEA REGION IN TURKEY**

**SUMMARY**

The aim of study is determined of growth performances and changes of morphological characteristics of horse chestnut seedlings at different light and moisture conditions at the nursery stage. This research carried out greenhouse conditions in the Gökçeşey Forest Nursery Directorate. In this context the same growing material (1/3sand+1/3clay+1/3natural organic humic material) was created in the greenhouse field. The 4 different light (30%, 50%, 60% and 80%) and moisture (40%, 60%, 80% and 90%) conditions were attempted on seedlings of horse chestnut in the greenhouse conditions. The results obtained from the research that a rapid growth of chestnut horse with the particularly responded quickly to changing light conditions, but can be continued until the response is determined about 80% intensity. On the other hand it reacts to changing humidity conditions against a chestnut horse, but was determined to show high growth in terms of numerical values, such as in this case, the light intensity. As well as root growth that the light intensity the resulting changes despite the rapidly increased to accommodate the growth that occurred and that this increase starts pause of approximately 70% light intensity, root length despite the changes in the moisture content was determined to continue to grow to about 90% moisture content. In this context light intensity of increased approximately 70% and performed upbringing process of horse chestnut trees in the moisture content up to 80%. However, both the bud growth, required for growth and development of fruit grown in 50% light 60% moisture content and their powerful and adaptations may be useful for the production of high horse chestnut trees.

**Keywords:** Horse chestnut, adaptation, light, moisture, seedling, greenhouse, growth

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

## INTRODUCTION

Horse Chestnut (*Aesculus hippocastanum* L.) is a large deciduous, rapidly-growing tree that can reach a height of 36 meters. It is native to the countries of the Balkan Peninsula, but because of its large, showy flower clusters the tree is cultivated worldwide for its beauty. Flowers are white or pink with a small red spot. Leaves are large, consisting of either five or seven leaflets and the fruit is round with a thick, green, spiny husk containing a glossy brown seed (chestnut or conker) (Anonymous, 2009). While the common name for the tree is horse chestnut, it is also known as buckeye, and like other buckeyes, is a member of the *Hippocastanaceae* family, rather than the chestnut family (*Castanea*) (Lorenz and Marek, 2009). The name, horse chestnut, is believed to be derived from the brown conkers that look similar to chestnuts and because a horseshoe shaped mark (complete with spots resembling horseshoe nails) is left on the twig when the leaves drop off in autumn (Pittler, 1998). Historically, the seed extract was used as a treatment for many ailments, including rheumatism, rectal complaints,<sup>3</sup> bladder and gastrointestinal disorders, fever (first written account in 1720), hemorrhoids (as early as 1886),<sup>4</sup> and leg cramps.<sup>5</sup> (Pearson and Vanhountte, 1993). Currently, horse chestnut seed extract (HCSE) is widely used in Europe for chronic venous insufficiency, hemorrhoids, post-operative edema, and topically for clearing skin conditions (Panigati, 1992). The aim of study is determined of growty performances and changes of morphological characteristics of horse chesnut seedlings at different light and moisture conditions at the nursery stage.

## MATERIAL AND METHODS

This research carried out greenhouse conditions in the Gökçeşey Forest Nursey Directorate. In this context the same growing material (1/3san+1/3clay+1/3natural organic humic material) was created in the greenhouse field. The 4 different light and moisture conditions were attempted on seedlings of horse chestnut in the grenhouse conditions. These different light and moisture conditions which were the attempted of this research were given Table1

Table 1. Different light and moisture conditions

Light Conditions (%)	Moisture Conditions (%)
30	40
50	60
60	80
80	90

The all treatments were used on 3 ages horse chestnut seedlings and measruments and observed about growth performances and morphological charecteristics after 1 growing season in the nursery. In this context height, root collar diameter, leaf area, root length and bud length state were determined

containing this research. The measured of 30 seedlings each parameter of seedlings. On the other hand total 200 seedlings were measured.

### **Statistical Analyses**

The ANOVA and Duncan Range Test were used in this research for determination of differences in all light and moisture conditions with respect to the growth performances and morphological characteristics. In this regard, the SPSS package statistical programme was used for analyses.

For identification, culture morphology, growth rate and conidial morphology were observed from 7-10 day-old cultures grown on PDA (Sutton, 1980). The shape, length and width of 100 conidia were determined; and mean length and width were calculated. Fungal isolates were sent to CABI-UK for species identification by sequencing the internal transcribed spacer (ITS) of the rDNA of two representative isolates.

## **RESULTS AND DISCUSSION**

### **Growth Performances of Seedlings**

The results obtained from different light and moisture conditions experiments were given in Table 2.

Table 2. Growth Performances of Seedlings

Growth Conditions Code	Light Conditions (%)	Moisture Conditions (%)	Height (cm)	Root Collar Diameter (mm)	Leaf Area (mm <sup>2</sup> )
1	30	40	12.3 <b>a</b> *	10.3 <b>a</b>	680.6 <b>a</b>
2	50	60	14.6 <b>b</b>	12.6 <b>b</b>	794.8 <b>a</b>
3	60	80	22.4 <b>c</b>	18.7 <b>b</b>	1052.7 <b>b</b>
4	80	90	28.6 <b>c</b>	23.2 <b>c</b>	1426.4 <b>c</b>

\*: Similar letters indicate the same group

The results of ANOVA and Duncan Range Test were applied on a dataset of growth parameters; there are significant differences at the 99% level for all parameters with respect to the different light and moisture conditions. In this study, the Duncan Range Test and results of this test occurring in 3 groups of growth variables. In this context, light and growth of the seedlings are growing rapidly due to the increase in humidity conditions. However, growth is slowing after the high humidity and light conditions and is emerging with small numerical differences between the growth rate. This type of growth has slowed down in other research, especially after the 80% light intensity and it is reported that then begin to decline at 60% humidity and fruit quality (Bazzoni et al., 1991; Bombardelli et al., 1996).

### **Morphological Characteristics of Seedlings**

Morphological characters related to growth such as stem length and bud length of horse chestnut in this study. The values obtained in the measurement results are given in Table 3.

Table 3. Morphological characteristics of seedlings at different light and moisture conditions

Growth Conditions Code	Light Conditions (%)	Moisture Conditions (%)	Root Length (cm)	Bud Length (mm)
1	30	40	28.4 <b>a</b> *	21.4 <b>a</b>
2	50	60	32.6 <b>b</b>	28.6 <b>a</b>
3	60	80	56.3 <b>c</b>	35.7 <b>b</b>
4	80	90	64.8 <b>d</b>	48.9 <b>c</b>

\*: Similar letters indicate the same group.

As a results of ANOVA and Duncan test at the root length of four characters in the group consisting of different growth conditions, and the bud length was determined that three different groups occurred. In this context, to give immediate response to differences in root length 50% humidity, and light intensity, but light buds length and remains unresponsive to differences in growth medium intensity up to 60% humidity. After this issue made especially moisture content of 40% root length of the horse chestnut trees in a similar research is increasing rapidly and it is emphasized that this case can easily be determined in light textured soils (Suter et al., 2006).

This from a young age, in the light of the results obtained from the research that a rapid growth of chestnut horse with the particularly responded quickly to changing light conditions, but can be continued until the response is determined about 80% intensity. On the other hand it reacts to changing humidity conditions against a chestnut horse, but was determined to show high growth in terms of numerical values, such as in this case, the light intensity. As well as root growth that the light intensity the resulting changes despite the rapidly increased to accommodate the growth that occurred and that this increase pause of approximately 70% light intensity, Root length despite the changes in the moisture content was determined to continue to grow to about 90% moisture content. In this context light intensity of increased approximately 70% and performed upbringing process of horse chestnut trees in the moisture content up to 80%. However, both the bud growth, required for growth and development of fruit grown in 50% light 60% moisture content and their strongly seedlings and adaptations may be useful for the production of high horse chestnut trees. Detailed the generation of the afforestation work done on it and kind of physiological studies for the pharmaceutical industry, as well as horse chestnut starting to find new areas is of great importance.

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## **THE EFFECT OF ORGANIC AND INORGANIC FERTILIZATION ON THE DEVELOPMENT AND YIELD OF THREE BRASSICA SPECIE**

### **SSUMMARY**

In the present study the effect of organic and inorganic fertilization on the development and yield of three Brassica species was examined. The experiment was conducted at Servota, Trikala during 2012-2013 growing season. Three brassica were studied [Broccoli: Grande F1, Cauliflower: Rex F1, Cabbage: Torpedo F1], whereas fertilization was implemented with the application of organic and inorganic fertilizers with plants getting the same amounts of nutrients at both occasions. Seeds from the three species were sown in seed trays containing peat and young seedlings were transplanted directly in the soil within an unheated plastic greenhouse. At the day of harvest plant features regarding plant development, such as the number of leaves, flower heads and main and second order shoots, total plant fresh weight, fresh weight of leaves and main and second order flower head and shoots, were recorded. From the results it is suggested that the fertilization method did not affected marketable yield for any of the studied species. The only features that were affected by fertilization method was leaf fresh and dry weight for cabbage and leaf dry weight for cauliflower, as well as shoot fresh weight for the same species. In conclusion, the fertilization method (organic or inorganic) does not affect yield of the studied species as soon as the plant nutrient requirements are covered.

**Keywords:** broccoli, cabbage, cauliflower, organic fertilization, conventional fertilization

### **INTRODUCTION**

The implementation of sustainable agricultural cultivation systems that are environmental friendly is a key factor for the increase of production of high nutritional value products, as well as the enhance in the added value of the final products that will allow the farmers for a higher profit. Moreover, it will contribute in the better and more rational management of natural resources that tend to be more and more scarce. In terms of nutritional value, Lima-Pallone et

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al. (2008) report that the application of organic agricultural systems is beneficial for the folic acid content in brassica species, whereas Meyer and Adam (2008) report a similar effect on the glyco Brassicidin content. In addition, nitrogen supply form can result in differences in the content of phytochemical substances such as glucosinolates, flavonoids, carotenoids and chlorophylls (Falovo *et al.*, 2011).

The application of organic fertilizers has been suggested to have many beneficial effects, since it results in an improvement of soil properties and an enhance in the microbial content (Dauda *et al.*, 2008; Suresh *et al.*, 2004). The development of innovative organic fertilizers has allowed farmers to apply balanced fertilization regimes that are similar to conventional fertilizers with all the beneficial effects (Naeem *et al.*, 2006). However, the main disadvantages of organic fertilizers are their high cost per nutrient unit and the fact that most of them are available in liquid form and therefore can only applied via irrigation water or in hydroponic systems.

In the present study we examined the effect of two fertilization regimes with the use of either organic or conventional fertilizers, on the yield and plant development of three brassica species (broccoli, cabbage and cauliflower).

## MATERIAL AND METHODS

The plant material used in the experiments was three hybrids of brassica species, namely broccoli [*Brassica oleracea* L. var. *italica* Plenck(Grande F1)], cabbage [*Brassicaoleracea* L. convar. *capitata* (L.) Alef. var *capitata* (Torpedo F1)], and cauliflower [*Brassica oleracea* convar. *botrytis* (L.) Alef. var. *botrytis* L. (Rex F1)]. Seeds of the three species were sown in seed trays filled with peat on October 19<sup>th</sup> and transplanted in soil 35 days after sowing (November 24<sup>th</sup>). Cultivation was carried out in an unheated plastic greenhouse in order to avoid depletion of fertilizers due to unexpected rainfall. The fertilization regime for both organic and conventional fertilization is presented in Tables 1 and 2. The conventional fertilizers implemented were the following: potassium nitrate (13-0-46), potassium sulfate (0-0-50), calcium nitrate (15.5-0-0 + 19% Ca), ammonium nitrate(34.5-0-0), mono potassium phosphate (0-52-34), borax and iron chelate (6%). Similarly, the organic fertilizers were: Avant Natur (5.5-0-0; Compo Expert GmbH), Fish-Fert (2-4-0.5; Humofert S.A.), Acadian (1-1-16; Humofert S.A.), borax and iron chelate (6%).

Fertilizers were applied throughout the cultivation season via irrigation water (fertigation), whereas after harvest plant development and yield was assessed (number of leaves, head and 2<sup>nd</sup> order shoots, total plant fresh weight, fresh weight of leaves and 1<sup>st</sup> order heads and 2<sup>nd</sup> order shoots and heads). Harvest was carried out when flower and leaf heads (for broccoli, cauliflower and cabbage respectively) reached the standard commercial size (March 6<sup>th</sup> to April 9<sup>th</sup> for broccoli, March 7<sup>th</sup> to April 16<sup>th</sup> for cabbage and March 19<sup>th</sup> to April 16<sup>th</sup> for cauliflower).



Table 1. Nutritional solution composition for conventional fertilization

Fertilizer type	Quantity (g per 10 L)	Nutrients (mg per litre)							
		N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Ca	Mg	S	B	Fe
13-0-46	4.87	63.3	289	-	-	-	-	-	-
0-0-50	0.236	-	10.6	-	-	-	4.25	-	-
15.5-0-0 +19% Ca	0.98	15.2	-	-	18.75	-	-	-	-
34.5-0-0	6.42	221.5	-	-	-	-	-	-	-
0-52-34	1.92	-	65.3	100	-	-	-	-	-
Borax	0.02	-	-	-	-	-	-	0.22	-
Iron chelate (6%)	0.19	-	-	-	-	-	-	-	1.12
Total	-	300	300	100	18.75	-	4.25	0.22	1.12

Table 2. Nutritional solution composition for organic fertilization

Fertilizer type	Quantity (g per 10 L)	Nutrients (mg per litre)							
		N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Ca	Mg	S	B	Fe
Avant Natur (5.5% N)	43.5	239.8	-	-	-	-	-	-	-
Fish-Fert (2-4-0.5)	20.5	41.0	10.25	82.0	15.37	0.82	3.48	-	-
1-1-16	18	18.75	287.5	18.75	-	-	-	-	-
Borax	0.02	-	-	-	-	-	-	0.22	-
Iron chelate (6%)	0.19	-	-	-	-	-	-	-	1.12
Total	-	299.5	297.75	100.7	15.37	0.82	3.48	0.22	1.12

The statistical design was a split-plot design, with main plots the fertilizer treatments and sub-plots the species. Each treatment was replicated four times (n=4) with 24 plots in total. Each plot was 4 m<sup>2</sup> (2 x 2 m) and plant distances were 50 cm between rows and 40 cm within each row (50,000 plants ha<sup>-1</sup>). Statistical analysis was carried out with statistical package Statgraphics Centurion (Statpoint Technologies Inc., USA).

## RESULTS AND DISCUSSION

From the results it is suggested that the application of either conventional or organic fertilizers did not result in significant differences in total biomass production as this is expressed by total plant fresh weight (Table 3). Similarly, yield features such as the 1st and 2nd order head fresh weight of broccoli did not differ significantly, whereas in the case of cauliflower the application of organic fertilizers resulted in higher weight for 2nd order heads without however obtaining a final marketable size (Table 4). Mohapatra et al. (2014) have also reported that the application of NPK fertilizers+bioinoculants, farm yard manure and vermicompost did not result in significant differences in the yield of broccoli

plants, whereas nutrient recovery was highest for vermicompost and the combination of vermicompost and farm yard manure.

Table 3. The effect of fertilizer regime (organic and conventional) on yield and morphology features of three brassica species

Species	Treatment	Total plant weight	2nd order shoots	Number of leaves	Leaves fresh weight	Leaves dry weight
Broccoli	Conventional	2651.04	5.21	69.68	1659.74	8.59
	Organic	2762.32	4.75	67.60	1748.01	8.58
	LSD	411.72	1.93	10.73	273.42	0.59
Cauliflower	Conventional	3450.46	1.33	19.8	2526.44	9.31b
	Organic	3431.51	1.00	19.8	2288.53	10.48a
	LSD	402.00	0.45	2.79	303.10	1.11
Cabbage	Conventional	4167.21	3.27	39.20	2843.82a	9.04a
	Organic	3822.01	2.31	35.55	2417.85b	8.48b
	LSD	429.79	1.27	8.43	354.02	0.47

\*Different latin letters represent significant differences between means of the same column and the same species according to Least Significant Differences test (LSD) at  $p=0.05$ .

In contrast, Zaki *et al.* (2012) reported that the combination of organic and inorganic fertilizers at a ratio of 75:25 resulted in higher plant growth comparing to organic or inorganic fertilizers alone

Table 4. The effect of fertilizer regime (organic and conventional) on yield and morphology features of three brassica species

Species	Treatment	1 <sup>st</sup> order head fresh weight	2 <sup>nd</sup> order head fresh weight	Head dry weight	Shoot fresh weight	2 <sup>nd</sup> order shoot fresh weight	Shoot dry weight
Broccoli	Conventional	430.43	22.06	9.25	336.66	176.33	6.25
	Organic	435.99	21.82	9.49	344.08	191.20	6.41
	LSD	109.05	4.78	0.74	47.39	56.26	0.44
Cauliflower	Conventional	667.86	4.03b	8.14	398.98b	48.97	6.82
	Organic	523.32	15.30a	8.86	607.84a	48.90	7.27
	LSD	188.48	3.32	1.03	139.28	5.63	0.95
Cabbage	Conventional	998.26	-	8.06	273.91a	-	9.00
	Organic	1146.51	-	7.61	212.38b	-	9.92
	LSD	198.44		0.50	40.39		1.99

\*Different latin letters represent significant differences between means of the same column and the same species according to Least Significant Differences test (LSD) at  $p=0.05$ .

These differences in the results may be due to the fact that they only used ammonium nitrate as an inorganic fertilizer plus the fact that they also applied bio-inoculation with various bacillus cultures. Moreover, Abou El-Magg *et al.*

(2014) reported that nitrogen rate combined with bio-nitrogen fertilizers can significantly affect broccoli yield as well as vegetative features such as plant weight, fresh weigh and number of leaves, dry matter content and mineral composition of heads, since bio-fertilizers can improve nitrogen fixation and consequently plant growth and development.

In general, the type of fertilizers (organic or conventional) did not affect most of the features assessed in our study, except for dry weight of leaves and shoot fresh weight of cauliflower and fresh and dry weight of leaves and shoot fresh weight of cabbage, with beneficial effect of organic and conventional fertilizers in the case of cauliflower and cabbage respectively (Tables 3 and 4). It could be suggested that as soon as plant nutrient requirements are sufficiently covered yield and plant growth potential could be fully expanded allowing for high yields regardless of fertilizer type. The fact that usually organic fertilization results in lower yields and plant growth comparing to inorganic fertilizers could be attributed to the fact that the used plant material has derived from conventional breeding programs where genotypes are evaluated under intensive farming systems and high input regimes. Therefore, there is a great need for crop breeding suitable for organic farming where usually inputs are low and plant requirements are not fully covered since sustainability and not the maximum yield is the ultimate goal (Lammerts van Bueren et al. 2011).

## CONCLUSIONS

From our study it is concluded that the type of applied fertilizers does not affect plant development and yield of the three tested Brassica species, as soon as plant nutrient requirements are sufficiently covered. However, these results need to be confirmed in the long term since organic fertilizers are more environment friendly and ideal for sustainable farming and have been suggested to improve soil properties, despite their disadvantages. Therefore, further research need to be conducted in order to have sufficient results for a solid conclusion regarding the type of fertilizers that farmers should apply in their crops. Moreover, plant breeding for new cultivars suitable for low inputs organic farming as well as the production of new and more efficient organic fertilizers is imperative in order to reduce production cost.

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## PRODUCTIVITY AND QUALITY OF SOME EARLY POTATO VARIETIES DEPENDING ON PLANTING RATE IN CONDITIONS OF MIDDLE PREDURALIE

### SUMMARY

The article deals with the data about productivity and quality of early varieties of potato: Zhukovskii early, Red Scarlett, Udacha, Rosalind depending on the planting rate. The trial was established on the sod-podzolic middle-loamy middle-cultivated soil in 2013 and 2014. The planned potato productivity of 35 t/ha was achieved in the variety Zhukovskii early and Rosalind 35.7 t/ha and 38.7 t/ha respectively, with the density of planting 71.4 thousand tubers/ha. Potato variety Rosalind also provided productivity of 35.8 t/ha in the variant with density of 57.1 thousand tubers/ha. Potato early maturity varieties *Red Scarlett* and *Udacha* formed up to 30 t/ha, and did not respond by the increase of tuber yield at densities from 41 to 71 thousand tubers/ha. The highest productivity was obtained by increasing the average potato tuber mass and increased density of potato plants on hectare.

The content of the marketable fraction in varieties did not differ and ranged from 76 to 81%. With planting overcrowding the varieties did not have a marketable fraction decrease in harvest. The starch content of the variants did not differ, and it was at the level of 11-13%.

Thus, it was found that on sod-podzolic middle-loamy soil to achieve the potato tuber yield of 35 t/ha in early potato variety Zhukovskii early the density of 71 thousand tubers/ha is needed, while for variety *Rosalind* density of 57 thousand tubers/ha is enough.

**Keywords:** potato, productivity, density, variety.

### INTRODUCTION

Potato in the non-chernozem area of Russia is one of the major food and industrial crops. The average productivity of potatoes in Russia is 15-17 t/ha, while the biological potential of this crop allows to obtain 30-40 t/ha and more. Increase of potato productivity up to 35 t/ha and more allows meeting the needs of current population in potatoes (Dubinin, 2013; Simakov, 2013).

The optimal density of potato plants is determined by the soil and climatic conditions, peculiarities of the variety. On the high agricultural background and with sufficient moisture provision the higher density level is more possible than

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on poor soils and with unstable water conditions. However, the excessive number of potato plants leads to the decrease of growth of productivity, economic efficiency of technique and the output of marketable fractions of tubers. This leads to the selection of certain optimal density, the value of which depends on the planting rate (Zamotaev, Galeev, 1964; Siniagin, 1966; Pavlov, 1968; Adamov, Shpiltskevich 1977, Iakimenko, 1982, Iusupov, 1984; Dmitrieva, Tsadko, 1990 Vakulenko, 2013). For new potato varieties the optimum planting density is not yet established.

### MATERIAL AND METHODS

In this regard in 2013-2014 we conducted the research, the purpose of which was to determine the optimum planting density of early potato varieties in order to reach the harvest of 35 t/ha. To achieve the goal the following tasks were done:

- To assess the variety reaction on planting density;
- Set the effect of planting density on the tubers quality.

To complete the tasks in the experimental field of Perm Agricultural Academy a two-factor field experiment was founded at the sod-podzolic middle-loamy soil with humus content of 4.2%, weak acid medium reaction (pHKCl 4.7), with a high content of mobile phosphorus and exchangeable potassium 181 mg/kg and 250 mg/kg of soil, respectively. Experimental design: Factor A – variety: A1 -Zhukovskii early (control); A2 - Red Scarlett; A3 – Udacha; A4 - Rosalind. Factor B - design (planting density), cm (thousands of tubers/ha): B1 -  $70 \times 35$  (40.8 thousands of tubers/ha); B2 -  $70 \times 30$  (47.6 thousands of tubers/ha) (control); B3 -  $70 \times 25$  (57.1 thousands of tubers/ha); B4 -  $70 \times 20$  (71.4 thousands of tubers/ha). Repeat 4 times. Experiment was carried out by method of split plots. The total area of the plot of the second order was 20 m<sup>2</sup>, accounting area was 15 m<sup>2</sup>. The length of the plot was 14.3 m, and the width of the plot 1.4 m (Dospikhov, 1985). The fore crop was barley. Agrotechnique was common for potatoes in the Permskii krai. Tillage included: in autumn - scuffing and underwinter plowing on a depth of arable layer; in spring - early spring harrowing and preplant cultivation with harrowing on a depth of 8-10 cm. Fertilizers were made in a dose of N90P90K120 for a pre-plant cultivation, the form of fertilizer is diamphosphoska (NPK 10:26: 26), ammonium nitrate (N-34), potassium chloride (K-60). Inter-cultivation included pre-emergence tillage and hilling. Harvesting was carried out manually with yellowing of lower leaves of potato.

Weather conditions in 2013 were unfavourable for the growth and development of the potato. Rainfall in June was near 60% of normal, and the temperature was higher than the long-term average annual value at 4.2 oC. In July the fallout was in large quantities, but uneven; it was hot weather; the temperature was higher than the long-term average annual value at 2.4 oC. In August rainfall and temperatures were close to long-term average annual values.

In general, the growing season was characterized by dry and hot weather, which negatively affected on the productivity of investigated early potato varieties.

Weather conditions in 2014 were favourable for the growth and development of the potato. Throughout the growing season cool weather with rainfall excess prevailed. The average monthly temperature in June was 15.0 °C, that on 1.4 °C is lower than normal, in July it was 14.4 °C, while normal is 18.4 C, and in August it was at 2 C higher than normal and was 17.1 °C. Rainfall in June was 84 mm, in July - 105 mm, which is 30% more than normal, in August - 58 mm. This has led to an increase in productivity of investigated early potato varieties.

### RESULTS AND DISCUSSION

Planned productivity of early potato varieties of 35 t/ha was achieved at Rosalind variety with density of 57 thousand tubers/ha (planting scheme 70 × 25 cm) and amounted to 35.8 t/ha, and at the varieties of Zhukovskii early and Rosalind at a planting rate of 71.4 thousand tubers/ha (planting scheme 70 × 20 cm) and amounted to 35.7 and 38.7 t/ha, respectively (Table 1).

Particular differences in planting rate revealed a significant increase in the productivity of 8.9 t/ha (LSD<sub>05</sub> - 4.6 t/ha) in Rosalind variety with a planting density of 71.4 thousand tubers/ha (planting scheme 70 × 20 cm) compared with the control rate of 47.6 thousand tubers/ha (planting scheme 70 × 30 cm). A significant decrease of productivity in comparison with the control, of 3.7 and 7.8 t/ha occurred in the variants with the planting rate 40.8 thousand tubers/ha (planting scheme 70 × 35 cm) by the varieties Zhukovskii early and Udacha, respectively.

Table 1. Productivity of potato early varieties depending on planting density, t/ha, 2013-2014

Planting rate, thousand tubers/ha (D)	Variety (A)				Average on B
	Zhukovskii early (control)	Red Scarlett	Udacha	Rosalind	
40.8	27.7	25.1	20.3	27.9	25.2
47.6	31.4	26.0	28.1	29.8	28.8
57.1	31.4	29.3	30.3	35.8	31.7
71.4	35.7	30.6	30.8	38.7	33.9
Average on	31.5	27.7	27.3	33.0	-
LSD <sub>05</sub> individual differences	On factor A		10.2		
	On factor B		5.7		
LSD <sub>05</sub> main effects	On factor A		5.1		
	On factor B		2.9		

Variety reaction on planting density is defined. Varieties Zhukovskii early and Udacha had the biggest yield at the planting rate of 47.6-71.4 thousand tubers/ha, variety Red Scarlett - 40.8-71.4 thousand tubers/ha, and variety Rosalind - 57.1-71.4 thousand tubers/ha.

Table 2. Productivity structure of early ripening varieties of potato depending on planting rate, t/ha, 2013-2014

Variety (A)	Planting rate, thousand/ha, (B)	Number of stems, pcs/m <sup>2</sup>	Number of primary stems	Tuber mass from a potato plant, g	Number of tubers in a potato plant, pcs	Average tuber mass, g	Average tuber number per plant pcs
Zhukovskii early	(40.8)	110.2	2.9	829	7.6	130	2.3
	(47.6)	153.9	2.9	859	7.3	132	1.8
	(57.1)	148.5	2.8	724	6.5	133	2.1
	(71.4)	208.8	3.2	760	7.2	141	1.9
Red Scarlett	(40.8)	158.1	3.6	746	7.5	124	1.5
	(47.6)	200.8	4.1	726	7.9	120	1.4
	(57.1)	252.7	3.9	694	7.9	120	1.2
	(71.4)	360.6	4.1	686	7.7	113	1.1
Udacha	(40.8)	114.4	3.1	716	7.0	105	2.4
	(47.6)	172.6	3.5	786	7.3	113	1.7
	(57.1)	197.0	3.2	758	6.8	109	1.7
	(71.4)	228.5	3.0	670	6.7	98	1.8
Rosalind	(40.8)	121.4	2.8	765	6.7	120	1.8
	(47.6)	128.5	2.7	740	6.9	134	1.7
	(57.1)	182.7	3.0	694	6.7	116	1.7
	(71.4)	189.2	2.9	754	7.3	122	2.2
Average on A <sub>1</sub>		155.3	2.9	793	7.1	134	2.0
Average on A <sub>2</sub>		243.0	3.9	713	7.8	119	1.3
Average on A <sub>3</sub>		178.1	3.2	732	7.0	107	1.9
Average on A <sub>4</sub>		155.5	2.9	738	6.9	123	1.8
LSD <sub>05</sub> individual differences	On factor A		0.85	165	1.9	32	0.6
	On factor B		0.67	108	1.4	13	0.4
LSD <sub>05</sub> main effects	On factor A		0.42	83	1.0	16	0.3
	On factor B		0.33	54	0.7	7	0.2

The main effects on the planting rate revealed a significant increase on 5.1 t/ha in the variant with a planting density of 71.4 thousand tubers/ha and a significant reduction in productivity on 3.6 t/ha in the variant with the planting density of 40.8 thousand tubers/ha (planting scheme 70 × 35 cm) in comparison



with the control –47.6 thousand tubers/ha. Success fully applied for species-level differentiation and names applied to data in GenBank are doubtful, as they were not linked to any type materials.

Data about productivity is confirmed by indicators of its productivity structure.

In the best variants of productivity – varieties of potato Zhukovskii early (40.5 t/ha) and Rosalind (43.3 t/ha), with a maximum density (71.4 thousand tubers/ha) as well as Rosalind at a density of 57 thousand tubers/ha with productivity 41.8 t/ha, the maximum productivity is obtained by means of the mass of tubers from the potato plant, as well as higher average weight of a tuber. Density of stems in these cases is the maximum in the experiment - 208.8 and 189.2 thousand stems/ha, respectively. The content of the marketable fraction in varieties did not differ and ranged from 76 to 81%. With overcrowding of planting the varieties are not marketable with marketable fraction decrease in harvest. The starch content of variants was not different and was at the level of 11-13%.

## CONCLUSIONS

It was found out that on sod-podzolic middle-loamy middle-cultivated soil of Preduralie of Russia the early potato varieties Zhukovskii early and Rosalind provide the planned productivity not less than 35 t/ha with a planting rate 71.4 and landing 57.1-71.4 thousand tubers/ha, respectively.

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## **CHARACTERISTICS OF FLOWERING AND FRUIT SET OF MAIN POMEGRANATE VARIETIES (*Punica granatum L.*) IN MONTENEGRO**

### **SUMMARY**

Morphological characteristics of flowers and flowering were studied in three main pomegranate varieties grown in Montenegro: 'Slatki barski', 'Šerbetaš' and 'Dividiš meke kore'. The varieties grown in three different locations: Dobra Voda and Tomba in the Bar coastal area, and in Golubovci area near Podgorica, were represented with five trees per locality. Results on dynamics of forming and falling of flowers and fruits are presented in the paper. The maximum length and width of a flowers in all varieties was at hermaphroditic type of flower (4.75 cm), a statistically significantly higher compared to the other two categories examined. Hermaphroditic type of flower has a statistically significantly longer pestle (13.58 mm), while the shortest in functionally male-type 3.93 mm. There was a highly significant correlation between the length of the pistil and diameter of a flower ( $r = 0.8022^{**}$ ), as well as the length of the flower ( $r = 0.7042^{**}$ ). Functionally male type of flower had in average the highest number of stamens (320), which is in the function of production of a sufficient amount of pollen and better fertilization, and the lowest was registered in hermaphrodite flower type (272). Pomegranate flowering lasted from two to almost three months in the cultivars 'Šerbetaš' and 'Slatki barski' from Dobra Voda in 2004 and 2003 respectively. The highest number of flowers per fruiting shoot was in variety 'Dividiš meke kore' (36.1) at locality Balabani in 2002. The lowest number of flowers (8.25) was formed in 'Šerbetaš' variety in 2004. Flowering maximum in the varieties grown in localities Tomba and Balabani was in the first half of June, while in Dobra Voda delayed in average 7-10 days, due to the climatic conditions in the orchard located at higher altitude.

**Keywords:** pomegranate, *Punica granatum L.*, flowering, falling of fruits

### **INTRODUCTION**

Pomegranate is considered one of the longest grown fruit species in the area with influence of the Mediterranean climate in Montenegro. Richness of pomegranate wild forms confirms that this area is one of gene-centers of this fruit species (Zohary and Hopf, 2000). Pomegranate blossoming depends on the agro-

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ecological conditions and lasts long, from March-April to June-August (Mars 2000). Pomegranate flower has a corolla intense red color, formed as single or in groups of 5-7 (Melgarejo, 1996). Number of stamens ranges from 200 to 350, and in complete flowers has 400 -1000 ovules (Derin and Eth, 2001). Blossoming takes place into two to four waves, with the best quality fruits originate from hermaphrodite flowers that are formed in the first flowering period (Mars, 2000; Buljko, 1974). Pomegranate forms, according to numerous authors, two types of flowers: type A- male flower with ovary that contains only a few ovules, and type B - hermaphrodite flower only that can bear fruit. Nalawadi et al. (1973), by examining pomegranate flower recognize three types of flowers: small, intermediate and hermaphrodite.

The objective of this study is to investigate the floral morphology, and dynamics of formation and falling of flowers and fruits in three most important pomegranate varieties grown in Montenegro.

### **MATERIAL AND METHODS**

The study was conducted on three prevalent pomegranate varieties in Montenegro: 'Slatki barski', 'Šerbetaš' and 'Dividiš meke kore' grown on three different locations. Localities Dobra Voda (DV) and Tomba (MN) are situated in Bar municipality in the coastal region, and Balabani near Golubovci (ZP) in Zeta-Bjelopavlici valley. Orchards in Dobra Voda are situated in the highest altitude (294 m). 'Slatki barski' variety is presented on each location, 'Šerbetaš' in Dobra voda and Tomba, while 'Dividiš meke kore' is grown only in Balabani village. Each of the varieties was presented with 5 shrubs per locality, and all shrubs were of the same age (13 and 14 years) and in the full productive period.

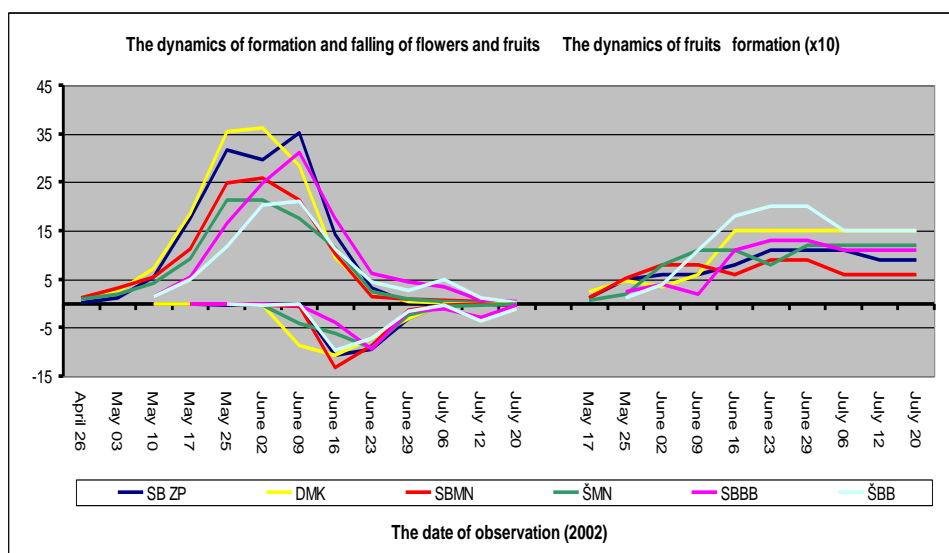
The study of the dynamics of developing, flower and fruitslets fall was followed on 5 randomly selected bearing branches per tree, recording changes every seven days until the moment of harvest. Anatomy features of flower were analyzed on a sample of 20 flowers per tree, where the flowers were divided into three groups: small, intermediate and hermaphrodite, according the length of the pistil and the ovary development. The flowers for analysis were taken in E3 advanced of F initial stage (Melgarejo et al., 1997). Statistical analysis of the observed flower characteristics was done by analysis of variance and the differences were compared by Takey test for the level of significance 95 and 99%, and by the Pearson's correlation analysis.

### **RESULTS AND DISCUSSION**

A In the observed years, similarities in the dynamics of the formation of flowers (Figures 1-3) are notable. Pomegranate flowering is the phenological phase that lasts long and in the tested localities usually occurs in one or two large waves.

The earliest formation of flowers was recorded in 2002 (26 April) on localities Tomba and Balabani. The first maximum of flowering in these locations was recorded in the period from 10-25 May in varieties 'Slatki barski'

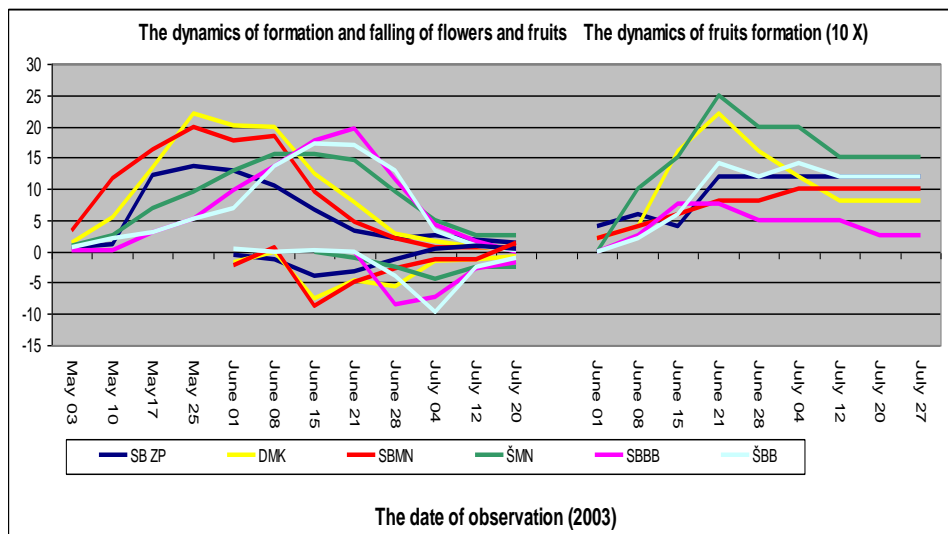
and 'Dividiš meke kore', while the second maximum was on 9 June. Variety 'Dividiš meke kore' had earlier picks, and in average formed the highest number of flowers. The lowest number of flowers formed variety 'Šerbetaš' in the site Tomba, but more presented hermaphroditic type. Certain discrepancies were registered in 2003, the year with somewhat different climate conditions. This is mostly related to the temperature, as ecological factor that has the greatest impact on the flowering (Adhikari and Adhikari, 2010). Flowering in 2003 was delayed due to lower air temperature in the period 6-9 April and slightly colder March. Very high temperatures in May compensated this delay in the later period of flowering. In 2003 it was registered one maximum for the studied cultivars; 25 May for the varieties on localities Tomba and Balabani, and 21 June on the locality Dobra Voda. In this year, there were much less formed flowers compared to other two years. The formation of flowers in 2004 had one maximum, 31 May for the varieties grown in Balabani and Tomba, and 13 June for the varieties in Dobra Voda. It is evident that flowering is delayed for two weeks in Dobra Voda, due to climatic conditions of the orchard which is at higher altitude.



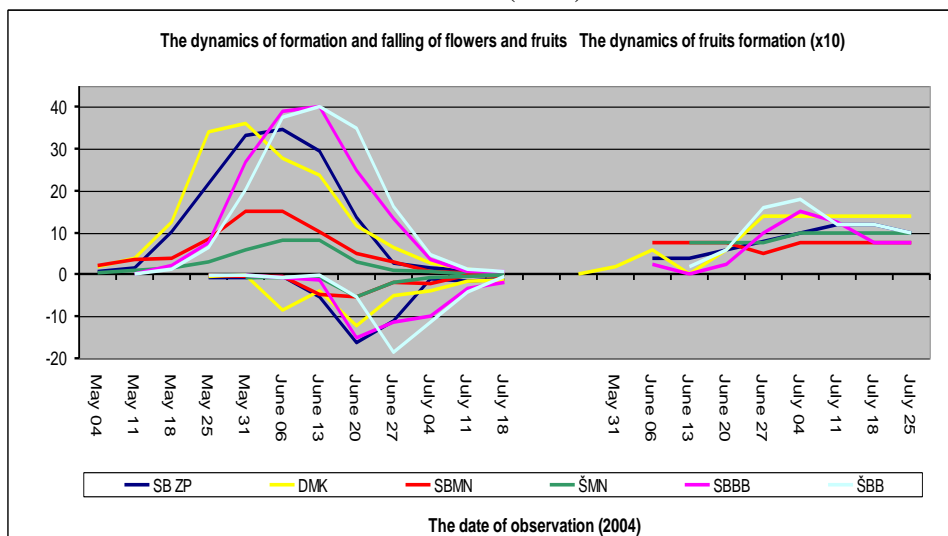
Graph.1. The dynamics of formation and falling of flowers and fruits, and fruits formation (2004)

The fall of flowers in average begins two weeks compared to their formation. Though, the maximum fall of flowers occurs later than the flowering maximum 14-17 days. This is consistent with the duration of the blooms stated by malgarejo et al., (1997). Falling of male flowers is a normal phenomenon, because the process of fertilization is completed. In addition to these also falls part of perfect flowers and formed fruitlets. Formation of fruits was registered early in 2002 (17 may) in cultivars grown in localities balabani and tomba and the latest in variety šebetaš in localities tomba and dobra voda (13 june 2004).

Formed fruits cease to fall by 24 July in average, and mature by the end of autumn. In average, the largest number of formed fruits per shoot was registered in variety 'šerbetaš' (1.5), which had a smaller number of flowers, but hermaphrodite in higher percentage.



Graph.2. The dynamics of formation and falling of flowers and fruits, and fruits formation (2003)



Graph.1. The dynamics of formation and falling of flowers and fruits, and fruits formation (2004)

The ratio of male and perfect flowers was studied by gozlecki and kaynak (2000) in the known turkish variety hicaznar. It was found variability in the

ration of hermaphroditic and male flowers, stating that perfect flowers were represented within 13.58 to 22.32%. Wetzstein et al., (2011) reported that in pomegranate changes periodically the ratio of male and hermaphroditic flowers, conditioned by environmental factors, age of the plant, which is an evolutionary advantage of pomegranate

Table 1. Floral morphology, comparison of differences

Variety/ treatment	Length of flower (cm)			Diameter of flower (cm)			No of petals			No of sepals			Length of pistils (mm)			No of stamens		
	M	I	H	M	I	H	M	I	H	M	I	H	M	I	H	M	I	H
Type of flower	M	I	H	M	I	H	M	I	H	M	I	H	M	I	H	M	I	H
Slatki barski DV	4.12	4.33	4.89	1.01	1.13	1.38	7.53	7.50	7.73	7.19	7.33	7.70	3.81	7.83	12.68	376.67	327.56	348.67
Slatki barski MN	3.84	4.19	4.73	0.95	1.12	1.47	7.37	8.25	6.67	7.18	8.12	6.67	3.17	6.62	12.67	341.59	246.00	333.38
Slatki barski ZP	3.69	3.96	4.71	0.90	1.04	1.46	6.73	7.35	6.83	6.59	7.01	6.75	4.25	7.71	13.22	273.81	241.19	305.03
Šerbetaš DV	4.11	4.35	5.10	1.05	1.15	1.69	8.33	8.21	7.61	7.03	7.97	7.45	3.44	7.94	14.84	381.39	315.78	360.48
Šerbetaš ZP	3.92	4.12	4.51	0.92	1.01	1.45	7.05	7.24	6.412	6.94	6.14	6.92	4.94	7.61	14.40	264.88	250.52	233.06
Dividiš meke kore	3.95	4.32	4.53	1.00	1.18	1.40	7.06	7.33	6.53	6.71	7.33	6.37	3.94	8.33	13.58	282.22	256.02	299.17
Average	3.94b	4.21b	4.75a	0.97b	1.10b	1.47a	7.35a	7.51a	7.10a	6.94a	7.32a	6.97a	3.93c	7.67b	13.57a	320.10a	313.30ab	272.84b
HSD <sub>0,05</sub>	0.3410			0.1397			0.6320			0.5838			1.2576			46.144		
HSD <sub>0,01</sub>	0.4346			0.1780			0.8055			0.7441			1.6029			58.811		
Legend	M - male flowers, I - intermediate flowers, H - heramfroditic (complete) flowers Abbreviations of localities DV- Dobra Voda, MN- Tomba, Bar, ZP- Golubovci, Podgorica																	

According to the results presented in table 1, hermaphrodite type of flower has a significantly longer and wider flower in relation to male and intermediate type of flower. This is the result of much larger ovary and longer pistil. Mars (2000) describes the male type of flower as a "bell-shaped", while the hermaphrodite as "vase-shaped". According to wetzstein et al. (2011), "male" flower of 'wonderful' variety was 2.7 cm long, and hermaphrodite 3.6 cm.



Figure 1. "Male", intermediate and hermaprodite type of flowers



Figure 2. Different stage of developm pomegranate type of flowers

According to martinez et al. (2000), in three studied clones the length and width of flower varied, although the authors point out that flowers were taken from the orchard only 4 years old. Calyx and petal number approximately is of the same values indicated by martinez et al. (2000). According to the correlation analysis these two features of flowers are statistically significantly conditioned (table 2), and as indicated by the coefficient of determination ( $r^2 = 0.4943$ ) they determine each other with 50%.

Table 2. Correlation matrix of floral morphology

	Length of flowers	Diameter of flowers	No of petals	No of sepals	Length of pistil
Diameter of flowers	$r=0.7031^{**}$ $r^2=0.4943$				
No of petals	$r=0.7116^{ns}$	$r=0.0916^{ns}$			
No of sepals	$r=0.2170^{ns}$	$r=0.1462^{ns}$	$r=0.8785^{**}$ $r^2=0.7718$		
Length of pistil	$r=0.5549^{**}$ $r^2=0.3079$	$r=0.8022^{**}$ $r^2=0.6435$	$r=-0.1024^{ns}$	$r=-0.0300^{ns}$	
No of stamens	$r=-0.1534^{ns}$	$r=0.1111^{ns}$	$r=0.6461^{**}$ $r^2=0.4174$	$r=0.6498^{**}$ $r^2=0.4224$	$r=-0.3153^{ns}$

\* Results are significant at  $p \leq 0.05$  and  $0.01$

Hermaphrodite type of flower has a significantly longer pistil in relation to the other two types of flowers. Pistil length is in statistically significant correlation to the length of the flower ( $r = 0.5549$ ), particularly in relation to the width of the flower ( $r = 0.5549$ ). Length of pistil is, according to the coefficient of determination, determined with 64% by the width of a flower. The largest



number of stamens was registered within male flower (320.10), significantly more than in hermaphrodite flower. Number of stamens is in statistically significant correlative dependence to the number of sepals and petals.

Examined cultivars did not show statistically significant differences in the flower structure; therefore, these features could not be used to determine and to distinguish varieties.

## CONCLUSIONS

Blossoming of pomegranate in conditions of montenegro occurs in one or two large waves. The most important factor influencing the dynamics of flowers formation is outside temperature.

The rapid flowering always occurs two weeks earlier in the localities tomba and balabani, while in the orchard at higher altitude was delayed for two weeks. The maximum fall of flowers and fruitlets happens 14 to 17 days in relation to the maximum of flower formation. Fall of formed fruitlets stops in the period 20-25 july.

Hermaphrodite flowers are larger in length and width, with a long pistil, which is a correlatively conditioned characteristic. Male flowers have significantly more stamens compared to the hermaphroditic flowers, which is in function of better fertilization.

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## UTILISATION OF INDIGENOUS KNOWLEDGE SYSTEMS FOR SUSTAINABLE VEGETABLE PRODUCTION IN EKITI STATE: IMPLICATIONS FOR SUSTAINABLE AGRICULTURAL DEVELOPMENT IN NIGERIA

### SUMMARY

Vegetable production is often faced with some challenges that borders on pests and diseases infestation and low yield. Therefore, this study explores indigenous knowledge system (IKS) approaches in vegetable production under tropical conditions. The following were examined: personal attributes of vegetable farmers, identification of various vegetables production stages with their corresponding IKS approaches, presentation of procedures used in developing some of the IKS amongst farmers, identification of reasons for using IKS in vegetable production and examination of benefits and problems associated with IKS utilization in vegetable production. Frequency distribution, percentages, mean and standard deviation were used to describe the data. Inferential statistics such as correlation was used in analysis. There was a relationship between IKS approaches used in vegetable production and personal attributes age, farm size, household size, and income. Reasons for using IKS included: hazards involved in utilisation of synthetic fertilizers, less financial commitment in processing of material, and it is environmental friendly. Benefits and problems associated with IKS were: 'it is profitable', 'it is cheaper than modern method', and 'it increases farm revenue'. Some IKS used in vegetable production include: bush fallow, trash burning, bed and heap making, soil incorporated with weathered poultry manure used to control nematode in tomatoes, extracts of occimum to control damping off. Others include application with lotion from black local soap and dusting with wood ash from Iroko tree (*Chlorophora excels*) to control insects. Constraints to production and IKS must be improved and to forestall IKS being supplanted for use in sustainable agricultural development in Nigeria.

**Keywords:** farmer, hazard, pests and diseases and agricultural development

### INTRODUCTION

Vegetable production has always been an integral part of traditional farming systems in developing countries. (Awujoola, 2007). Vegetables are

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needed in households in the tropics to enhance, or improve, intake of balanced diets and they serve as a source of income to small producers (Rubsihayo, 1994). Demand for vegetables has increased as a result of urbanization, industrialization, growth in population and diversification of eating habits (Rubaihayo, 1992).

Vegetable gardening practices promote variety in diets and improve household nutrition of rural populations. In spite of vegetable improvement programs which produce high yielding, early maturing, disease resistant varieties and extension services to boost production in developing nations, there is reluctance by producers to adopt scientific knowledge.

Atteh(1990) and Bamigboye & Kuponiyi (2009) noted that most scientific knowledge failed because of assumed superiority of western technologies and the body of knowledge by small scale producers. Kolawole (2002) claimed that rural people, to whom all research development efforts are directed, have their own body of knowledge that enables them to arrive at decision which would help better their lots. Brokensha et al. (1980) stated "to ignore rural peoples knowledge is to ensure failure in development". This is because local people respects the expertise of indigenous people which is regarded as a major contribution to development (Osunade 1996). The development and use of local knowledge including agricultural practices is generally believed to conform to ecologically sound land management systems.

The study was undertaken to investigate utilisation of indigenous knowledge systems for sustainable vegetable production in Ekiti State, Nigeria. Specifically it was undertaken to:

- a) describe personal attributes of vegetable farmers in the study area,
- b) identify various vegetable production stages and their corresponding IKS approaches,
- c) present procedures used in developing and utilising some of the IKS among farmers,
- d) identify reasons for using IKS in vegetable production and examine benefits and problems associated with IKS utilization in vegetable production.

## **MATERIAL AND METHODS**

The study area was Ekiti state in south-western Nigeria located between 40° 20' and 50° 40' east longitude and between 6° 20' and 8° 10' north latitude. Ekiti state is comprised of 16 Local Government Areas (LGA). Farming is a widespread occupation

A multistage sampling technique was used to select respondents. The first step involved purposive selection of 5 LGAs from the 16 LGAs in the state based on the concentration of vegetable farmers. Two communities were then randomly selected from each of the LGA, making ten communities. Using simple random sampling technique, 10 respondents were sampled all 100 vegetable farmers were

sampled Validated and pretested structured interview schedule was used to elicit requisite information from the respondents between April and May, 2014.

#### Data analysis procedure

The simple descriptive statistics frequency, percentage, mean and standard deviation were used to describe the data while Pearson Correlation was used to make inferential deductions on the relationship between IKS utilization and personal characteristics of the vegetable farmers

### **RESULTS AND DISCUSSION**

Personal characteristics of respondents (Table 1) varied. The majority of respondents are in the active years of life. There are more female vegetable farmers than males, and (corroborate) findings of (Rubaihajo, 1994). The average family size varied but suggested it was made up of husband, wife, children and dependant relatives, Most respondents had a below average family income. Almost all respondents had some education.

Table 1. Frequency and percentage distribution of socio-economic characteristics of sampled vegetable farmers in Ekiti state, Nigeria

<b>Socio-economic characteristics</b>	<b>Frequency</b>	<b>Percentage n=100</b>
<b>Age</b>		
Below 35	20	20
Between 49 and 59	64	64
64+	16	16
Total	100	100
<b>Gender</b>		
Male	30	30
Female	70	70
Total	100	100
<b>Household size</b>		
1 to 5	60	60
6 to 10	25	25
11+	15	15
Total	100	100
<b>Income (Naira)</b>		
93,580	58	58
58,966.5	19	19
24,351	15	15
> 100, 000	8	8
Total	100	100
<b>Education</b>		
Primary education	36	36
Post secondary	25	25
Secondary	29	29
None	10	10
Total	100	100

The IKS utilized in vegetable production varied (Table 2). The great majority utilized slashing and trash burning for land clearing. The [assumption] was that during burning most soil dwelling insects would have been destroyed and the refuse would add potash to the soil. Other methods were employed for soil fertility management. A minority used ogirisoko lotion to treat seed before planting to protect against soil dwelling insects.

Control of nematodes and pest of okra and tomato (*Solanum lycopersicum* L) were used, and other methods were used for above ground insects. Methods were used to control damping off a fungal disease of tomatoes and pepper (*Capsicum annuum* L.) The IKS are highly utilized in vegetable production in the study area. The finding support the report of Kolawole (2002) that no matter the degree of modernity, people will still use what is known to them.

Table 2. Distribution of respondents by the types of IKS utilize in vegetable production (2013)

Stages of production	Corresponding IKS	Frequency	Percentage
Land preparation Slashing without burning Slashing and trash burning Heap and bed making	Cutlass	25	25
		80	80
	Hoe and shovel	65	65
		35	35
Sowing Seed	Treated seeds with ogirisoko portion to guide against soil dwelling insects	40	40
Soil fertility management	Organic manuring	60	60
	Bush fallow	72	72
	Cover cropping	40	40
	Crop rotation	5	5
Weed control	Manual (using hoe)	100	100
Pest and nematode control	Soil incorporated with weathered poultry manure	59	59
	Extract of siam weed and neem	15	15
Defoliating insect Control	Dusting from iroko wood ash	50	50
Disease control Damping off tomatoe and pepper	Black local soap	45	45
	Spray with occimum portion	48	48

The majority of respondents indicated that IKS was less hazardous had less financial commitment are available as reasons for using it in vegetable production (Table 3). A smaller subset use IK it is environmentally friendly.

Table 3. Reasons for using IKS

Reasons for using IKS	Frequency	Percent
Less hazardous	80	80
Less financial commitment in processing of material involved	62	62
It is environmental friendly	40	40
It is readily available	65	65

Better than half of respondents indicated that energy is dissipated during preparation and utilization of most IKS causing a problem to its utilization (Table 4). A smaller subset indicated that elders who are the major custodians of the IKS are not willing to divulge the knowledge. The serious implication is that most of the IKS are not being documented and can be lost. A little better than half felt that the odor is disagreeable, this may be curtailed through proper standardization.

Table 4. Problems associated with the use of IKS

Reason	Frequency	Percent
The odor could be offensive at times e.g. neem during extraction	55	55
A great of energy consumed during preparation and utilization	60	60
Custodians of indigenous knowledge are not willing to knowledge	45	45

Multiple responses

Source: Field survey, 2014

Four personal characteristics of vegetable farmers had positive and significant relationships with IKS utilization (Table 5). The positive correlation of age, household size, income and farm size indicated that the greater the variation in these variables, the more IKS producers utilized in vegetable production.

Table 5. Correlation analysis showing relationships between IKS utilization and socio-economic characteristics of vegetable farmers

Socio-economic characteristic of vegetable farmers	Correlation coefficient	Coefficient of determination $r^2$ (COD)	% Contribution
Age	0.359	0.1288*	12.8
Household size	0.118	0.0139**	1.39
Income	0.322	0.159**	15.9
Farm size	0.380	0.144*	14.44
Education level	-0.076	0.0057NS	0.58

\*Significant at 0.05 \*\* significant at 0.01

The implications are: since experience is a product of age, the older the farmer is the more experience he had in farming and the more local methods were used. Also, the larger the household size (which may serve as farm support) the more IKSs were used. The large with a tendency of variation of crops. The higher the income generated the more IKS practiced indicating that if something works it would be adapted. The negative correlation between education and IKS utilization would tends towards western methods of farming (Deji et al., 2005; Bamigboye, 2008).

### CONCLUSIONS

The types of IKS utilized in the vegetable production in the study area were: soil incorporated with weathered poultry manure, extracts of occimum to control damping off and dusting with wood ash from *Chlorophora excels*. Some of the principal problems militating against the use of IKS in vegetable production were: custodian of indigenous knowledge are not willing to diverge their knowledge and the odor could offensive during preparation and usage It is therefore recommended that scientists should mainstream indigenous people into research cycle for holistic rural development.

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## **CEREALS BREEDING & SEED BUSINESS IN RUSSIA: CURRENT DEVELOPMENT AND PROSPECTS**

### **SUMMARY**

Winter wheat is cultivated at 13.1 ha in Russia. Volume of the domestic seed market is estimated at \$2 bln, while the share of commercial seeds is \$1 bln. The highest yields (about 5 tons per ha) are harvested by farmers of Southern region. Production risks are much higher in Central part, and Middle Volga. There are 292 winter wheat varieties listed in the National Register in 2015; 98% of them are local ones. Krasnodar Institute is on the top of winter wheat breeder's rating list, out of about 50 domestic breeders. SWOT analysis of national breeding programs showed, their strengths are reach gene pool, and traditional selection methods. Institution network in range of zones, varieties adaptation especially in low-, and average income segments; intellectual property IP rights similarity to European ones, and others are also strengths. Weaknesses are old infrastructure and equipment's; limited access to modern technologies; autocratic management; poor breeders motivation etc. Opportunities are possible collaboration development; trailing in different climate zones; seed market potential growth, etc. Realty as subject of raiders attacks; agricultural policy unpredictability; "dyeing" some breeding programs due to cost; erosion of genetic resources; mutual sanctions; availability of know-how and hi-tech in breeding are threats. Full life cycle of variety lasts from 26 to 30 years. Royalty market of winter wheat certified seeds are assessed as \$3.4 m

**Keywords:** breeding programs, winter wheat, seed market, variety life circle, royalty rate.

### **INTRODUCTION**

Russia has got 9% of the world's arable land with 26% of the most fertile soils globally [Alabushev A. 2010; Medvedev A.2009; Shamaev V. 2014]. Agricultural sector accounts for 4.1% of the country's GDP as of 2013, which declined from 5,6% in 2010. Grain production is a key sector of agriculture with gross wheat yields 58 m tons in 2014, and as a result, Russia is of the important grain exporter. However, country should play a greater role on the world markets of food and feed supply in near future because of available agro-climatic resources.

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Main constrains in the development of agriculture is low market financing of arable land [Shamaev V. 2014]. State regulation of the grain market is controversial; the allocation of resources between agriculture, processing, trade, banks is not optimal, and at the expense of agricultural production. Limitation of commodity wheat exports by imposing export duties in 2015, and devaluation of local currency reduce market funding farmers, exacerbated by the increased interest rates. The domestic market is not able to compensate the farmers loses from the restriction of export facilities, which reduce intensification (and cost of seeds).

The objective of the article is to assess competitiveness of Russian crop breeding and seed production complex on the example of winter wheat (*Triticum aestivum* L.)

### MATERIAL AND METHODS

National Registers of Belorussia, Germany, Kazakhstan, Russia, and Ukraine, official statistics of Russia and USDA , results of author's market researches, and market assumptions were used as sources of information. Domestic breeding programs are assessed with SWOT analysis. Commercial variety life circle calculation is based on author's methodology [Goncharov S. 2013].

### RESULTS AND DISCUSSION

Average yields of winter wheat grew up about 5 Dt per ha in Russia for last 30 years, what is significantly less, then in USA (+7 Dt/ha), and in China (+20 Dt/ha) mainly due to laggard in breeding (fig.1).

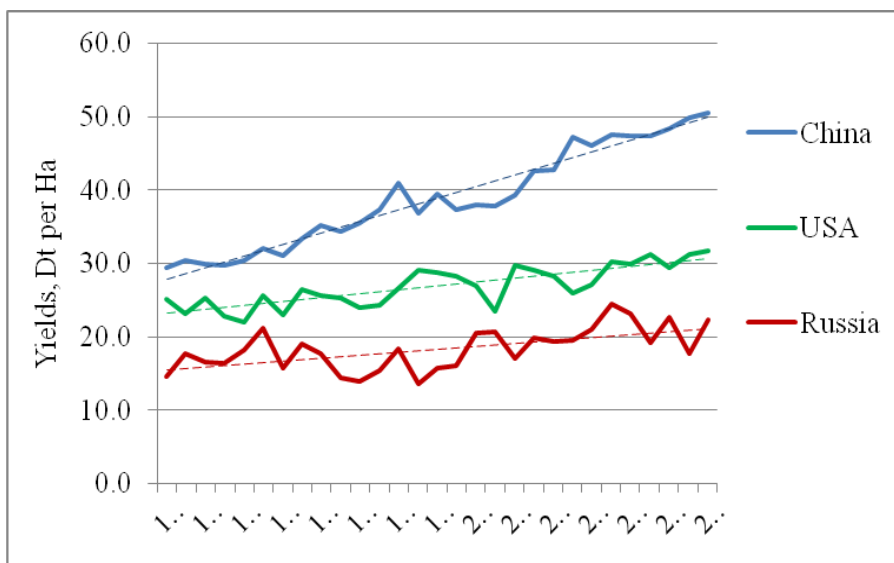


Fig.1 – Wheat yields in China, USA, and Russia, Dt per Ha  
(source <http://faostat3.fao.org/download/Q/QC/E>)

Seed is a key factor of agricultural prospects, as other agricultural inputs efficacy depends on them in a big scale. Appropriate seeds quality is required to meet the demand of diverse climate conditions and cropping systems. Sustainable agricultural production depends on adapted varieties flow, crops production and efficient channels of quality seeds supply to the farmers [Medvedev A.2009; Alabushev A. 2010; Goncharov S. 2013]. Volume of the domestic seed market is estimated at \$2 bln, while the share of commercial (marketed) seeds does not exceed \$1 bbln.

The seed sector of ex-Soviet Union was well organized and was controlled by State planned economy. Despite current attempts to reform it, the overall situation remains practically unchanged. Seed industry faces a major challenge in terms of deteriorating material and technological base for the breeding and lack of infrastructure required to supply quality seeds for the farmers. Developing import substitution policy, the State declares breeding & seeds business support as much as 41,4 bln rubles [RUR] in 2015-2020.

The share of sowing seeds having local origin is high only for cereals and legumes which accounted for 96% of the total market. Wheat production provides turnover as 570 \$/ha in South of the country (June 2015), but only 300 \$/ha in Siberia due to difference in yields, and commodity prices. Wheat yields in Krasnodar region are highest in the country compare to Stavropol and Rostov ones (located in South as well), but even more compare to yields in Voronezh and Belgorod ones (Central Region). Average yields in Krasnodar region improved +20 Dt per ha within period since 1996 to 2014, compare to these in Voronezh region (+8 Dt per ha) (fig.2).

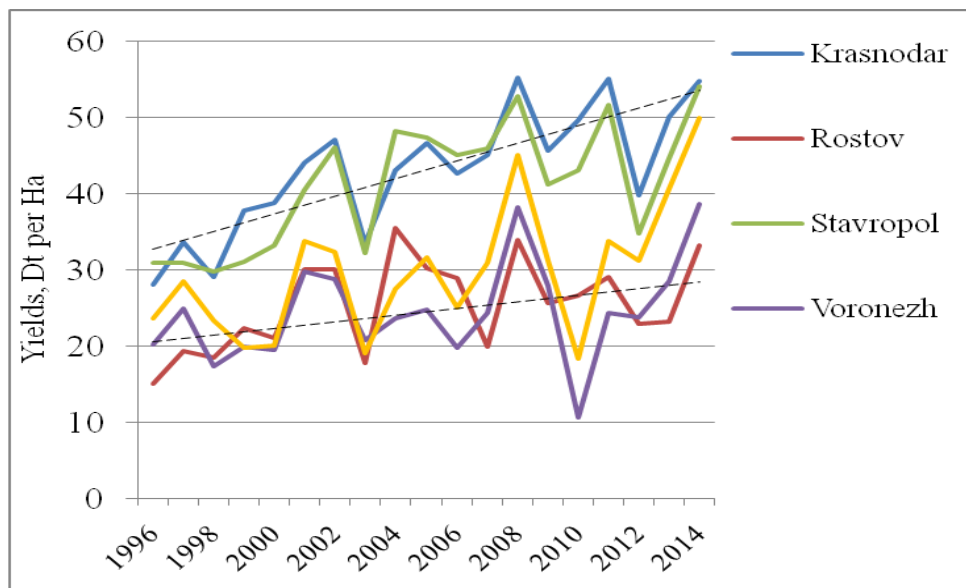


Fig. 2 - Winter wheat yields in regions of Russia, Dt per ha  
(source [http://www.gks.ru/bgd/regl/b08\\_11](http://www.gks.ru/bgd/regl/b08_11))

South of Russia is the “grain basket” of the country by cultivation areas, number of listed winter wheat varieties, and substantial yields as well. There are 292 winter wheat varieties from about more than 50 applicants in the National Register; 98% are local ones. Out of them 10 top-leaders are grown at 40% territory of the crop acreage. Central Russia and Middle Volga are also zones of winter wheat production, but with higher risks.

Varieties of market leader Krasnodar Institute by Luk’yanenko name (KNIISH) are cultivated at 98% winter wheat area (1.2 m ha) of Krasnodar region, at 50% area of Stavropol region (1.7 m ha), about 20% of Rostov region (2 m ha). KNIISH is only breeder of 44 listed wheat varieties, and is additionally co-author of about 30 joint bred ones. Bred 25% of the varieties listed in National Register (Grom, Irishka, Juka etc.) KNIISH puts in registration up to 10 applications annually: 4-5 of them will be listed then. KNIISH is also commercializes their varieties in Ukraine, Armenia, Azerbaijan, and Turkey. Its varieties are cultivated about at 24% areas of winter wheat in this country (fig.3). Academic L.A. Bespalov leads wheat breeding program there [3].

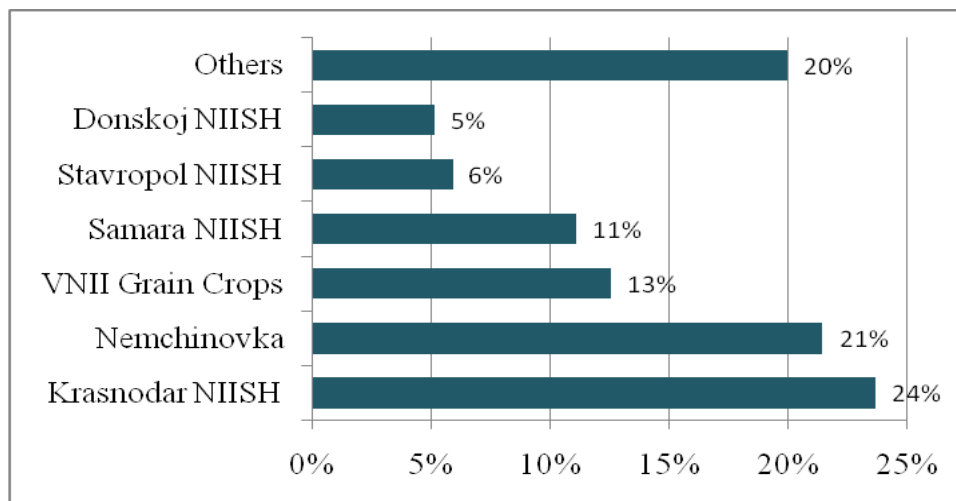


Fig.3 – shares of winter wheat top breeders in Russia, % of cultivated areas

All 12 listed varieties of the Moscow Research Institute of Agriculture "Nemchinovka" domain in the non-Chernozem zone, in the northern and central part of the Central Chernozem region. They are cultivated at 95% areas in Oryol region (out of 0.3 m ha), at 40% in Lipetsk region (0.35 m ha), almost at 60% in Tambov region (0.32 m ha), and 12% in Voronezh region (0.6 m .ha). Such varieties as Moskovskaya 39, Moskovskaya 56, Galina are still market leaders there. Academic B.I. Sanhukhadze is the senior breeder.

33 listed varieties bred by National Research Institute of Grain Crops by Kalinenko name (VNII Grain Crops) domain in Rostov region, occupy 14% of winter wheat areas in Stavropol region, and about 25% in Voronezh one (0.6 m

ha). Its market share is declining due to resign of Dr. V.I. Kovtun, the ex-head of breeding department, who is employed by Stavropol NIISH now. Such varieties, as Severodonskaya jubilejnaya, Don 95, Donskoj sjurpriz, Donskaya bezostaya bred by VNII Grain Crops, used to be market-leaders in different years.

The Donskoj Zone Institute distributes seeds of its 16 listed varieties (Gubernator Dona, Donako, Donskaya Lira etc.) in centraland eastern parts of European Russia. Samara Research Institute (Samara NIISH) and Stavropol Research Institute (StNIISH) are in top-6 list of national winter wheat breeders, and their products are well presented in Mid-Volga and Southern regions correspondingly.

The success of local breeding programs took place in xx century due to genetics development and the implementation of crossing and selection methods. However, backlog, accumulated the last 30 years as only a few local breeding programs relay on innovative technologies. Using the methods of dna sequencing, genetic distance study, marker selection and di-haploids production in the connections with conventional breeding provide their owner the competitive advantages. higher adding value of “innovated” varieties provides their better competitiveness in commercial use.

They are sulpho-, and imidozalinon-tolerant hybrids and conventional varieties, “stay green” effect of corn, “nully-lox” malting barley varieties etc. Further yield potential growth due to hetherosis effect is expected by cereals hybrids commercialization. As a breeding is low business in this country, private breeding programs due to high interest rate (15-20%), and inflation (8%).

Traditional sources of local breeding programs funding are 1) the state budget, which recoups staff salaries expenses; 2) off-budget funds are spent on breeding program running (equipment and facilities, fuel, fertilizers, plant protection, etc.) Off-budget funds are also including royalties. Share of the off-budget is not sufficient.

Strengths of domestic breeders are: diverse genetic resources; the network of research stations and universities in different soil-climatic zones and, as a consequence, adaptation of varieties to local conditions, stress-, and diseases tolerance. The approved IP rights system is similar to the European one. The traditions and continuity of scientific schools, which were developed in the USSR, are also there.

Weaknesses are outdated equipment and facilities, an excessive bureaucracy. Breeders are poor motivated, and aged scientific staff is common. Commercial funding is not satisfactory; institutes share profits from the use of breeding varieties (not breeders), so investments in research and development (r&d) are insufficient. There is a lack of modern methods of increasing the efficiency of traditional selection. Official trialing don't provide objective results due to low-, and average input there. There is a lack of the necessary

infrastructure to ensure farmers get quality seeds, varietal weakness policy and legislative framework. Breeders are poorly informed about the processors and grain trader needs in value chains.

Table 1. SWOT analysis of domestic breeding programs

<p><b>Strength</b></p> <ul style="list-style-type: none"> <li>-Gene pool</li> <li>-Traditional selection</li> <li>-Institution network in range of zones</li> <li>-Varieties adaptation</li> <li>-Low-, and average income segments</li> <li>-UPOV membership</li> <li>-IP similar to European ones</li> <li>-Scholarship &amp; traditions</li> <li>-State support</li> </ul>	<p><b>Weakness</b></p> <ul style="list-style-type: none"> <li>-Old infrastructure and equipment's</li> <li>-Limited access to modern technologies</li> <li>-Burocratic management</li> <li>-Poor motivation of breeders</li> <li>-Shortage of market funding</li> <li>-Registration (timing, transparency, low input)</li> <li>-Seed business constrains</li> <li>-Pain point &amp; needs of processors uncertainty by breeders</li> </ul>
<p><b>Opportunities</b></p> <ul style="list-style-type: none"> <li>-Collaboration development</li> <li>-Trialing in different climate zones</li> <li>-Seed market potential (growth commercial seed share)</li> <li>-Private investments in breeding programs</li> <li>-CIS market demand growth</li> <li>-Privatization of local institutes</li> <li>-Import substitution program</li> </ul>	<p><b>Threats</b></p> <ul style="list-style-type: none"> <li>-Realty as subject of raiders attacks</li> <li>-Agricultural policy unpredictable</li> <li>-Some breeding programs to stop due to low effecacy</li> <li>-Erosion of genetic resources</li> <li>-Mutual sanctions: availability of know-how and hi-tech in breeding</li> <li>-Breeding programs to stop</li> </ul>

Opportunities of the local breeding programs are in development of cooperation, particularly in the trialing in different zones. Collaboration with western seeds companies facilitates market approach. Growing capacity of the grain market increases demand of new varieties and high quality seeds by farmers. Share of commercial seeds is going to grow up since 8% nowadays to 12-15% in 10 years. Private seed companies make attempts to invest in crop breeding. New domestic varieties have good chances to be distributed in other republics of the cis cue to similarity of geographical, cultural and economic conditions. About ¼ varieties in the state register have 2 or more of originator (institutes), what proves needs of trialing network in different climate conditions and regions; that's another prospect of possible cooperation development. Further r&d institutes privatization will give an impetus to their development. Current state policy of import substitution is additional support of local breeding varieties. There are a range of threats in this country. Breeders are not



independent; they are part of institutes or stations. Most of local r&d centers possess properties (land, realty etc. From ex-ussr times).raider schemes for disposing of valuable properties are the greatest threat for the local r&d, and for breeding programs as well, as many institutes are located in the territory of big cities. So, price of realty is high. Lobbying of domestic varieties and hybrids leads to possible breeding postponing due to shortage of competitiveness. Other threats are uncertainty of agricultural policy in general and the unpredictable effects "manual control" of the economy, and thus legal uncertainty. Government doesn't allow bio-tech, gm-researches to be developed properly. Substantial number of breeding programs may be ceased due to insufficient return of investment.

Commercialization of awheat variety begins together with seeds sale, which coincides with its registration in the state register, and overs with its exception, termination of seed production and seeds sale. Currently the average period of commercial use, or winter wheat life circles in this country is 13 years. It is shorter, then 17 years for spring barley, and 20 ones spring wheat [goncharov s. 2013]. Generally, life circle of winter wheat variety in russia has the sameduration, as in germany, but longer compare to belorussia, and ukraine (fig. 4)

Taking in consideration duration of selection of the winter wheat variety is 10-12 years, the registration lasts for 3-4 years, and commercial use doesfor 13 years, a full cycle of a variety is from 26 to 30 years on average. Breeder should for eese needs of consumers and processors as well at least 15-25 years forward.

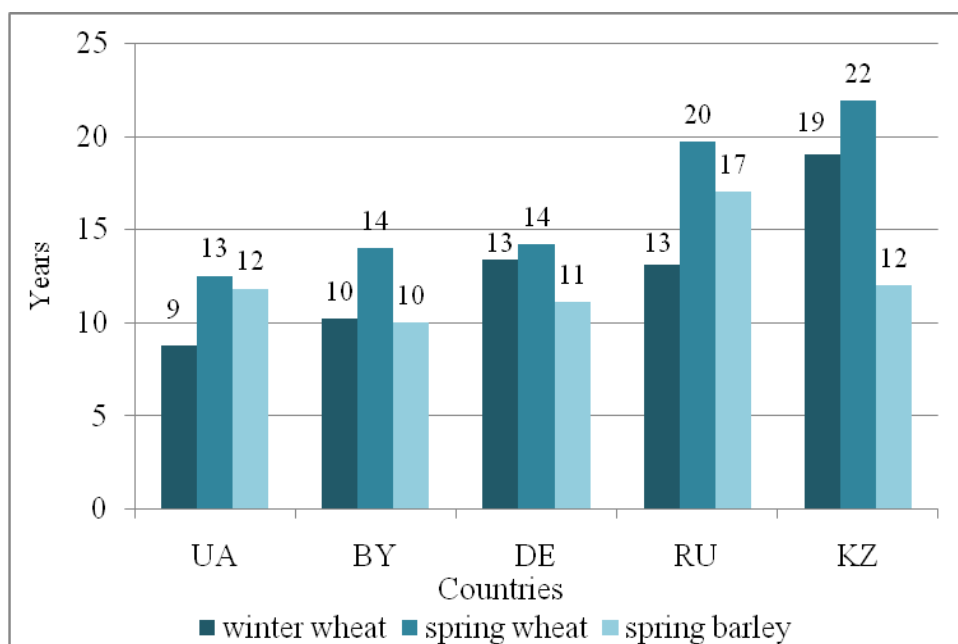


Fig. 4.–Commercial life circle length of cereals varieties in different countries, years [4].

Winter wheat acreage reaches 13.1 m ha in Russia, and with seeding rate as 0.23 t/ha, the seeds demand is 2.97 m tons. However, with 8% share of the commercial seed, actual demand is 0.24 m tons. Considering average price of selling seeds as 15 th.rur./t, and average royalty rate as 5% of 1 ton of seed price, the potential of royalty market is 180 m rur(\$3.4 m). Royalties for farm saved seeds are not common in this country. For comparison, winter wheat area of Germany as 3.2 m ha with seeding rate as 0.14 t/ha needs 0.45 m tons of seeds (Goncharov, 2013). Considering 45% share of the commercial seeds, their volume amounted as 0.20 m tons. Multiplying the amount of commercial seed at the average royalty rate (73 \$/t) get market volume royalties as \$14.6 m (without farm saved seeds -fss royalties).

There are more than 50 local winter wheat breeding institutes and stations in Russia compared to 13 ones in Germany. Thus, royalty income of German breeder exceeds those in Russia, what allows to select more varieties with higher added value through the use of modern methods and hi-tech approaches. Up to 10-15% of the turnover of German seed companies invested in R&D.

Low market financing of arable land (per hectare revenues in the production of wheat) in Russia has direct impact on sustainable development of breeding programs. Seed market development may be imposed via:

- commercial seeds share rising from 8% nowadays till 12-15% in 10 years;
- privatization of breeding institutes, and stations;
- state investment in R&D, and in high-tech especially;
- concentration and industrialization of seed business; about 20 cereals processing plants with capacity 250 th.tons were built for last 15 years.
- legal adjustment (fss royalty collection);

Competitive advantages of cereals seeds may be shorter variety life cycle, higher added value of varieties, switch to cereals hybrids due to quick return of investment in breeding, and adequate business-models. But portfolio management is implemented usually by institute's authority, which don't have sufficient marketing knowledge.

Domestic R&D needs substantial investment to fill information gaps, to conduct researches and to ensure the implementation of environmental technologies in agriculture. Climatic changes increase the level of investment needs required to reach food security mainly via crop breeding. This requires additional state investments, as private capital prefers to invest in short-term projects with quick return. The government should improve the data collection mechanisms and to ensure farmers know-how and seeds for their adaptation to new challenges.

## CONCLUSIONS

1) Status of breeding and seed production complex of the country depends on the efficiency of agriculture in general and from market funding of arable land in particular.

- 2) National cereals breeding programs don't meet new challenges in a full scale due to poor State and market financing (high FSS, limited royalty market etc.)
- 3) Commercial life circle of wheat variety lasts 13 years on average.
- 4) Winter wheat royalty market value is \$3.4 m.
- 5) Institute privatization, and State investments in high-tech as shares of R&D reforming should be implemented to improve competitiveness of crop breeding.

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## SHORT-TERM EFFECTS OF GREEN COVER ON SOIL QUALITY AND PLANT BIODIVERSITY OF MEDITERRANEAN ORGANIC OLIVE GROVES

### SUMMARY

Environmental impacts, low yields and high production costs of conventional agriculture threaten the ancient olive groves which are an important Mediterranean benchmark. Implementation of low-impact and cost-effective agricultural practices and evaluation of their short-term effects under organic management are challenges for sustainable management. This work aimed to evaluate the short-term effects of green cover on soil quality and plant biodiversity, focusing on selecting sensitive indicators by comparing organic and conventional management systems. Two green covers - mixed cover crop species (ORG-MCCS) and natural cover (ORG-NATVEG) - were compared with conventional groves (CONV). Soil quality and plant biodiversity were evaluated before and after green cover application. The results showed that certain physical and chemical and most measured biological soil parameters differed significantly between treatments. Moreover, ORG-MCCS performed better than ORG-NATVEG. The parameters were selected to be sensitive indicators. In conclusion, the present work gives further information on the effects of management systems and green cover application on olive orchards. Impact assessment of agricultural practices on plant and soil biodiversity and testing of the selected indicators in similar studies could help in designing sustainable olive-growing practices.

**Keywords:** Grassing, organic olive groves, sustainability, indicators, biodiversity

### INTRODUCTION

The environmental, socio-cultural and economic values are among the most perceived important values in the Mediterranean Region. Natural value, environmental quality and cultural heritage linked to extensive farming practices

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would allow characterizing them as “high natural-value farmlands” (Calabrese et al., 2015). These qualities positively or negatively are affected by agricultural practices depending on cropping systems and farm conditions. The Mediterranean ancient olive orchards (AOOs) play an important role for the Mediterranean basin to be one of the 34-biodiversity hot spots on Earth. Olive tree is one of the oldest known cultivated trees are a cultural and historical symbol of the basin (Kabourakis, 2012). Traditionally, olives were management with extensive agricultural practices and recognized as high ecological and cultural values (Calabrese et al., 2015). Environmental impacts, low yields and high production costs of conventional agriculture threaten the AOOs, which are an important Mediterranean benchmark. Intensification and implication of agricultural practices to reduce the cost of production has led to the widespread bare-soil practice. This can result in detrimental impacts on soil quality and plant biodiversity (Calabrese et al., 2015; CENT.OLI.MED, 2012). Implementing alternative farming practices, which are environmentally sound – conserve natural resources (Soil, water and biodiversity) and minimize the cost of production under organic management are promising. Application of cover crop using mixture of species is among the best alternative and beneficial practices (Clark 2007). Implementation of low-impact and cost-effective agricultural practices and evaluation of their short-term effects under organic management are challenges for sustainable management. This work hence aimed to evaluate the short-term effects of green cover on soil quality and plant biodiversity, focusing on selecting sensitive indicators by comparing organic and conventional management systems.

## MATERIAL AND METHODS

The short-term effect of soil green cover practice –“grassing” on soil physical, chemical and plant biodiversity parameters on AOOS under organic management system during autumn 2011 to autumn 2012 was successfully investigated. The study was conducted in Torre Guaceto State Nature Reserve, Apulia Region (South Italy) (Fig.1A) with the aerial photo of the surveyed fields (Fig.1B).

This study quested alternative low-impact, cost-effective alternative to the environmentally detrimental practice of leaving the soil bare in monumental olive orchards. Two types of “Grassing” practices- mixed cover crop species (ORG-MCCS) and spontaneous natural cover (ORG-NATVEG) – under organic management were compared with conventional orchards (CONV). Several soil quality and plant biodiversity parameters were evaluated before (T0) and after (T1) green cover application during autumn 2011 and autumn 2012 (Table 1). Twenty six commercially mixed native species from seven different families mostly (80%) from the Fabaceae family (Table 2) were sown on ORG-MCCS\_3a & 4a while ORG-NATVEG\_3b & 4b were allowed to grow on (Fields 3b and 4b) at the same time the CONV orchards are managed as widespread practices that leaves the soil bare (Fig.1)

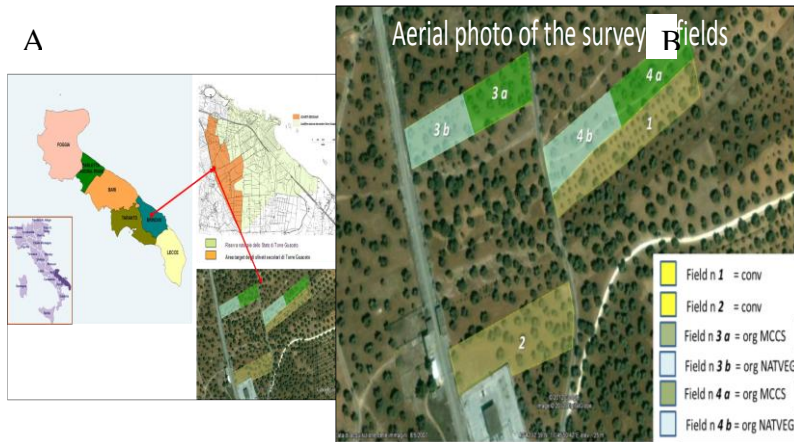


Figure 1 Torre Guaceto State Nature Reserve, Apulia Region (South Italy). In the box at the top on the right, Torre Guaceto State Nature Reserve is highlighted in green and AOOs in orange (A); Aerial photo the studied orchards (B) indicated as (fields) [Fields n1 and n2 –CONV\_1 and CONV\_2 and Fiends n 3a & n 4a - ORG-MCCS\_3a & 4a and Fiends n 3b & n 4b–ORG-NATVEG\_3b & 4b respectively]

Table 1 Potential indicators to evaluate soil quality and plant biodiversity

Sir.No.	Category	Indicator	Acronym	Unit
1	Soil quality	Soil Texture (Sand, Clay or Silt)	TXT	%
2		Bulky Density	BD	$\text{g cm}^{-3}$
3		Rock Fragments	RF	%
4		Field Capacity	FC	%
5		Erodibility	KE	$\text{t ha}^{-1} \text{MJ}^{-1} \text{mm}^{-1}$
6		Annual Soil Loss	ASL	$\text{t ha}^{-1} \text{yr}^{-1}$
7		pH (H <sub>2</sub> O)	pH	-
8		Electrical conductivity	EC	$\text{mS cm}^{-1}$
9		Total Nitrogen	TN	$\text{g kg}^{-1}$
10		Available Phosphorus	PAS	$\text{mg kg}^{-1}$
11		Exchangeable Potassium	KX	$\text{mg kg}^{-1}$
12		Exchangeable Sodium	NaX	$\text{mg kg}^{-1}$
13		Exchangeable Calcium	CaX	$\text{mg kg}^{-1}$
14		Exchangeable Magnesium	MgX	$\text{mg kg}^{-1}$
15		Carbon –Nitrogen Ratio	C/N	-
16		Cation exchange capacity	CEC	$\text{m e } 100 \text{ g}^{-1}$
17		Soil Organic Matter	SOM	$\text{g kg}^{-1}$
18	Plant biodiversity	Shannon diversity index	H'	-
19		Equitability	E	-
20		Number of species	N	-
21		Richness index	RI	-

Table 2. Commercially mixed cover crop species sown on the org-mccs\_3a &amp; 4a fields with family names,% in the mix, and their common names

Sr. No.	Family names	%	Species Names	Common names
1	Apiaceae	1.00	<i>Calendula officinalis L.</i>	pot marigold
2		0.20	<i>Carum carvi L.</i>	caraway
3		0.05	<i>Anethum graveolens L.</i>	smelly dill
4		0.05	<i>Daucus carota L.</i>	wild carrot /queen Anne's lace
5		0.05	<i>Foeniculum vulgare Mill.</i>	sweet fennel
6		0.05	<i>Pastinaca sativa L.</i>	wild parsnip
7		0.05	<i>Coriandrum sativum L.</i>	coriander
8		0.05	<i>Centaurea cyanus L.</i>	garden cornflower
9		0.04	<i>Cichorium endivia L.</i>	cultivated endive
10		Boraginaceae	0.05	<i>Borago officinalis L.</i>
11	6.00		<i>Raphanus sativus L.</i>	cultivated radish
12	Caryophyllaceae	0.16	<i>Agrostemma githago L.</i>	common corn cockle
13	Fabaceae	20.00	<i>Vicia sativa L.</i>	garden vetch
14		12.00	<i>Onobrychis viciifolia Scop.</i>	sainfoin
15		8.00	<i>Trifolium incarnatum</i>	crimson clover / Italian clover
16		8.00	<i>Trifolium resupinatum L.</i>	persian clover
17		7.00	<i>Trifolium alexandrinum L.</i>	egyptian clover/berseem clover
18		7.00	<i>Melilotus officinalis (L.) Lam</i>	sweet clover
19		5.00	<i>Medicago lupulina</i>	black Medick
20		4.50	<i>Medicago sativa- Eugenia</i>	
21		4.50	<i>Medicago sativa L.</i>	alfalfa
22		2.00	<i>Trifolium hybridum</i>	alsike clover
23	1.00	<i>Ornithopus sativus Brot.</i>	common bird's-foot	
24	Hydrophyllaceae	3.00	<i>Phacelia tanacetifolia Benth.</i>	lacy phacelia
25	Malvaceae	1.00	<i>Malva sylvestris L.</i>	high mallow
26	Polygonaceae	7.00	<i>Fagopyrum esculentum Moench</i>	buckwheat

According to the weather data in the area, most of the recorded rainfall of 2011-2012 was concentrated in December to March where it peaked in February 2012 (data not shown). The soil texture of the fields ranged from sandy clay loam, loam and clay loam. Parameters were compared in reference to the conv orchards at t0 and t1. Values were normalized to the condition of conv orchard before the application of the green cover (conv\_1 (t0) =100). Results were then displayed using a spider diagram. Additionally, a one-way analysis of variance (ANOVA) of all collected data but soil biological parameters was run. Significant differences in parameters at t0 and t1 were separated using Tukey's test (HSD)  $p < 0.05$  using Excel statistical software. Finally, significant variables were selected as sensitive



minimum data set (mds) of indicators to evaluate the short-term impacts of management and green cover practices of aofs.

## RESULTS AND DISCUSSION

### Effect of green cover on soil quality and plant biodiversity parameters

Any comparison of the impacts of organic and conventional farming systems on biodiversity (and soil biodiversity) is likely to be problematic, largely as a result of the complexity of, and interactions between, the range of farming practices that comprise the two systems (Hole et al., 2005). Results of the present study show that some soil physical, chemical, and almost all the plant biodiversity parameters showed already clear difference between management systems and grassing practices. Figure 2 shows the soil physical parameters at T0 and T1. It was very clear that the physical soil parameters change at difference times especially with most sensitive parameters such FC, which is very visible the moisture content of the soil, is extremely low regardless of management practices or cover to the soil. Another important an important soil physical property is the ASL significantly higher with CONV management but also with ORG-MCCS\_4a probably influenced by the first tillage practices to sow the MCCS and adds to the higher the estimated ASL (Fig 2). Interestingly important soil physical parameters - FC and ASL had significantly better values with the ORG management systems and the grassing to slightly help in reducing ASL and hence soil erosion (Fig 2). Almost similar values of BD were observed. BD affects porosity and resistance to root penetration and gases and water exchange. It is therefore usual to use it as an index of soil compactness (USDA-NRCS 2008).

Like to the soil physical parameters, the soil chemical parameters were also variable between the systems at the two surveying times. Certainly, SOM, TN, MgX significantly improved with the ORG management and in ORG-MCCS practices (Fig.3). Practical experiences indicate maximal benefits of cover crop application can be obtained by using mixtures grasses and legume species with rotates over time and space (Clark 2007). The plant biodiversity was noticeably improved with ORG-MCCS than ORG-MCCS during the 2011-2012 in terms of both diversity and resilience of plant biodiversity (Table 3). It was clear that H' and N subsequently the RI were improved under the ORG system and ORG-MCCS practices. Although the dynamics of plant species can be variable over time due to many biotic and abiotic factors especially in Mediterranean climate. However, during the following year (2012-2013) using few selected species did not improve the diversity and resilience in same area (Calabrese et al., 2015).

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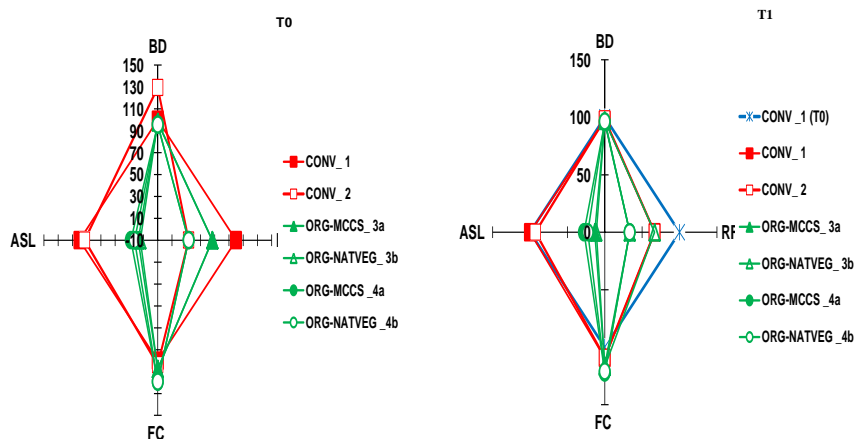


Figure 2 Soil physical parameters at T0 and T1, in reference to field 1 (CONV 1) at T0 (= 100); [Legend: BD= bulk density; RF = rock fragments; FC= field capacity; ASL = annual soil los

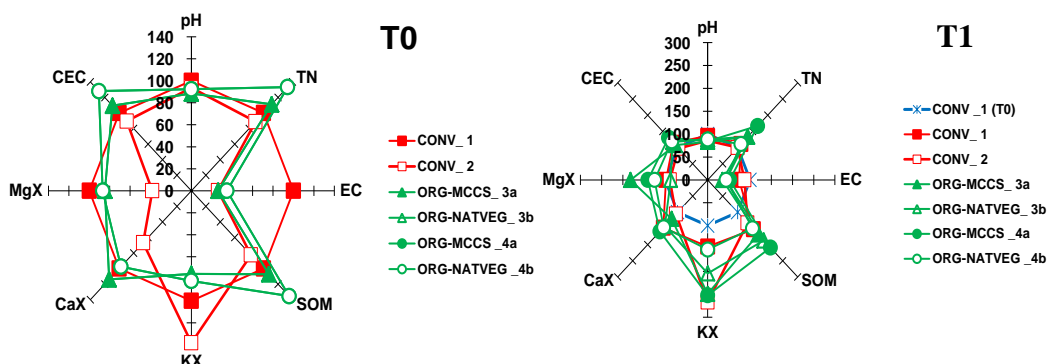


Figure 3 Chemical soil parameters compared before (T0) and after grassing (T1) with reference to (CONV\_1 (T0) =100); [Legend: TN = total nitrogen; EC = electrical conductivity; SOM= soil organic matter; KX = exchangeable Potassium; CaX= exchangeable calcium; MgX = exchangeable magnesium; CEC – cation exchange capacity].

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during the following year (2012-2013) using few selected species did not improve the diversity and resilience in same area (Calabrese et al., 2015).

Table 3. Plant biodiversity parameters measured before (T0) and after (T1) grassing application on AOOS (Autumn 2011 – Autumn 2012)

Management system/ practices	T0				T1			
	H'	E	N	RI	H'	E	N	RI
CONV_1 (T0)	-	-	-	-	100.00	100.00	100.00	100.00
CONV_1	100.00	100.00	100.00	100.00	147.37	108.86	44.44	78.87
CONV_2	91.39	57.59	66.67	82.40	0.00	0.00	0.00	0.00
ORG-MCCS_3a	156.94	80.19	107.41	82.40	190.43	88.61	125.93	110.57
ORG-NATVEG_3b	156.94	80.19	107.41	82.40	84.21	50.633	66.67	68.68
ORG-MCCS_4a	164.59	82.28	125.93	96.83	232.54	104.557	140.74	132.08
ORG-NATVEG_4b	164.59	82.28	125.93	96.83	244.98	111.39	133.33	173.58

[Legend: H' = Shannon diversity index; E = Equitability; N = number of species; RI = richness index]

This work provided early years effects of soil management practices on the aoos which are recognized high as ecological and cultural value crops especially in the south-eastern apulia region (Calabrese et al., 2015). The authors have been searching over the last three years for alternative low-impact, cost-effective agricultural practices to the widespread environmentally detrimental practice of leaving the soil bare in monumental olive orchards.

Table 4. Sensitive indicators selected as MDS to evaluate short-term impacts of management systems or grassing practices

Indicator category	Indicator	Management system		Green cover practices	
		ORG	CONV	MCCS	NATVEG
Soil quality	KE	0.28 a	0.20 b	0.27 a	0.25 b
	ASL	0.22 a	0.94 b	0.20 b	0.58 a
	pH	6.83 b	7.33 a	6.55 b	7.15 a
	TN	1.00 a	0.90 b	1.35 a	1.00 b
	MgX	215.67 a	149.00 b	295.50 a	162.38 b
	CEC	21.48 a	18.15 b	21.55 a	19.80 b
Plant biodiversity	H'	1.87 a	0.89 b	2.21 a	1.29 b
	N	15.75 a	7.13 b	18.00 a	10.86 b

## CONCLUSIONS

In conclusion, the present work gives further information on the effects of management systems and green cover application on olive orchards. Some soil physical and chemical and plant biodiversity parameters showed differences between the two grassing types. Moreover, a positive effect of organic management was already observed from the first year survey. Impact assessment of agricultural practices on plant and soil biodiversity and testing of the selected

indicators in similar studies could help in designing sustainable olive-growing practice.

### ACKNOWLEDGEMENTS

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**IDENTIFYING SOME HERBICIDE RESISTANT GRASSES,  
MONITORING THEIR NATURE AND DISTRIBUTION IN  
SOUTHWESTERN PART OF ALEPPO CITY, SYRIA**

**SUMMARY**

It is common to say that weeds in Syria have developed resistance to herbicides, but this needs to be confirmed by research studies which are rare in the area. This study is run to describe and diagnose those weeds, and to monitor their nature and allocate their distribution in the Quaik river area in the southwestern part of Aleppo city in Syria. Seeds of suspected resistant grasses have been collected during the 2010 summer from Al-Eis, Al-Hadher and Banes, which were theoretically identified as resistant biotype to (ACCase) herbicides. In addition to seeds of grasses collected from ICARDA Tel Hadya station which were expected as sensitive biotype, taking into account that the seeds were preserved properly for the 2011/2012 season. In this experiment four herbicides have been used, two ACCase inhibitors, Ralon super (fenoxaprop-p-ethyl) and Topik (clodinafobpropargyl), the other two ALS inhibitors, Atlantis (mesosulfuron + iodosulfuron) and Pallas (pyroxsulam). The results showed that, the short spike canarygrass (*Phalaris brachystachys* L.) was the most risky in this area which have developed resistance to the (ACCase) in all locations including Tel Hadya station, the hood canarygrass (*Phalaris paradoxa* L.) came second in its resistance shown to the (ACCase) at all locations except Tel Hadya station. The wild oat (*Avena fatua* L.) came third, the results shown developed resistance to the (ACCase) in two locations Al-Hadher and Banes, sensitive in Tel Hadya station, while the results at Al-Eis were uncertain.

**Keywords:** Bioassay, discriminating dose, herbicides resistance, seed sampling, site of action.

**INTRODUCTION**

Herbicide resistance is the genetic capacity of weed population to survive herbicides treatment that, under normal use conditions, would effectively control that weed population. It is an example of evolution happening at an accelerated pace and an illustration of the "survival of the fittest" principle. Applying the same herbicide in the same field year after year will select resistant plants. The resistant weeds may eventually dominate the population. However, the first herbicide-resistant weed was discovered in 1968 (Ryan, 1970). In the next two decades, there have been numerous reports of weed biotypes exhibiting

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resistance to photosystem II inhibiting herbicides (Bandeem et al, 1982). Ian Heap presented a graphs were made from data accessed from the survey website (WSSA, 2014) show the chronological increase in unique cases of herbicide resistant weeds (almost 440 cases). Grasses which is our interest in this study are involving the inhibition of acetyl-CoA carboxylase (ACCase). Lipids are involved in the biogenesis and function of various membranes, cellular signal transduction and other physiological functions (Browse and Somerville, 1991). Herbicide resistant, related (WSSA, 2004) doesn't mentioned any case of herbicide resistant in Syria, while the same reference referred to two cases in Turkey, and one case in Saudi Arabia, both registered as ACCase inhibitors, as well as 19 cases in (Israel), 2 registered as ACCase inhibitors. If the objective of a survey is to determine the occurrence of resistance in one or more weed species to a particular herbicide, fields treated with these herbicides in the growing season when the survey is conducted can be identified by randomly contacting growers in the study area (Beckie et al. 1999a, 1999b). Actually this is well-matched with the farmers of Euphrates river area as they have complained from grasses herbicides, the main complaint was wild oat. The rationale reason was this grass became herbicide-resistant for ACCase inhibitors, because this herbicide group was commonly used since decades. However, new herbicides have been available in Syria recently to control weeds in cereals, by different mode of action, ALS inhibitors. Several demonstration trials applied in 2006 and 2007 in that region clearly indicated the efficiency of herbicide (ALS) in comparison with the conventional herbicides that prevailed for so long (ACCase). The phenomenon was repeated later in the southwestern region of Aleppo, where farmers complained from the herbicides, which were used to control grasses in broad leaf crops, those herbicides are ACCase inhibitors. The Canary grass was the most troublesome weed in this region and obviously was putative resistant to those herbicides. Several demonstration trials applied in 2007, 2008 and 2009 in Quaik river region clearly indicated the efficiency of herbicide (ALS) in comparison to (ACCase).

Heavy infestations of a single weed species surviving herbicide treatment provide the best indication of resistance (Beckie et al, 2000). These are the situation of some grasses in Syria, but despite the simplicity and ease of justification such cases, scientific experiment to confirm herbicide-resistance to (ACCase) in our region is required. The experiment theoretically suppose that, the seeds which were selected from ICARDA center at Tel-Hadya putative susceptible biotype, , this is because of integrated crop management practices are followed in the station, and those collected from other locations putative resistant biotype for ACCase inhibitors, then the effectiveness results after application will prove or deny those hypotheses. Plant response to the herbicide is based on visual evaluation, mortality, or growth inhibition comparative to untreated grasses. Resistance is confirmed if the dose response of the biotypes is statistically different and the putative resistant biotype is not controlled at doses that control susceptible plants (Beckie et al, 2000). If the farmers cannot be convinced that

herbicide-resistance in weeds will have a significant impact on their profits, they will not adopt measures to prevent its development. The cost of resistance is not only related to higher herbicide costs, we should note the costs involved with the additional number of times herbicides have to be applied, also there is further costs resulting from reduced yields because of the poorer weed control. If we calculate the theoretically estimated cost of herbicide-resistance at the national level in Syria, we will find that a huge amount of money was lost and was spent in vain, based on this fact we must persuade farmers to adopt anti-resistance strategies where the cost is much lower in comparison to the cost of resistance. The adoption of such strategies requires first identifying herbicide-resistant, monitoring their nature and distribution in our region. This article is one of the first studies on the herbicide-resistance in Syria that may tackle this phenomenon in details, and that can be further generalized on wider scale in future.

### MATERIAL AND METHODS

Selection of a field site for collecting suspect weeds depends upon the objective. In some instances, specific areas are sampled in response to poor herbicide performance. Alternatively, site selection can be based on past herbicide use or cropping history (Beckie et al, 2000).

Seeds of grasses collected in 2010 summer from Quaik river flat near ICARDA from Al-Eis, Al-Hadher, and Banès, which assumed as herbicide resistant (ACCase) and from inside Tel-Hadya ICARDA center, which expected to be sensitive biotype, those seeds were preserved in paper bags at normal temperature and low humidity room, later they putted in the fridge to break dormancy phase. For many species, seeds are stored at room temperature at low humidity (40% or less) for 1 to 4 mo (Adkins et al. 1997). For some species, storage at temperatures below freezing is more conducive to breaking dormancy (Anderson et al. 1998).

Locations had chosen upon practical experience in this region, as well as the demonstrations trials results that directly referred to herbicide-resistant. Wild oat, hood canary grass and short spike canary grass had collected from southwestern of Aleppo. Seeds collected in the previous year had planted in December 1, 2011 in sterile soil pots, each pot had coded to reflect the type of grass and collecting origin, Table 1.

Grasses	Al-Eis	Al-Hadher	Banes	Tel-Hadya
<i>Avena fatua</i> .	AVEFA-R1	AVEFA-R2	AVEFA-R3	AVEFA-N
<i>Phalaris paradoxa</i>	PHAPA- R1	PHAPA-R2		PHAPA-N
<i>Phalaris brachystachys</i>	PHABR- R1	PHABR-R2		PHABR-N

Each grass type encoded by location and species. It planted in 15 pots, then 10 pots had chosen because of good germination to facilitate the process of spraying. Five treatments including untreated control had applied. Two of those

herbicides were ACCase inhibitors, Ralon Super (fenoxaprop-P-ethyl 69g/L) recommended dose (41.4 g a.i. ha<sup>-1</sup>) and Topik (clodinafobpropargyl 240 g ) dose (60.0 g a.i. ha<sup>-1</sup>), and two herbicides were ALS inhibitors, Atlantis OD (Mesosulfuron-methyl 10g + Iodosulfuron-methyl 2g /L) dose (10.0 g a.i. + 2.0 g a.i. ha<sup>-1</sup>), and Pallas OD (Pyroxsulam 45g) dose (22.5 g a.i. ha<sup>-1</sup>) the untreated control sprayed by water only.

The discriminating dose is not defined as the recommended field (label) rate, although the latter may equal the former in some cases (Heap 1994). So the discriminating doses for this experiment are followed the label recommendation as well as the local experience in this region. Application date was April 3, 2012 where Azote Knapsack sprayer fitted with 4 flat fan nozzles 80.02, boom wide 2m, 250 liter/ha. Grasses stages were B22 – B30 (Uwe Meier, 2001).

The experiment carried out in strip-plot design in two replicates for two treatment factors, the biotypes from different sites and the herbicides, using pots as an experiment unit. This design adopted for its role in reducing the impact of leakage of chemical treatment applied between pots. Data were analyzed using analysis of variance (ANOVA) to assess main effects and interactions of the two factors, herbicide treatments and grasses, taking into account the error structure under the strip plot design in RCBD. Furthermore, residual plots were examined to support the ANOVA assumptions. Pairs of treatments of interest were compared using least significant differences (LSD) at 5% level of significance. In order to study the similarity of herbicides in term of biotypes responses to herbicides treatments, we used hierarchical clustering analysis using Euclidean distance to form the similarity matrix and furthest neighbor (i.e. complete link) as clustering method.

Plant response to the herbicide is evaluated based on visual evaluation, mortality, and growth inhibition relative to untreated grasses. Resistance is confirmed if the dose response of the biotypes shows significant differences and the putative resistance biotype is not controlled at doses that control sensitive plants.

American method was used as an estimating scale (i.e. 0 = Health Plant - 100 = Full Death). Commercial formulation of the herbicide was applied using accurate sprayer. The efficacy evaluations have been taken 23 days after application.

## RESULTS AND DISCUSSION

The primary hypothesis expected to find no resistance at Tel-Hadya's location, while herbicide-resistant for ACCase inhibitors are developed in other three locations. Results of efficacy revealed this expectation and show significant differences between the collect location treatments (Fpr <0.001). Grasses have been collected from all locations clearly reflects the good response of using (ALS) herbicide and the results were excellent, generally the efficacy results were > 85% with high level of similarity, this results are confirming that no resistant for ALS inhibitors.



The (ACCCase) herbicide reflected variable responses in term of locations; Tel-Hadya site (AVEFA-N) presented acceptable response to all herbicides, the efficacy results were  $> 60\%$  which mean no herbicide-resistant for ACCCase inhibitors. The other two locations Al-Hadher (AVEFA-R2) and Banes (AVEFA-R3) reflected bad efficacy response, the efficacy were  $< 40\%$ , which mean clearly resistance for (ACCCase) as we expected. Abnormal response was at Al-Eis location (AVEFA-R1), because of good response to the Topik herbicide results, which was almost  $75\%$  while Ralon was almost  $40\%$ , obviously the herbicide-resistant in Al-Eis location was confusing, this contradicts the hypothesis, so it is appropriate to recommend to keep monitoring the wild oats in Al-Eis location.

In some areas of Syria *Phalaris* spp. can be found with high density and represented as dominant grass, Quaik river area can be considered as good example, canarygrasses can be noticed easily in wheat fields where the conventional methods are adopted, like the multiple tillage, continuously planting cereal and the rotation with cereals every second year at best probability, so the annual weeds are considered the most harmful comparing with perennial.

The (ACCCase) herbicide reflected variable responses on hood canarygrass in term of collected locations. The Topik efficacy results was  $> 85\%$ , while Ralon was  $< 25\%$  for seeds collected from Tel-Hadya site (PHAPA-N). As we know in advance Ralon is normally weak in controlling this grass (previous experience), so we can accept this outcome that function of (ACCCase) will be represented only by Topik, therefor we can say that, no herbicide-resistant for ACCCase inhibitors in this location. The other locations Al-Eis (PHAPA-R1) and Al-Hadher (PHAPA-R2) reflected bad efficacy  $< 40\%$ , which means clear resistance for (ACCCase) as we assumed in advance.

The (ACCCase) herbicide reflected bad response on short spike canarygrass for all locations, including Tel-Hadya site. The efficacy results for Topik and Ralon were  $< 45\%$ , this contradicts with the hypothesis. Herbicide-resistant for ACCCase inhibitors in Tel-Hadya (PHABR-N) location may be the most important conclusion and unexpected result in this trial, while the results were bad as assumed in the other locations Al-Eis (PHABR-R1) and Al-Hadher (PHABR-R2).

The wild oat results in different locations were encoded with herbicide's name, example (Banes-A), which means wild oat sampling from Banes and treated by Atlantis. The hierarchical clustering analyses clearly separated the sensitive biotypes (S) than resistant biotypes (R). The results reflects an excellent responses when using Atlantis and Pallas herbicides in all locations, those results confirm that no resistant for ALS inhibitors found. On the other hand variable responses when using Topik and Ralon herbicides related to different locations has been noticed.

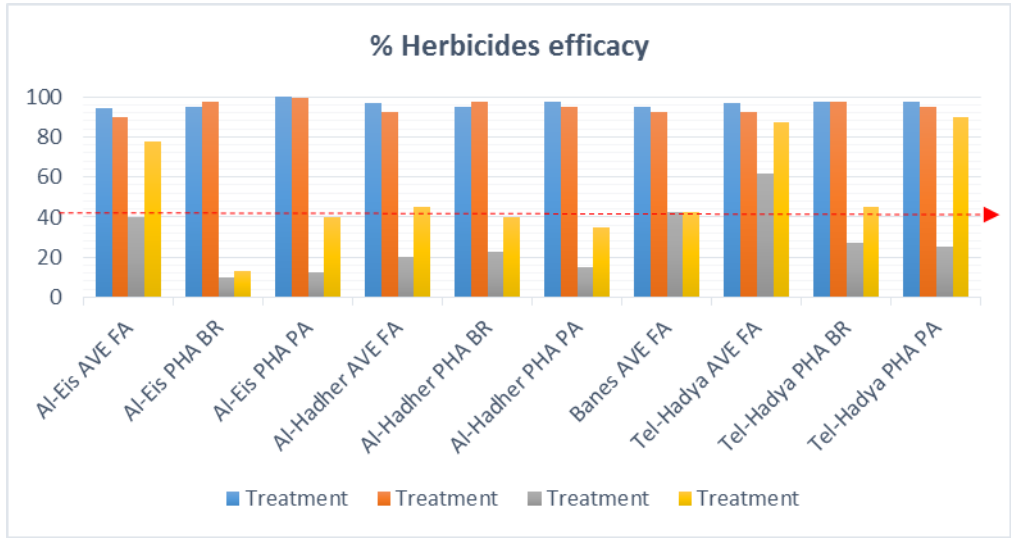


Figure 1. Herbicides efficacy in controlling grasses form different locations.

ACCase inhibitors works well in Tel-Hadya location, so it takes place in sensitive group (left), but the wild oat sampling from Al-Hadher and Banes had been located clearly in resistant group (right). The wild oat sampled from Al-Eis was still confusing, it takes place in two groups due to the use of Ralon or Topik, so we cannot confirm or deny the resistance.

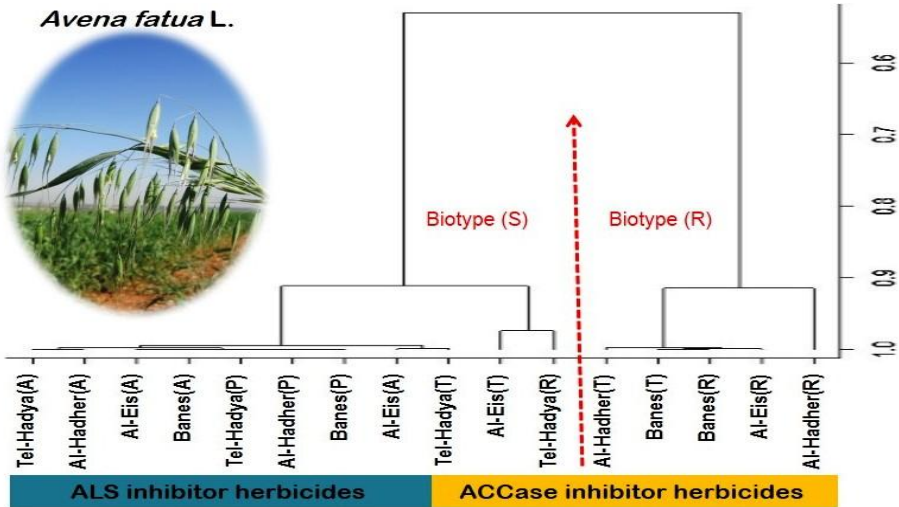


Figure 2. Level of similarity depends on encoded name (AVEFA) with herbicide related and activity results.

The hierarchical clustering analyses for hood canarygrass have presented two separate groups clearly, the sensitive biotypes (S) (left), and the resistant biotypes (R) (right). The results reflect the excellent responses when using Atlantis and Pallas herbicides at all locations, this confirms no resistance for ALS inhibitors. While ACCase herbicide showed variable responses related to different locations. Tel-Hadya hood canarygrass is still sensitive when treated by Topik, and this is consistent with the theory. On the other hand, the same biotype which was treated by Ralon located in the resistant group, this is normal as we know the weakness of this product in controlling *Phalaris* sp. The hood canarygrass had been collected from Al-Eis and Al-Hadher clearly located in the resistant group.

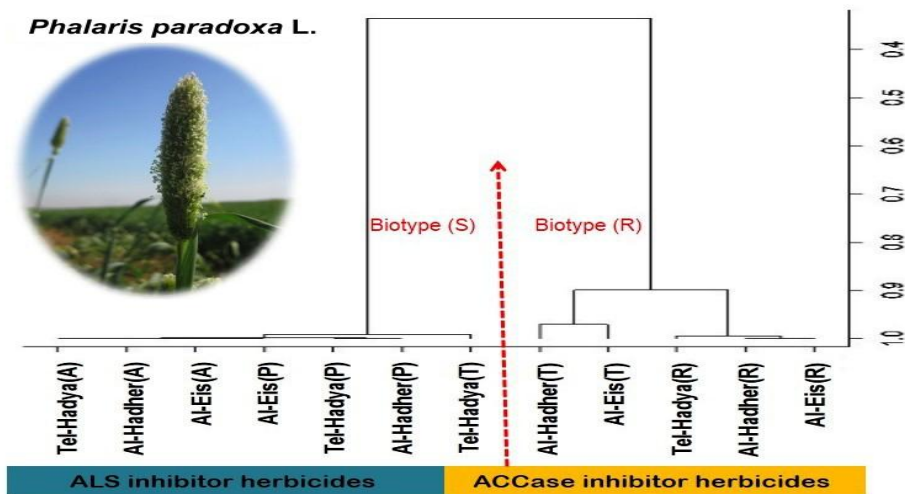


Figure 4. Level of similarity depends on seed code name PHABR with herbicide related activity results.

## CONCLUSIONS

According to results, it can be concluded that the short spike canarygrass is the most dangerous in this area related to the resistant issue, where resistance was demonstrated to (ACCase) in all locations including Tel-Hadya station. The hood canarygrass comes second in terms of severity, as demonstrated by its resistance to (ACCase) at all locations except Tel-Hadya station. Finally, the wild oat comes third in terms of severity, as demonstrated by its resistance to (ACCase) in both locations Al-Hadher and Banias while Al-Eis site was doubtful, hence Tel-Hadya station grass was sensitive.

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**VARIABILITY IN THE CONCENTRATION OF TOTAL PROTEINS  
AND ACTIVITIES OF PEROXIDASE ENZYME IN TWO-YEAR-OLD  
NEEDLES OF AUSTRIAN PINE (*Pinus nigra* Arnold)**

**SUMMARY**

Variability in the concentration of total proteins and activities of peroxidases enzyme in two-year-old needles of Austrian pine (*Pinus nigra* Arnold), were studied in the needles of eight different half-sib lines of Austrian pine. The samples were taken from a twenty-two-year-old seedling seed orchard on Jelova Gora (Western Serbia). The results of this study show the pronounced variability of the concentration of total proteins, and enzyme activity of peroxidases in the study half-sib lines. This infers that proteins have different levels of expression in individual Austrian pine lines. Also the enzyme of peroxidases shows different activity depending on the genetic constitution of the trees of the analyzed half-sib lines. Based on the seed orchard size and the planting pattern, it can be assumed that the effect of external factors is reduced to a minimum. The peroxidases present as soluble in the apoplast or with covalent bond to cell wall, have an important role in lignification, suberisation, healing of injuries, defense against pathogens and atmospheric pollutants.

**Keywords:** peroxidases enzyme, proteins, needles, Austrian pine

**INTRODUCTION**

The influence of genetic variability on some enzymes and the occurrence of isoenzymes which are specific for some varieties of plant species have been known from earlier studies (Schmidtling & Hipkins, 1998; Leinemann, 2000). Question is still posed how great the influence of genetic diversity is on these biomacromolecules in comparison with the effect of various environmental factors. In order to familiarize with the effect of genetic potential and diversity on one of the key enzymes involved in the stress response, which is also responsible for lignification process in cell wall, enzyme activity of peroxidases was monitored in the needles of Austrian pine (*Pinus nigra* Arnold) half-sib lines. Genetic diversity causes various expression and reactivity of the enzyme, as well

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as various isoenzyme forms. In addition, activity of this enzyme in dependence on medium acidity was monitored. By means of isoelectrofocusing, isoenzymes were determined which participate in the mentioned reactions and processes in eight selected Austrian pine lines incorporated in generative seed orchard.

Generative seed orchard of Austrian pine on Jelova Gora mountain has metapopulation structure and area of 3.10 ha. It was established with 40 half-sib lines of Austrian pine, with the total of 5700 plants. On the basis of relatively small orchard area, it can be assumed that environmental factors are more or less uniform, whereby almost the same conditions for the growth and development of the trees of all half-sib lines are provided. Therefore, the results obtained from the analyses which were carried out to the greatest extent reflect the influence of genetic diversity of the half sib lines.

In two-year-old needles, the activity of soluble peroxidases (POD) present in cytoplasm and apoplast was measured, as well as the concentration of total proteins in both fractions. In addition to enzyme activity, isoenzyme profile on polyacrylamide gel was also monitored.

### **MATERIAL AND METHODS**

Experiments were done on two-year-old needles of Austrian pine (*Pinus nigra*). Needles were taken from ten-year-old Austrian pine trees. Material was frozen and transported to laboratory where the analyses were performed. Samples for the extraction of enzymes were homogenized, 1 g each. Activities of peroxidase enzyme were analyzed. Isoenzyme profile of this enzyme was determined in soluble fraction. In addition, the concentration of total proteins was determined. Concentration of proteins was determined with the use of Bradford (Bradford, 1976) method. This method is based on binding Coomassie Brilliant Blue G 250 to aromatic amino acid residue, whereby maximum color absorption is moved from 465 nm to 595 nm. The color is developed after 2 min and the increase of absorbance is measured at 595 nm.

### **RESULTS AND DISCUSSION**

Genetic diversity causes various expression and reactivity of the enzyme, as well as a variety of isoenzyme forms. In order to obtain an answer to the question how large the effect of genetic diversity is on one of the key enzymes involved in the stress response, which is also responsible for the process of lignification in cell wall, the enzyme activity of peroxidases was monitored. In addition, activity of this enzyme in dependence on medium acidity was monitored. By means of isoelectrofocusing, isoenzymes were determined which participate in physiology processes in the needles of the tested Austrian pine lines.

Specific enzyme activity of peroxidases was first tested with regard to different pH values of medium, i. e. buffer on which the reaction was measured. In that way, optimum pH value was precisely determined for the enzyme reaction of total peroxidases in the needles of Austrian pine. It was determined that

optimum value of pH is 5.5 for peroxidase reaction (Figure 1). Reaction was performed in the mixture of isocitrate-phosphate buffers with various pH values (3 – 8). PH optimum of the reaction is the same as for peroxidases isolated from spruce needles.

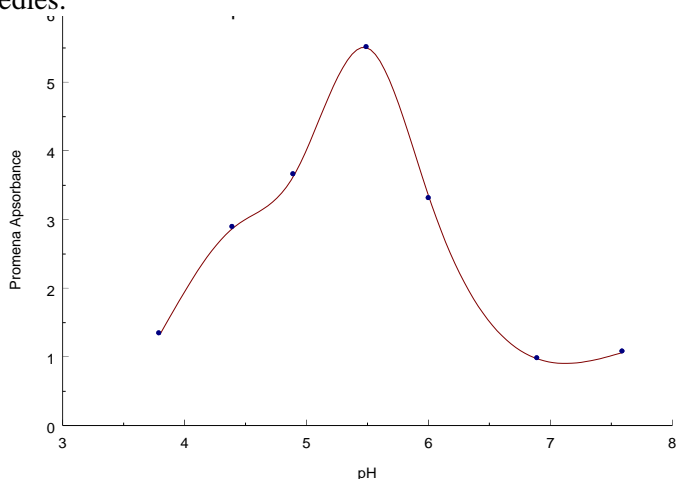


Figure 1. Optimum pH value for the enzyme reaction of total peroxidases from the needles of Austrian pine

Figure 2 shows the concentration of total proteins in the isolate of austrian pine needles. Protein content in different lines ranges from 0.05 to 0.20mg/ml. The lowest protein content was measured in the needles of the line 23, and the highest in the line 32. In the figure, statistically significant differences among the lines are marked. Statistically, the lines 23 and 40 differ from the other lines to the greatest extent. Synthesis of total proteins depends on many factors (external and internal).

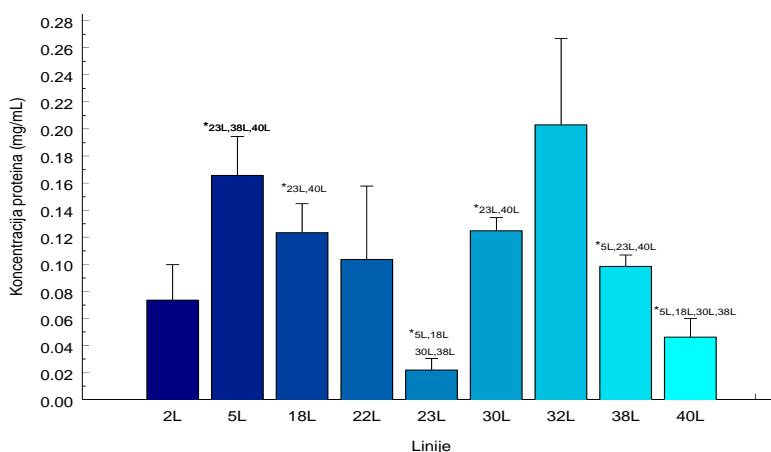


Figure 2. Concentration of proteins in the needles of various Austrian pine lines.expressed in mg per ml

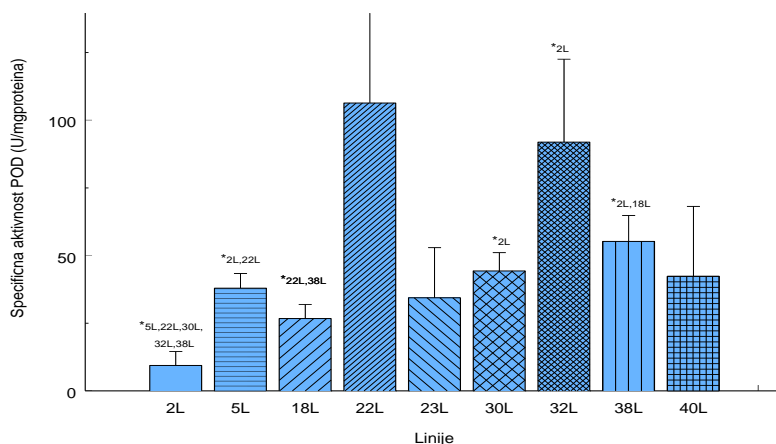


Figure 3. Enzyme activity of peroxidases expressed as specific activity (U/mg of proteins) in the needles of various Austrian pine lines

Figure 3 shows the results of specific enzyme activity of peroxidases in the needles of austrian pine. The lowest value was measured in the line 2, and it amounted to 9.5u/mg of proteins, and the highest in the line 22, which amounted to 110u/mg of proteins. To the greatest extent, statistically significant difference was demonstrated between the lines 2 and 22.

The results of studies showed that the change in the concentration of total proteins and activities of peroxidase enzyme in the needles of austrian pine lines, wherefrom the seed orchard of this species was established on jelova gora mountain near užice, significantly differed (figures 2 and 3). Proteins have various expression level in the individual austrian pine lines, while at the same time peroxidase enzyme shows different activity which can be considered to be under direct genetic control, having in mind that the influence of external factor was minimized due to relatively small seed orchard area. Various activities of enzymes and proteins which have been recorded show that genetic variation of analyzed austrian pine half-sib lines is highly significant for the further directed use of the genetic potential of this species. Other authors, e.g. Leinemann, demonstrated that the composition of isoenzymes of other enzymes, such as glucose-6-phosphate dehydrogenase (g-6-pdh) or isocitrate dehydrogenase (idh) had special importance during the separation of various subspecies of *prunus spinosa* (leinemann, 2000). This method is currently successfully applied for reliable determination of genetic base in species and subspecies variability of trees.

Results of the analyses performed with spruce needles, radotić et al. 2000, showed that the occurrence of new isoenzymes of peroxidases in spruce needles was an indicator of stress caused by heavy metals (cd, ni, cu). This poses new questions for future researches in the sense – are the other enzymes reliable



indicators of divergences inside species and could some enzymes of antioxidant defense be reliably used in order to monitor environmental pollution?

### CONCLUSIONS

Results of the performed analyses show the presence of pronounced variability in the concentration of total proteins, content of heavy metals and activities of peroxidase enzyme in two-year-old needles of analyzed Austrian pine half-sib lines. Concentration of total proteins and activities of peroxidase enzyme are significantly different in various Austrian pine half-sib lines (Figures 2 and 3) while the proteins have various level of expression in individual lines.

In addition, peroxidase enzyme shows different activity which depends on genetic constitution of the trees from analyzed half-sib lines, because based on the seed orchard size and the planting pattern, it can be assumed that the effect of external factors is reduced to minimum. Peroxidases present as soluble in apoplast, or covalently bound to cell wall, play an important role in lignification, suberization, healing injuries, defense against pathogens and airborne pollutants.

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## **IMPACT OF SOIL WATER DEFICIT ON SOME PHYSIOLOGICAL PARAMETERS OF DURUM AND BREAD WHEAT GENOTYPES**

### **SUMMARY**

Drought is a worldwide issue that impacts seriously on the security of food production. The aim of this research was to study the effect of soil water deficit on some physiological parameters of durum and bread wheat genotypes. Gas exchange parameters of flag leaf measured by using LI-COR 6400-XT Portable Photosynthesis System. Drought caused of reduction photosynthesis rate (Pn), stomatal conductance (gs), transpiration rate (E), mesophyll conductance (gm), photosynthetic pigments content, leaf area (LA), dry weight (DW), relative water content (RWC) of flag leaf. Leaf specific mass (LSM) was increased under rain-fed condition. Strong relationships were detected between gs and E, between gm and Pn. The Pn was positively and significantly correlated with LA, RWC, and DW but non-significantly correlated with Chl content. Physiological traits can be used as selection criteria for drought resistance.

**Keywords:** wheat, soil water deficit, gas exchange parameters, yield

### **INTRODUCTION**

Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair plant growth and productivity worldwide (Anjum et al., 2011). Drought, being the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor (Shao et al., 2009). Up to 26% from the usable areas of the Earth is subjected to drought (Blum, 1986). Wheat is one of the four (maize, rice, wheat, soybean) most important crop plant for cultivation area and production in the world. In the field main development stages of wheat (stem elongation, heading-flowering, grain filling) occur when the water deficit in the soil increases in rain fed regions. Wheat is one of the widely cultivated crops in Azerbaijan, where drought is the main limiting factor for its production (Aliyev, 2001). Up to 35% of the 650.000 hectare wheat grown areas is under rain-fed conditions.

Drought affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition, stomata closure with consecutive reduction of transpiration, decrease in chlorophyll content and

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inhibition of photosynthesis and protein changes (Lawlor and Cornic, 2002; Yordanov et al., 2003) to cope with osmotic changes in their tissues. Leaf gas exchange is very susceptible to drought stress. The reduction of Pn results from the closure of stomata due to water deficit, since decrease of  $g_s$  is the most efficient way to reduce water loss, and parallel with this the CO<sub>2</sub> diffusion into the leaves restricted, resulting in a decrease in  $C_i$  (Cornic 2000). The limitation of CO<sub>2</sub> fixation during water deficit is also influenced by the diffusion of CO<sub>2</sub> from the intercellular spaces to chloroplasts (Loreto et al., 2003; Molnar et al., 2005), and by metabolic factors such as ATP-limited regeneration of ribulose-1,5-bisphosphate (Flexas, Medrano, 2002; Lawlor and Cornic, 2002). The stomatal function in drought-tolerant species is controlled to allow some carbon fixation even in stress conditions; hence, the water use efficiency increases (Brestic and Zivcak, 2013).

Reduced plant size, leaf area, and leaf area index are a major mechanism for moderating water use and reducing injury under drought stress (Mitchell et al., 1998). Drought leads to a decrease in water content, dry biomass, chlorophyll content of leaves of wheat genotypes (Dulai et al., 2006; Changhai et al., 2010). Proline, which is one of the amino acids highly synthesized under drought stress as a messenger, is considered to be one of the first metabolic responses to stress (Hare and Cress, 1997).

The ability of crop plants to acclimate to different environments is directly or indirectly associated with their ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and, consequently, the growth and yield of the whole plant (Chandra, 2003). Under field conditions identifying of wheat genotypes with optimal heading time, high photosynthetic activity, well-resistance and superior yield is very important task for researches. The purpose of this research was to study the effect of soil water deficit on some physiological parameters of durum and bread wheat genotypes and to determine physiological traits which can be used for identification high productive and tolerant wheat genotypes.

## MATERIAL AND METHODS

Field experiment was carried out in the research area of Plant Physiology and Biotechnology Department of Research Institute of Crop Husbandry located in Absheron peninsula, Baku, during the 2012-2013 growing season. Six durum wheat genotypes (Garagylchyg 2, Vugar, Shiraslan 23, Barakatli 95, Alinja 84, Tartar), seven bread wheat genotypes (Gobustan, Giymatli 2/17, Gyrgyzgul 1, Azamatli 95, Tale 38, 12<sup>nd</sup> FAWWONN<sub>97</sub>, 4<sup>th</sup> FEFWSNN<sub>50</sub>) were used for this study. Sowing was done at an average density 400 seeds m<sup>-2</sup> with self-propelled mechanical planter in 1 m x 10 m plots, consisting of 7 rows with 15 cm apart. Each genotype was sown with three replications both in irrigated and rain-fed conditions. Irrigated plots were watered at stem elongation, flowering and grain filling stages. Fertilization was applied as N<sub>120</sub>P<sub>60</sub>K<sub>60</sub> per hectare. 30% of the nitrogen applied at planting and the rest at the beginning of stem elongation.

The Pn, gs, Ci, and E were measured with a Portable Photosynthesis System LI-6400 XT (LI-COR Biosciences, Lincoln, NE, USA) during postanthesis grain formation stage.

Photosynthetic pigments content ( $\text{mg g}^{-1}$  DW) was determined following the method of Lichtenthaler (1987) with modifications. About 0.1 g fresh leaves were ground in 96% ethanol for the extraction of chlorophyll and carotenoids. Absorbance of the supernatant was recorded at 664, 648, and 470 nm spectrophotometrically (Genesys 20, Thermo Scientific, USA). Pigments content calculated by the following formulas.

$$\text{Chla}=(13.36 \cdot A_{664}-5.19 \cdot A_{648}) \cdot 25 / \text{DW} \quad \text{Chlb}=(27.43 \cdot A_{648}-12 \cdot A_{664}) \cdot 25 / \text{DW}$$

$$\text{Chl (a+b)}=(5.24 \cdot A_{664}+22.24 \cdot A_{648}) \cdot 25 / \text{DW}$$

$$\text{Car(x+c)}=(4.785 \cdot A_{470}+3.657 \cdot A_{664}-12.76 \cdot A_{648}) \cdot 25 / \text{DW}$$

Proline content was measured spectrophotometrically according to Bates et al. (1973) with modifications. About 0.5g leaves homogenized in a pre-chilled pestle and mortar with 5ml of 3% sulphosalicylic acid. Then, homogenate centrifuged at 3500 g (HERMLE Z 400K, Germany) for 15 min at 4°C. The supernatant (0.2 ml) was transferred to plastic tube containing 3% ninhydrin (0.4 ml), and 0.2 ml of 96% acetic acid and 0.2 ml of 3% sulphosalicylic acid were added. Tubes were incubated for 1 h at 96°C in a water-bath and 2 ml of toluene were added to each tube, then stirred, and centrifuged at 3500 g for 15 min at 4°C. The absorbance of the upper phase was measured at 520 nm. Determination of proline was carried out by a calibration curve in 0.01-0.2 mm proline.

Leaf area (LA,  $\text{sm}^2$ ) was measured with an area meter (AAC-400, Hayashi Denkon Co., LTD, Japan). Leaf dry weight was then determined, and Leaf Specific Mass (LSM, leaf dry matter per unit leaf area,  $\text{mg mm}^{-2}$ ) was calculated. The relative water content (RWC) was determined gravimetrically. Immediately after cutting at the base of lamina, leaves were preserved within plastic tubes and in time transferred to the laboratory. Fresh weight (FW) was determined after removal and turgid weight (TW) was measured after saturating leaves in distilled water for 24 h at room temperature. After saturating, leaves were carefully blotted dried with tissue paper. Dry weight (DW) was measured after oven drying the leaves samples at 105°C for 24 h. RWC was calculated by using the following formula:  $\text{RWC}(\%) = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$ .

## RESULTS AND DISCUSSION

Effect of drought stress on gas exchange parameters. Water deficit significantly affected leaf gas exchange parameters (Table 1). A higher Pn was observed in flag leaf of genotypes Barakatli 95, Alinja 84, Tartar, Giymatli 2\17, Tale 38, 4<sup>th</sup>FEFWSNSN $\approx$ 50 under normal water supply. Water stress caused considerable reduction of Pn in most of genotypes. Less reduction of Pn was observed in genotypes Garagylchyg 2, Giymatli 2\17, Tale 38, 4<sup>th</sup>FEFWSNSN $\approx$ 50. A higher gs of non-stressed plants was observed in flag leaf of durum wheat genotypes and in bread wheat genotypes of Gobustan, Tale 38 and 4<sup>th</sup>FEFWSNSN $\approx$ 50. Drought stress led to deep reduction of gs (37-88%) of wheat genotypes. Drought also led to a decrease in Ci. We observed an increase

of  $C_i$  in genotypes Giymatli 2\17 and Azamatli 95 under drought stress. Higher E was detected in flag leaf of durum wheat genotypes Barakatli 95, Tartar, Vugar, Shraslan 23, bread wheat genotypes Tale 38 and 4<sup>th</sup>FEFWSNSN№50 under irrigated condition. Water deficit caused strong reduction of E especially in genotypes Shiraslan 23, Gobustan, Gyrgyzy gul 1. The genotype 12<sup>nd</sup>FAWWONN№97 with the smallest leaf area showed lowest  $P_n$ ,  $g_s$  and E. The mesophyll conductance ( $g_m$ ) was calculated as the ratio of  $P_n$  to  $C_i$ , water use efficiency (WUE) was calculated as the ratio of  $P_n$  to E. The  $g_m$  decreased, but the WUE increased under the influence of water stress. An increase in WUE could be due to more reduction in E than  $P_n$  by water deficit. A sharp increase in the WUE of genotypes Garagylchyg 2, Shiraslan 23, Gobustan, Gyrgyzy gul 1 indicates a strong decrease in the E. Table 2 shows correlation between gas exchange parameters and calculated  $g_m$  and WUE under irrigated and rain-fed conditions. Positive and significant correlations were found between  $P_n$  and  $g_s$ , E,  $g_m$ . There was more strong correlation between the  $P_n$  and  $g_m$ , than the  $P_n$  and  $g_s$ , indicating the dominance of  $g_m$  in reducing of  $P_n$ . Negative correlation was observed between  $P_n$  and  $C_i$ . Positive correlations were observed between  $g_s$  and  $C_i$ , E. Correlation between E and  $g_m$  was positive and significant. Negative and significant correlation was observed between E and WUE.

Effect of water deficit on RWC. Although RWC was higher in non-stressed plants than stressed ones, there were no significant differences between cultivars at these levels of RWC (Fig.1). Higher RWC was observed in genotypes Barakatli 95, Alinja 84, Tartar, Gyrgyzy gul 1, Tale 38, 12<sup>nd</sup>FAWWONN№97, and 4<sup>th</sup>FEFWSN№50.

The genotypes Tartar, Gyrgyzy gul 1, Tale 38, 12<sup>nd</sup>FAWWONN№97, and 4<sup>th</sup>FEFWSN№50 were late heading, and their younger flag leaves contained relatively more water. Lower RWC was observed in genotypes Shiraslan 23, Gobustan, Giymatli 2/17, and Azamatli 95. Should be noted that the genotypes Azamatli 95 and Gobustan were the earliest heading. Under the influence of water stress significant reduction of RWC was found in genotypes Garagylchyg 2 (12%), and Giymatli 2/17(14%). A slight decrease of RWC was observed in genotypes Vugar, Alinja 84, Gobustan, Gyrgyzy gul 1, Azamatli 95, Tale 38, 12<sup>nd</sup>FAWWONN№97, non-significant reduction in genotypes Shiraslan 23, Barakatli 95, and 4<sup>th</sup>FEFWSN№50. The difference in RWC of irrigated and rain-fed plants was almost imperceptible in genotype Tartar. In the field, strengthening of water stress occurs gradually, it allows plants to develop various mechanisms of adaptation to resist to water scarcity. Effect of water stress on flag leaf area. Water stress limits the growth of assimilating surface area of flag leaf of tested wheat genotypes (Fig.2). The reduction in leaf size which results in smaller transpiring area, is an adaptive response to water deficit (Tardieu, 2005). A significant decrease in the flag leaf area was observed in all genotypes. More profound reduction of flag leaf area was observed in genotypes Shiraslan 23 (44%) and Vugar (35%), Gyrgyzy gul 1(37%), Tale 38 (34%), Garagylchyg 2 (31%), Barakatli 95 (31%), 4<sup>th</sup>FEFWSN№50 (30%), 12<sup>nd</sup>FAWWONN№97 (28%), Tartar (28%).

Table 1. Gas exchange parameters of *T.durum* Desf. and *T.aestivum* L. genotypes in response to drought stress

Wheat genotypes	Gas exchange of wheat genotypes under irrigated (I) and rainfed (R) conditions						
	per units area and time ( $\text{m}^{-2} \text{s}^{-1}$ )					$C_i$ , $\mu\text{mol CO}_2 \text{mol}^{-1}$	WUE, $\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$
		$P_n$ , $\mu\text{mol CO}_2$	$g_s$ , $\text{mol H}_2\text{O}$	$E$ , $\text{mmol H}_2\text{O}$	$g_m$ , $\text{mol CO}_2$		
<i>Triticum durum</i> Desf.							
Garagylchyg 2	I	18.1	0.529	6.13	0.059	303	2.95
	R	16.6	0.223	3.81	0.067	246	4.36
Vugar	I	19.8	0.551	7.31	0.069	288	2.71
	R	12.5	0.135	3.24	0.056	226	3.85
Shiraslan 23	I	16.3	0.568	7.25	0.053	310	2.25
	R	10.8	0.087	2.24	0.037	291	4.82
Barakatli 95	I	22.0	0.555	8.13	0.073	302	2.71
	R	14.3	0.173	4.10	0.064	225	3.49
Alinja 84	I	21.5	0.492	6.94	0.079	273	3.10
	R	13.8	0.144	3.04	0.064	214	4.54
Tartar	I	22.8	0.645	8.50	0.079	289	2.68
	R	16.2	0.173	4.21	0.083	195	3.85
<i>Triticum aestivum</i> L.							
Gobustan	I	16.5	0.717	6.57	0.049	338	2.51
	R	10.4	0.086	1.71	0.033	314	6.08
Giymatli-2/17	I	19.4	0.364	4.78	0.070	279	4.06
	R	16.2	0.209	3.33	0.057	286	4.86
Gyrmyzy gull	I	14.3	0.366	5.33	0.047	306	2.68
	R	10.3	0.141	1.57	0.039	266	6.56
Azamatli 95	I	17.1	0.325	5.69	0.063	273	3.01
	R	9.8	0.206	3.35	0.034	276	2.80
Tale-38	I	20.7	0.598	6.82	0.066	313	3.04
	R	17.6	0.308	5.36	0.068	256	3.28
12 <sup>nd</sup> FAWWO N97	I	13.4	0.352	3.55	0.043	310	3.77
	R	9.04	0.118	2.32	0.030	299	3.90
4 <sup>th</sup> FEFWSN N50	I	22.0	0.531	7.02	0.078	281	3.13
	R	17.8	0.298	6.47	0.075	236	2.75

Irrigated	Parameters	$P_n$	$g_s$	$C_i$	E	$g_m$	WUE	Rain-fed
	$P_n$	1	0.433**	-0.070	0.819**	0.778**	0.058	
	$g_s$	0.341**	1	0.592**	0.592**	0.019	-0.271*	
	$C_i$	-	0.459**	1	0.156	-	0.399**	
	E	0.800**	0.366**	-0.305*	1	0.535**	0.445**	
	$g_m$	0.975**	0.196	-0.622*	0.766**	1	0.244*	
	WUE	0.130	-0.161	-0.228	-	0.163	1	
					0.458**			

\*\* , Correlation is significant at the 0.01 level; \* , Correlation is significant at the 0.05 level

Deep reduction can be explained to the fact that the formation of the flag leaf of late-heading wheat genotypes (Vugar, Shiraslan 23, Tartar, Gyrgyz gull, Tale 38, 4thFEFWSN№50, and 12ndFAWWON№97) occurs at a severe water shortage. A more profound reduction of flag leaf area in these genotypes was compensated with conservation of RWC at high level.

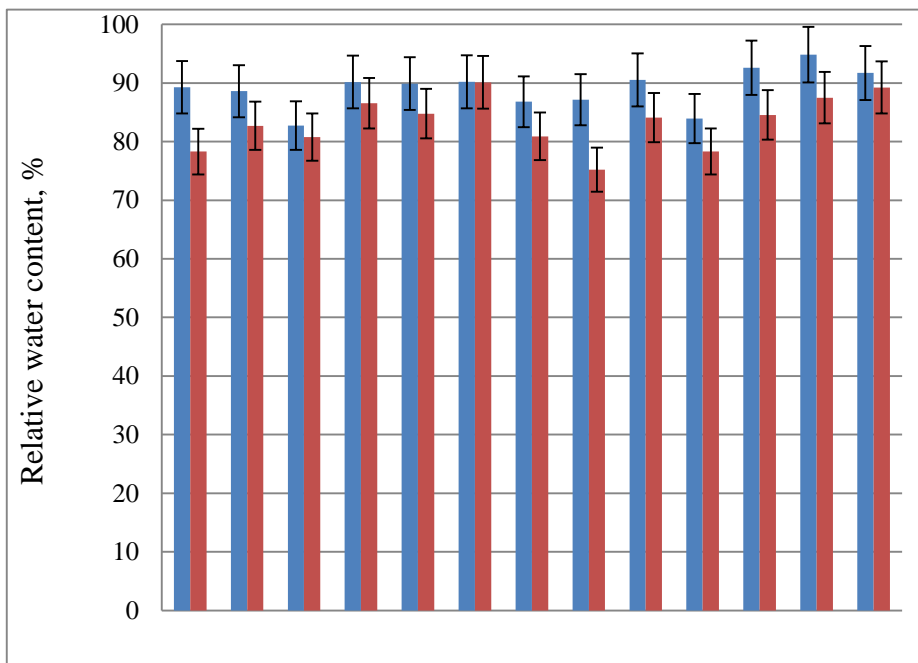


Figure 1. Effect of water stress on flag leaf RWC



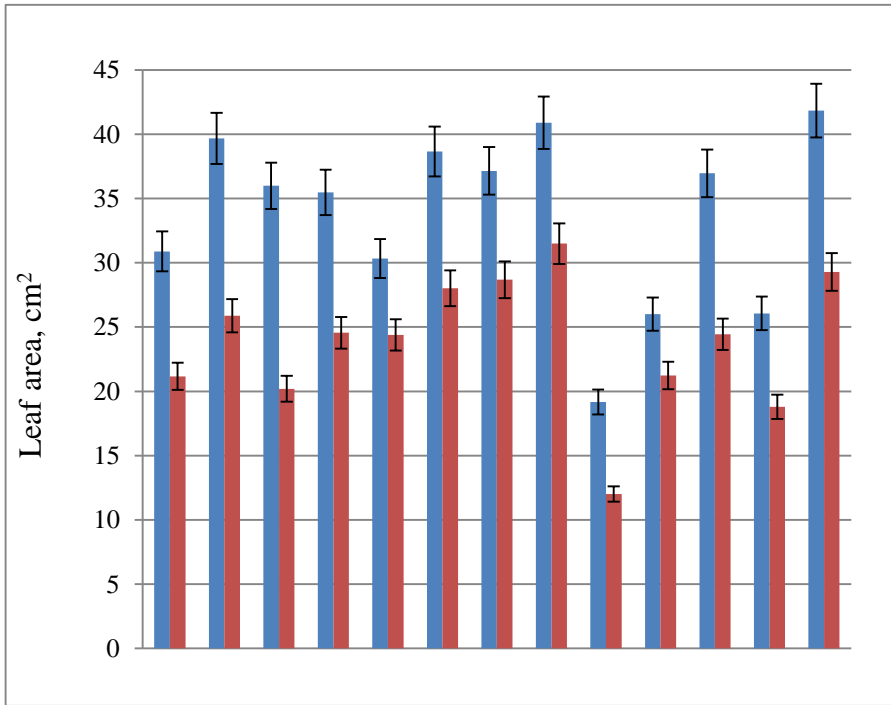


Figure 2. Effect of water stress on flag leaf area

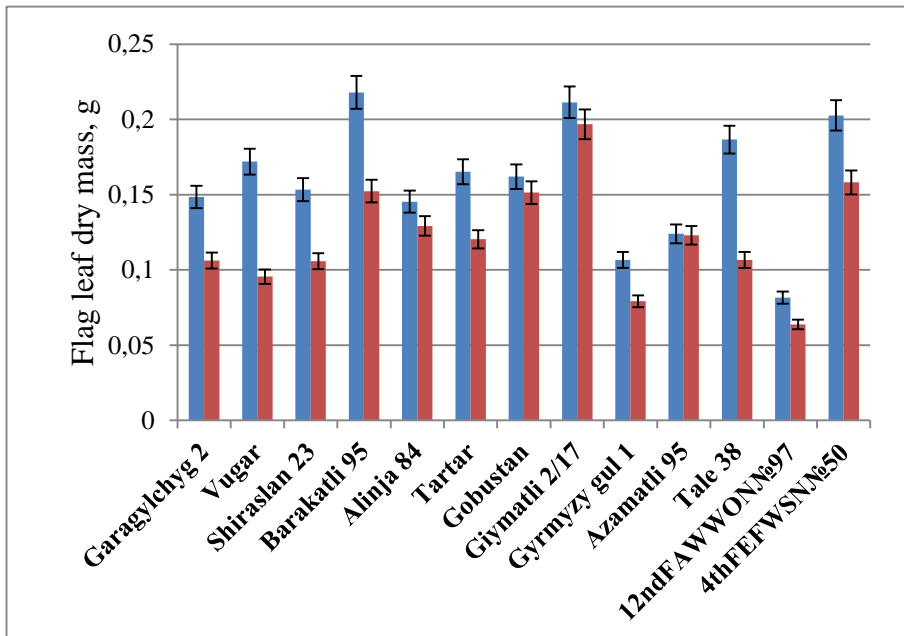


Figure 3. Effect of water stress on flag leaf dry mass

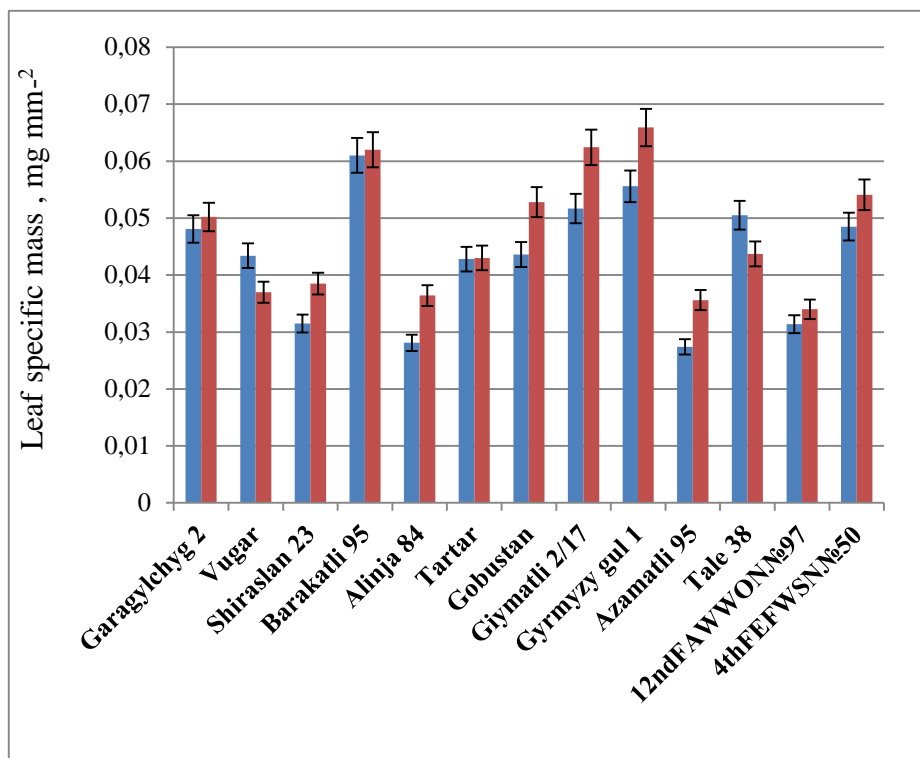


Figure 4. Effect of water stress on leaf specific mass

**\*Note:** blue bars correspond irrigated plants, red bars correspond rain-fed plants

A more profound reduction of flag leaf area in these genotypes was compensated with conservation of RWC at high level.

Effect of water stress on flag leaf dry biomass. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Zhao et al, 2006). Water scarcity causes a decrease of dry biomass of flag leaf (Fig. 3). As in the case of leaf area, a strong reduction of dry biomass was observed in all genotypes of durum wheat, with exception of Alinja 84, in bread wheat genotypes Gyrmyzy gul 1, Tale 38, 12ndFAWWON№97, 4thFEFWSN№50. A smaller reduction of flag leaf dry biomass under water stress was observed in genotypes Azamatli 95, Gobustan, Giymatli 2/17, Alinja 84. A more profound reduction of flag leaf dry mass was detected in genotypes Vugar (44%) and Tale 38 (43%).

Effect of water stress on Leaf Specific Mass (LSM). LSM was calculated from the ratio of flag leaf dry mass to flag leaf area and it is inverse leaf specific area (LSA). LSM is considered to reflect relative carbon accumulation, at lower nutrient or moisture availabilities or at higher light irradiances, leaves tended to be smaller, with higher LSM, density and thickness (Witkowski and Byron,

1991). It was revealed an increase of LSM under water stress in most wheat genotypes (Fig. 4). Such an increase in the LSM is probably adaptive response to drought and is due to the relatively greater reduction in leaf area than the dry mass. A reduction of LSM was observed in genotypes Vugar and Tale 38, because of the greater reduction in dry mass. A higher LSM was observed in genotypes Barakatli 95, Gyrgyzy gul 1, Giymatli 2/17, Tale 38, 4<sup>th</sup>FEFWSN№50, Garagylchyg 2, lower LSM was observed in genotypes Azamatli 95, Alinja 84, 12<sup>nd</sup>FAWWON№97, Shiraslan 23. A slight increase in LSM was observed in genotypes Garagylchyg 2, Barakatli 95, Tartar, more profound increase was observed in genotypes Azamatli 95, Alinja 84, Shiraslan 23, Giymatli 2/17, Gobustan, Gyrgyzy gul 1.

Effect of water stress on photosynthetic pigments content. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers (Anjum et al., 2011). In general, water stress caused significant declines in photosynthetic pigments content, in the ratio of  $\text{Chl}(a+b)/\text{Car}(x+c)$  and an increase in the ratio of  $\text{Chl}a/b$  (Table 3). The decrease in chlorophyll content under drought stress may be the result of pigment photo-oxidation and chlorophyll degradation. Lower values of the ratio  $\text{Chl}(a+b)/\text{Car}(x+c)$  indicates water stress damage to the photosynthetic apparatus, which is expressed by faster breakdown of chlorophylls than carotenoids. Photosynthetic pigments were higher among bread wheat genotypes than durum wheat ones. Higher decrease of chlorophyll content was observed in genotypes Vugar (35%), Shiraslan 23 (29%), Barakatli 95 (21%), Gobustan (29%), Giymatli 2/17 (31%), Azamatli 95 (37%), and 4<sup>th</sup>FEFWSN№50 (28%). A slight decrease was observed in genotypes Gyrgyzy gul1, 12<sup>nd</sup>FAWWON№97, Alinja 84, Tale 38 and Garagylchyg 2. An increase in  $\text{Chl} a/b$  could be due to more reduction in  $\text{Chl} b$  than  $\text{Chl} a$  by water deficit.

Correlations between physiological parameters. Table 4 shows correlations between studied physiological parameters. The  $P_n$  was positively and significantly correlated with RWC, LA, and DW.

Correlation between LA and DW was positive and significant, correlation between LA and Chl was positive but non-significant. The DW was positively, non-significantly correlated with LSM. Effect of water stress on proline content. Drought stress increased proline content about eight-tenfold in flag leaf of genotypes Vugar, Alinja 84, Gyrgyzy gul 1 and more than tenfold in genotype Tale 38, this increasing role as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in wheat (Fig.5).

Photosynthesis is the primary source of dry matter production and grain yield of crop plants (Shao et al., 2005). Leaf photosynthesis may vary with leaf age, position, leaf surface, and general plant and development stage (Richards, 2000). Variations in daily time course of weather parameters such as light intensity, temperature, relative humidity, etc. also affect leaf gas exchange. According to our results under drought stress  $g_s$  plays an important role in the regulation of  $P_n$ , E and  $C_i$ . The  $P_n$  is less limited than E under drought stress.

Table 3. Changes of Chl a, b and Chl (a+b) contents, Car (x+c) content, Chl a/b and Chl (a+b)/Car (x+c) of wheat genotypes under drought stress.

Wheat genotypes		Pigments content of wheat genotypes under irrigated (I) and rainfed (R) conditions					
		mg per g dry weight of leaf				Chl a/b	Chl (a+b)/Car(x+c)
		Chl a	Chl b	Chl (a+b)	Car(x+c)		
<i>T. durum</i> Desf.							
Garagylchyg 2	I	7.14	3.34	10.48	1.76	2.14	5.96
	R	5.50	3.06	8.56	1.18	1.80	7.25
Vugar	I	6.02	2.93	8.95	1.45	2.06	6.16
	R	4.00	1.86	5.86	0.98	2.15	5.97
Shiraslan 23	I	5.68	2.68	8.36	1.41	2.12	5.93
	R	4.08	1.89	5.97	1.02	2.15	5.84
Barakatli 95	I	6.08	2.81	8.89	1.54	2.16	5.76
	R	4.83	2.19	7.02	1.15	2.21	6.09
Alinja 84	I	5.10	2.66	7.76	1.24	1.92	6.26
	R	4.46	2.01	6.47	1.16	2.22	5.57
Tartar	I	4.90	2.51	7.41	1.17	1.96	6.34
	R	6.23	2.69	8.92	1.58	2.32	5.66
<i>T. aestivum</i> L.							
Gobustan	I	6.78	3.30	10.08	1.58	2.06	6.37
	R	5.08	2.57	7.65	1.20	1.98	6.35
Giymatli 2/17	I	5.85	2.68	8.53	1.38	2.18	6.17
	R	4.07	1.84	5.91	1.12	2.21	5.26
Gyrmyzy gul 1	I	7.19	3.22	10.41	1.86	2.23	5.60
	R	7.17	3.06	10.24	1.93	2.34	5.31
Azamatli 95	I	6.68	3.70	10.38	1.38	1.81	7.50
	R	4.43	2.06	6.49	1.12	2.15	5.82
Tale 38	I	7.68	3.54	11.22	1.84	2.17	6.08
	R	6.44	3.13	9.57	1.60	2.06	5.99
12 <sup>nd</sup> FAWWON №97	I	6.80	3.57	10.37	1.67	1.98	6.21
	R	6.68	3.29	9.97	1.65	2.03	5.98
4 <sup>th</sup> FEFWSN №50	I	7.14	3.49	10.63	1.80	2.04	5.92
	R	5.20	2.49	7.69	1.34	2.08	5.75

Table 4. Correlations between different physiological parameters

Parameters	P <sub>n</sub>	RWC	LA	DW	LSM	Chl
P <sub>n</sub>	1					
RWC	0.527**	1				
LA	0.798**	0.321	1			
DW	0.674**	0.116	0.845**	1		
LSM	-0.171	-0.327	-0.201	0.330	1	
Chl	0.274	0.623**	0.113	-0.043	-0.235	1

\*\* Correlation is significant at the 0.01 level

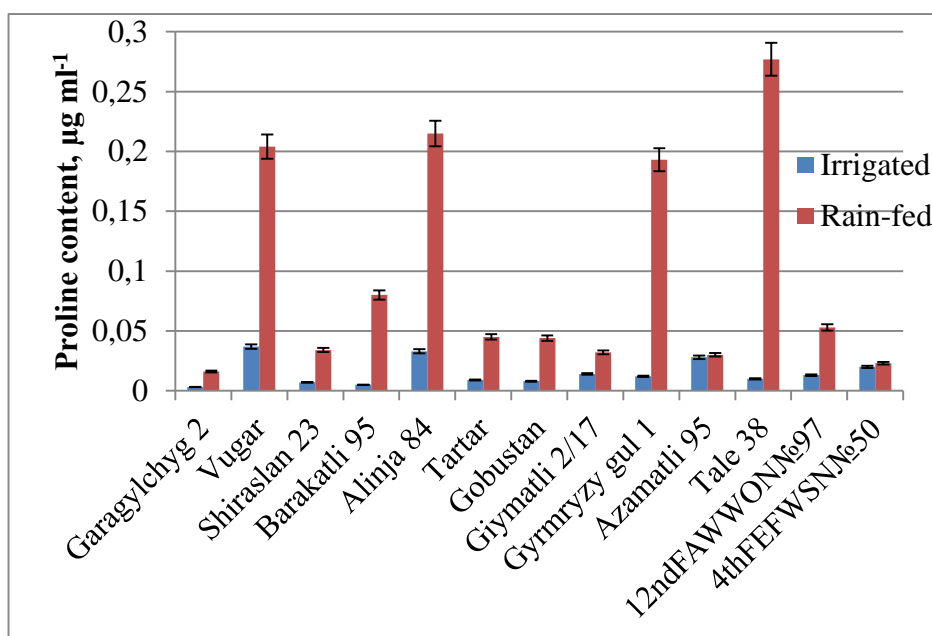


Figure 5. Effect of water stress on proline content

The  $g_m$  has a dominance role in the regulation of  $P_n$ . This result was in agreement with result of Siddique et al., (1999). During the post anthesis grain formation stage when the drought strengthened the decrease in the  $P_n$  could be associated with a reduction of  $CO_2$  in the intercellular spaces. Bread wheat genotypes Tale 38 and 4<sup>th</sup>FEFWSN№50 were characterized by high  $g_s$ ,  $P_n$  and E.

Despite the fact that the gas exchange parameters, leaf area and dry mass strongly influenced by drought, RWC in the flag leaf remained relatively high. This means that the genotypes use different mechanisms of adaptation to maintain the RWC. Water scarcity led to a greater reduction in LA than the DW,

as a result of LSM increased. Our result was in agreement with result of Witkowski and Byron (1991), but according to result Bogale et al., (2011) the LSA of durum wheat genotypes was also increased under water deficit. We found a stronger reduction of the area and dry weight of flag leaf in durum wheat genotypes. Genotypes with higher LSM probably have more photosynthesizing cells and chloroplasts per unit leaf area. The Chl content was higher in the flag leaf of bread wheat genotypes than durum wheat. However flag leaf of durum wheat genotypes retain green color for a longer period than bread wheat genotypes (SPAD units). Drought stress leads to more reduction of Chl b than Chl a. This may be due to the fact that Chl b is a main component of photosystem II, disruption of electron flow and formation of oxidizing radicals under drought stress results in the more decrease of this pigment. The RWC decreased non-significantly, but Chl (a+b) content increased in genotype Tartar under water stress. Perhaps this was associated with more accumulation of antocianes in spike and leaves of this genotype under drought stress. The individual leaf traits play an important role in drought avoidance of wheat genotypes. A smaller area and erect orientation of flag leaf of genotypes Gyrgyzy gul 1, Azamatli 95 (in addition leaf rolling), 12<sup>nd</sup>FAWWON№97, and leaf waxiness of Giymatli 2\17 allows plant to avoid damaging leaf water potential in leaves by reducing the water flow through the leaf surface. Relatively high flag leaf RWC of genotypes Vugar, Barakatli 95, Alinja 84, Tartar, Gyrgyzy gul 1, Tale 38, 12<sup>nd</sup>FAWWON№97 under drought stress perhaps was associated with strong accumulation of proline. Our results showed that strong reduction of flag leaf area and dry mass occurs in genotypes with late heading time. But late heading genotypes have an advantage-photosynthesis in younger flag leaf take place at higher photosynthetically active radiation and this leads to the formation of more assimilates. The  $P_n$  was positively and significantly correlated with LA, RWC, DW but non-significantly correlated with Chl content. The relatively high  $P_n$  was detected in genotypes Garagylchyg 2, Tartar, Giymatli 2/17, Tale 38, 4<sup>th</sup>FEFWSN№50 under rain-fed condition. Strong reduction of LA of genotypes Vugar, Barakatli, 95, Alinja 84, Tartar, Gobustan, Gyrgyzy gul 1, Tale 38, 12<sup>nd</sup>FAWWON№97 and 4<sup>th</sup>FEFWSN№50 allowed to retain the high RWC and non-strong reduction of Chl (a+b) content under rain-fed conditions. These genotypes also showed a relatively high increase in proline content. According to our results the genotypes Vugar, Shiraslan 23, Gobustan and Tale-38 were more sensitive to drought stress. The genotypes Tartar, Gyrgyzy gyl-1, Azamatli- 95, 4<sup>th</sup>FEFWSN№50 were resistance to drought stress

## CONCLUSIONS

Drought causes of adaptive changes of physiological parameters. Wheat genotypes survive drought stress through reduction of leaf area, dry mass, gas exchange parameters, photosynthetic pigments. In response to a lack of water stomatal conductance decreases which leads to decrease in the concentration of CO<sub>2</sub> in the intercellular spaces, photosynthesis rate and transpiration rate.

Increasing the area and dry mass of leaves is delayed due to the suppression of photosynthesis. The relative water content positively correlated with photosynthesis rate, leaf area, dry mass and chlorophyll content. This trait can be used as selection criteria for drought resistance.

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## FEATURES OF PINE STANDS FUNCTION IN DNEIPER NORTH STEPPE, UKRAINE

### SUMMARY

The article is devoted to distribution of *Pinus sylvestris* stands areas within North Steppe on the basis of forestry biometric indexes. The research objective was analysis of current state of *Pinus sylvestris* stands within Dnieper North Steppe on the main silvicultural and forest inventory parameters. The forest stands within Steppe zone are formed under conditions extremely unfavorable for arboreal plants growth. Occupying a very wide area, *Pinus sylvestris* has a large number of ecological forms, and it is both tolerant to low and high temperatures, it is winter-hardy and low-demanding to soil fertility and moisture. It was noted the irregular age structure in pine plantations under regional condition. Area of *Pinus sylvestris* stands within North Dnieper Steppe is 21472 ha with the total growing stock 45711 m<sup>3</sup>·ha<sup>-1</sup>. According to age structure, majority of the stands from *Pinus sylvestris* were middle-aged within the studied region, with the greatest representation in the IV-VI age classes. *Pinus sylvestris* distribution by structure rate showed significant dominance of pure stands. *Pinus sylvestris* It was established the prevailing types of site forest conditions on the basis of the edaphic factors (soil fertility and humidity). *Pinus sylvestris* forests mainly formed under pine mixed forest site type, in dry and mesic hygrotopes. The largest area of the analyzed species was occupied by stands of II and above forest capacity classes. The obtained results show predominance of high-productive pine stands within studied area. It was found that the maximum area of pine stands have high relative density.

**Keywords:** forest fund, *Pinus sylvestris*, growing stock, indexes of forest biometrics

### INTRODUCTION

In recent times, particularly after the United Nations Conference on Environment and Development 1992 (UNCED), there have taken place a substantial changes in priorities of relationships between individual and forest, which involve transition from the use of forest as a resource to the management

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of processes within forest ecosystems. The most important criteria for sustainable forest management are considered to be support and preservation of forests productive capacity, their protective and social functions, and biodiversity (Hensiruk, 1992; Caspersen and Pacala, 2001; Scherer-Lorenzen et al., 2005) because dramatic changes associated with human activities on environment effect negatively on the stands condition and functioning (Bormann, 1985; FAO, 1993). As a major component of forests, arboreal plants reflect the overall status of forest ecosystems, the features of their functioning and the main trends of their development. These issues were investigated by the authors in scientific publications (Loza and Nazarenko, 2006; Bezrodnova et al., 2013; Lovinska, 2014; Sytnyk et al., 2015).

It is known that in the Steppe zone forest stands are formed under conditions extremely unfavorable for arboreal plants growth. Only a few species are able to adapt to the complex of stress factors typical for this natural area. One of them is Scots pine (*Pinus sylvestris* L.), is known for its high resistance (Belgard, 1971; Vakuliuk, 2006; Siryk, 1991; Sychev, 2011). Significant distribution and penetration of single populations outside the forest zone are proved by high adaptive capacity of such species. Occupying a very wide area, Scots pine has a large number of ecological forms, therefore, this species is both tolerant to low and high temperatures, it is winter-hardy and low-demanding to soil fertility and moisture. Usage the not scientifically substantiated methods of forest management had led to deterioration in the forest stands quality, loss of their environmental-forming, resource, and recreational functions. For this reason, it is very important to obtain the complete and reliable information regarding status of forest-forming species within the stands for effective management and conservation of forest ecological and social functions (Alekseyev, 2003; Gulchak, 2011). The forest management process should begin with investigation of the forest stand characteristics.

The state and productivity of such characteristics are influenced by a complex of factors, first of all, age of forest-forming species, forest site condition, capacity, and stand density. As the research objective, it was analysis of the current state of Scots pine stands within Dnieper North (ravine) Steppe on their main silvicultural and forest inventory parameters.

## **MATERIAL AND METHODS**

The objects of the survey were pine stands of North Dnieper Steppe within the Dnipropetrovsk oblast. The survey of silvicultural taxational characters in pine stands was performed using information of stratum database "Ukrderzhlesproekt" and conventional methods of forest inventory (Anuchin, 1982; Gulchak, 2011) in 2009-2014. On the basis of this database, it was processed 4790 stratum in accordance to the relevant indexes within the existing age groups of trees. The analysis was subjected to such characteristics of Scots pine stands as forest type, composition, age of stand, mean height and diameter, timber resource, forest capacity, and relative density.

The classification schemes of trophotopes and gygotopes is based on the edaphic factors, such as soil fertility and humidity. Soil fertility is characterized by trophogenic sequence and denoted by the letters A, B, C, and D. These individual units of trophogenic sequence called trophotopes. Each trophotope is represented by the forest site with equal soil fertility within self boundaries, distinguished from the next by one gradation (Belgard, 1971). Trophotope "A", "bor" or pure pine forest, indicate very poor soil conditions, predominantly with sandy soil, sometimes loamy sands with a short rhizosphere zone; gritty consistency stipulate their poorness. Trophotope "B", "subor" or mixed pine forest is characterized by relatively poor soils. It is represented by loamy sand or sandy soil with thin sandy-loam or loamy layers, or with heavy layers of that at a considerable depth. Trophotope "C", "sugrud" or oak mixed forest has a relatively rich habitat conditions. Soils are represented by sandy-loam, sometimes sand with layers of loam and sandy-loam, or denuded shallow grey forest soil, sometimes brown soil with the little humus horizon, skeletal, derived from volcanic rocks and sandstones. Trophotope "D", "grud" or oak pure forest has the most fertile habitat conditions. The soils are loam with the heavy (greater than 0.8 m) rhizosphere, more rarely sandy and sandy-loam soils with the layers of loam and clay, available to plant roots. Sometimes the sandy and sandy-loam soils occur with shallow horizon of flow "mineralized" groundwater.

Whereas, the trophotopes are divided by six gygrogene sequences: gygotopes with indicators of soil moisture 0 to 5. Gygotopes of 0 range respond to very dry (xerophilic) conditions; 1 – dry (meso-xerophilic); 2 – mesic (mesophilic); 3 – mesic-wet (meso-gygraphilic); 4 – wet (gygraphilic); 5 – swamp (ultragygraphilic).

According to the classification scheme, each site of forest is characterized by two classification units: trophotope and hygotope. In the surveyed forest stands we selected 16 sampling units for Scots pine in existing grades of trophotopes and hygotopes within stands of different ages. The delimitation of sampling units was carried out instrumentally with surface marking and binding to net of rides. The size of sampling units was ranged from 0.25 to 0.5 hectares. Within the sampling units it was determined the total growing stock with the model trees by way of step representation. Model trees were selected for each level of thickness. Work on the sampling units was performed in the following order:

- 1) complete tree enumeration;
- 2) determination of the mean diameter of the stands with usage of average cross-sectional area according to tally sheet, by dividing the sum of basal areas on the total number of trees within the forest stand;
- 3) selection of three medium-size and shape sample trees in the each level of thickness;
- 4) measurement of diameter of the sample tree accurate to 0.1 cm;

- 5) measurement of height of the sample tree accurate to 0.1 m;
- 6) cutting of the sample trees;
- 7) determination of volume of the sample tree by the formula:

$$V_{\text{aver}} = g_{\text{aver}} \times h_{\text{aver}} \times f_{\text{aver}}$$

where:  $g_{\text{aver}}$  – cross-sectional area of the average sample tree;  $h_{\text{aver}}$  – average height of the average sample tree,  $f_{\text{aver}}$  – form factor of the average sample tree;

- 8) determination of stock in the sampling unit according to the formula:

$$M = V_{\text{aver}} \times N$$

where:  $M$  – growing stock,  $V_{\text{aver}}$  – volume of the average sample tree,  $N$  – number of trees within the stand.

Values of the growing stock obtained on the sampling unit for investigated tree species was transferred on 1 ha according to the formula:

$$M = M_{\text{samp.un}} \times F_{\text{conv}}$$

by multiplying it on the conversion factor  $F_{\text{conv}} = 1 \text{ ha}/S_{\text{samp.u.}}$

According to the materials of forest management, there were found the area of Scots pine stands belonging to a particular age class, and the total growing stock of this species within surveyed areas. The age class Scots pine was 10 years. In the stands of the same age, determination of dependence of growing stock formation on the type of trophotope and hygrotrope was determined by usage of the average growing stock by the formula:

$$M_{\text{aver}} = M_{\text{total age cl}} / S_{\text{stand age cl}}$$

where  $M_{\text{total age cl}}$  is the total growing stock in the same age class stands,  $S_{\text{stand age cl}}$  is the area of the stand of given age class.

## RESULTS AND DISCUSSION

According to our silvicultural and forest inventory analysis, the total area of Scots pine plantations within North Dnieper Steppe was 21472.9 ha with a growing stock  $4571 \text{ m}^3 \cdot \text{ha}^{-1}$ . The native pine stands occupy the area of 3693.8 ha (17.2 %), while artificial pine forest stands were located on 17779.1 ha (82.8% respectively) of the pine plantations total area. Age of stands has a great biological and economic importance; it is associated with growth stages of the stands. In forestry, it is known the "normal forest" concept, which is characterized by a uniform distribution of stands by age groups. It is the most favorable from the standpoint of economic benefit. According to actual data, it was established that the Scots pine stands were considerable asymmetric within the age groups. The middle-aged stands were predominated in 58.5 % as well as the young stands occupied 32.7% of the total area (Table 1). The mean age of dominant group was 60 years, and the average young group age was 23 years, with average growing stock 292 and  $92 \text{ m}^3 \cdot \text{ha}^{-1}$  respectively. Amount of the pine stands area in other age groups was not exceeding 10 %.

Table 1. The pine stands distribution, stock and average valuation indexes according to age groups

Age groups	Area		Growing stock			Average parameters		
	ha	%	ths.m <sup>3</sup>	%	m <sup>3</sup> /ha	D, cm	H, m	A, yrs
Young-age	7037.1	32.7	768.1	16.7	92	9.9	8.3	23
Middle-age	12556.8	58.5	3353.8	73.4	292	25.2	20.7	60
Maturing	1344.6	6.3	341.8	7.5	273	34.0	23.7	82
Mature	528.0	2.4	106.1	2.3	223	40.6	24.1	104
Overmature	6.4	0.1	1.3	0.1	225	46.5	24.1	153
Total	21472.9	100	4571.1	100	221	31.2	20.9	84

Age amplitude of pine forest stands in North Dnieper Steppe covers 15 age classes (1 to 14 and 18) (Fig. 1).

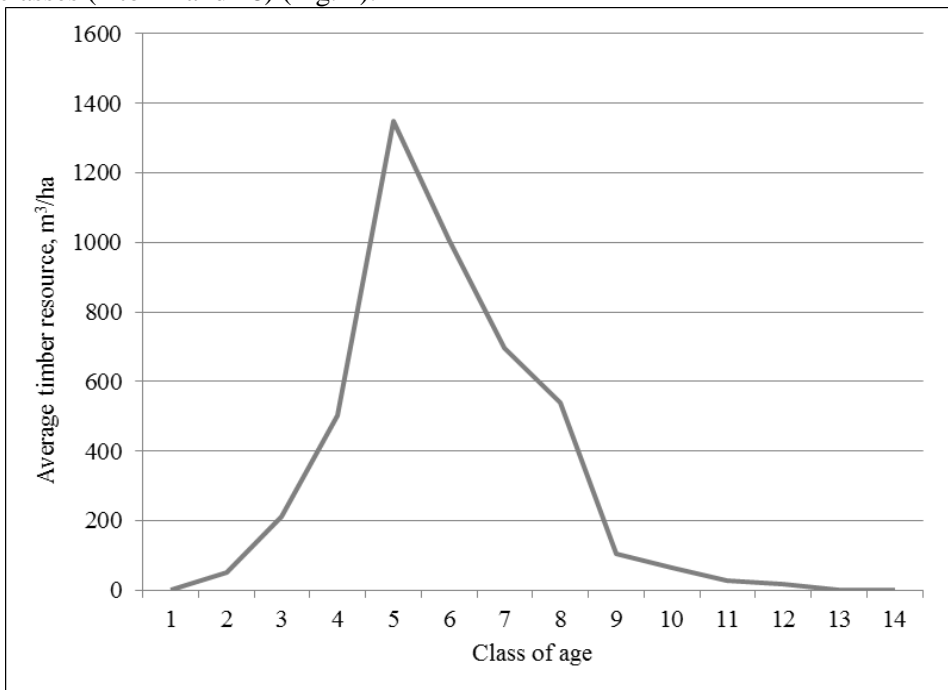


Figure 1. Growing stock of Scots pine stands depending on age classes

The largest area of Scots pine stands within studied territory was occupied by trees of III-VII age classes. Chare of such stands rises gradually from class III

(11.1 %), reaches its maximum (26.1 %) in class V, and further it goes into decline (10.5%, class VII). The average growing stock per unit of area rise with increasing of the stand age (98.6 to 316.4 m<sup>3</sup>·ha<sup>-1</sup>). Starting from age class IX, it has been fixed the relative minimum of the total area of overmature age category. As can be seen from the submitted graph, the total stock of timber varied proportionally with variation of stands area within different age classes (Fig. 1).

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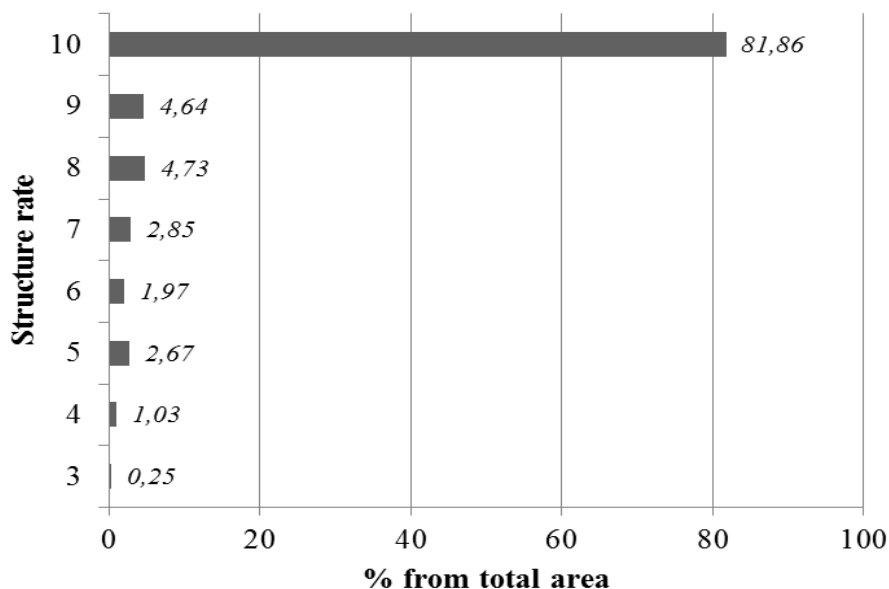


Figure 2. Distribution of the pine stands areas by structure rate, %

By type of forest site, we have identified quite wide limits to Scots pine growth under condition of North Dnieper Steppe. Stands from Scots pine are formed on 17 types of edatopes: A<sub>0-3</sub>, B<sub>1-4</sub>, C<sub>0-4</sub>, D<sub>0-3</sub>. By way of interpretation of forest site type data for Scots pine stands, it has been found that dry and mesic conditions dominated within the studied territories, and were 41.1 and 56.5 %, respectively; and 2/3 of its total area was concentrated in mixed pine forests (Fig.3).

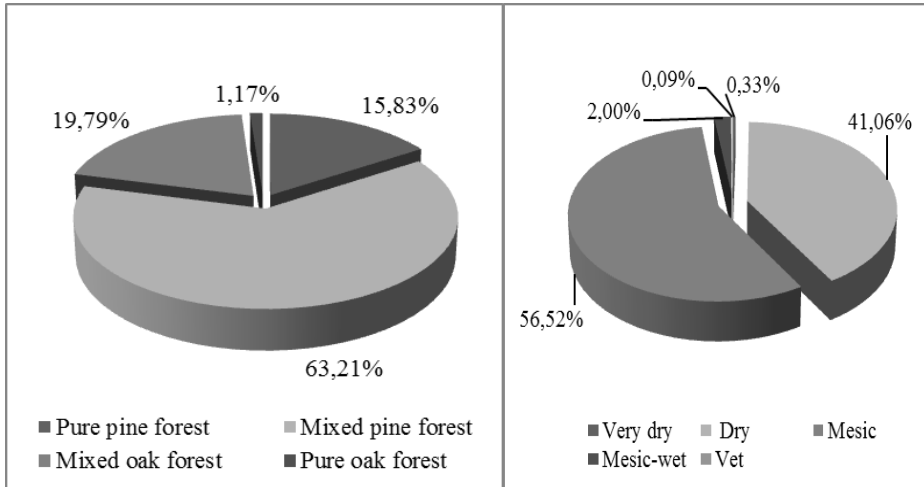


Figure 3. Distribution of the pine stands areas by trophogenic (A) and hygrogenic (B) ranges, %

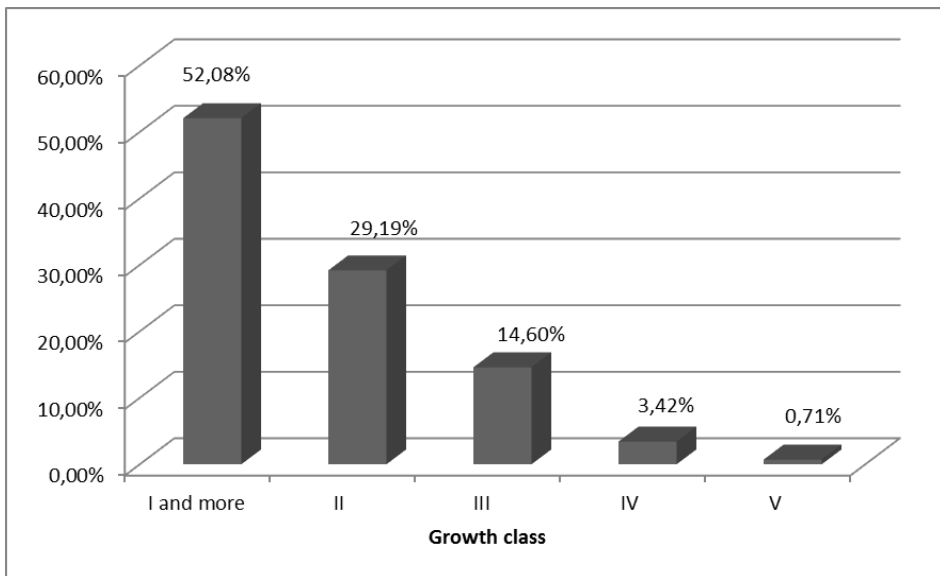


Figure 4. Distribution of the pine stands area by growth classes, %

Shares of other 11 forest types, when Scots pine grows were less than 10.0 % for each type. Within the other 25 forest types, the Scots pine stands had minimum areas 5.0%. Taking into account the biological features of Scots pine, its light-demanding and middle demanding to the soil fertility, the prevailing conditions are quite favorable for such species growth.

Analysis of the areas distribution by forest capacity classes showed that the Scots pine stands are considered to be high-productive because this species

grows in I and above forest capacity class, on the square 11183 hectares, according to Orlov (1911), accounting respectively for 52.1 % (Fig. 4).

According to submitted data, the second largest area was occupied by pine stands of II forest capacity class, and the only 4.1% of area were occupied by poor stands of IV and V classes.

The degree of stocking is an important indicator of the stand condition because on the basis of this parameter it can be evaluated effectiveness of forest management aimed to improving the forest productivity. According to distribution of the Scots pine stands area by relative density, this species forms high-density stands on  $\sim \frac{1}{2}$  of total square (11778.6 ha); middle-density stands on 7569.6 ha, and poor and open stands occupied only 9.9% of the total area (Fig. 5).

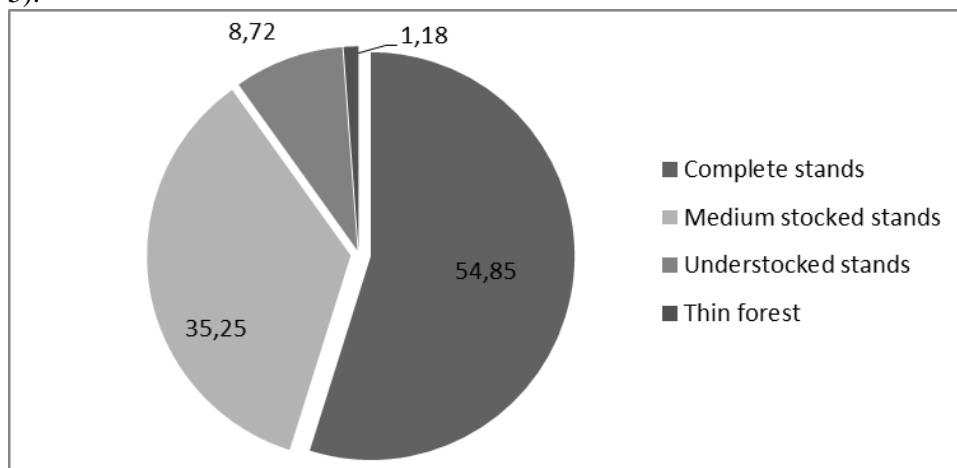


Figure 5. Distribution of the pine stands area on degree of stocking, %

The average relative density of pine stands within the north dnierper steppe was quite high and amounted 0.71. It largely depends on the age, significantly decreasing in overmature stands.

## CONCLUSIONS

Area of Scots pine stands within North Dnieper Steppe is 21472 ha with the total growing stock 45711 m<sup>3</sup>·ha<sup>-1</sup>. According to age structure, majority of the Scots pine stands within the studied region were middle-aged, with the greatest representation in the IV-VI age classes. Scots pine distribution by structure rate showed significant dominance of pure stands (81.8 %). Within North Dnieper Steppe, the Scots pine forests mainly formed under pine mixed forest site type, in dry and mesic hygrotopes. The largest area of the analyzed species was occupied by stands of II and above forest capacity classes. Stands of Scots pine in  $\sim \frac{1}{2}$  of the total square were high-density, whereas only 10% of the total pine forests areas were low-density and open. The results of this survey can



be used to improve forestry activity in the pine stands within the studied region, in particular, when performing cleaning cutting, forest sanitation, and in determining the measures to create a highly productive, biologically sustainable plantations with appropriate regime of forestry activity.

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*Viktoria LOVINSKA, Svitlana SYTNYK,  
Mykola KHARYTONOV, Irina LOZA*<sup>1</sup>

## FEATURES OF PINE STANDS FUNCTION IN DNEIPER NORTH STEPPE, UKRAINE

### SUMMARY

The article is devoted to distribution of *Pinus sylvestris* stands areas within North Steppe on the basis of forestry biometric indexes. The research objective was analysis of current state of *Pinus sylvestris* stands within Dnieper North Steppe on the main silvicultural and forest inventory parameters. The forest stands within Steppe zone are formed under conditions extremely unfavorable for arboreal plants growth. Occupying a very wide area, *Pinus sylvestris* has a large number of ecological forms, and it is both tolerant to low and high temperatures, it is winter-hardy and low-demanding to soil fertility and moisture. It was noted the irregular age structure in pine plantations under regional condition. Area of *Pinus sylvestris* stands within North Dnieper Steppe is 21472 ha with the total growing stock 45711 m<sup>3</sup>·ha<sup>-1</sup>. According to age structure, majority of the stands from *Pinus sylvestris* were middle-aged within the studied region, with the greatest representation in the IV-VI age classes. *Pinus sylvestris* distribution by structure rate showed significant dominance of pure stands. *Pinus sylvestris* It was established the prevailing types of site forest conditions on the basis of the edaphic factors (soil fertility and humidity). *Pinus sylvestris* forests mainly formed under pine mixed forest site type, in dry and mesic hygrotopes. The largest area of the analyzed species was occupied by stands of II and above forest capacity classes. The obtained results show predominance of high-productive pine stands within studied area. It was found that the maximum area of pine stands have high relative density.

**Keywords:** forest fund, *Pinus sylvestris*, growing stock, indexes of forest biometrics

### INTRODUCTION

In recent times, particularly after the United Nations Conference on Environment and Development 1992 (UNCED), there have taken place a substantial changes in priorities of relationships between individual and forest, which involve transition from the use of forest as a resource to the management

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

of processes within forest ecosystems. The most important criteria for sustainable forest management are considered to be support and preservation of forests productive capacity, their protective and social functions, and biodiversity (Hensiruk, 1992; Caspersen and Pacala, 2001; Scherer-Lorenzen et al., 2005) because dramatic changes associated with human activities on environment effect negatively on the stands condition and functioning (Bormann, 1985; FAO, 1993). As a major component of forests, arboreal plants reflect the overall status of forest ecosystems, the features of their functioning and the main trends of their development. These issues were investigated by the authors in scientific publications (Loza and Nazarenko, 2006; Bezrodnova et al., 2013; Lovinska, 2014; Sytnyk et al., 2015).

It is known that in the Steppe zone forest stands are formed under conditions extremely unfavorable for arboreal plants growth. Only a few species are able to adapt to the complex of stress factors typical for this natural area. One of them is Scots pine (*Pinus sylvestris* L.), is known for its high resistance (Belgard, 1971; Vakuliuk, 2006; Siryk, 1991; Sychev, 2011). Significant distribution and penetration of single populations outside the forest zone are proved by high adaptive capacity of such species. Occupying a very wide area, Scots pine has a large number of ecological forms, therefore, this species is both tolerant to low and high temperatures, it is winter-hardy and low-demanding to soil fertility and moisture. Usage the not scientifically substantiated methods of forest management had led to deterioration in the forest stands quality, loss of their environmental-forming, resource, and recreational functions. For this reason, it is very important to obtain the complete and reliable information regarding status of forest-forming species within the stands for effective management and conservation of forest ecological and social functions (Alekseyev, 2003; Gulchak, 2011). The forest management process should begin with investigation of the forest stand characteristics.

The state and productivity of such characteristics are influenced by a complex of factors, first of all, age of forest-forming species, forest site condition, capacity, and stand density. As the research objective, it was analysis of the current state of Scots pine stands within Dnieper North (ravine) Steppe on their main silvicultural and forest inventory parameters.

## **MATERIAL AND METHODS**

The objects of the survey were pine stands of North Dnieper Steppe within the Dnipropetrovsk oblast. The survey of silvicultural taxational characters in pine stands was performed using information of stratum database "Ukrderzhlesproekt" and conventional methods of forest inventory (Anuchin, 1982; Gulchak, 2011) in 2009-2014. On the basis of this database, it was processed 4790 stratum in accordance to the relevant indexes within the existing age groups of trees. The analysis was subjected to such characteristics of Scots pine stands as forest type, composition, age of stand, mean height and diameter, timber resource, forest capacity, and relative density.

The classification schemes of trophotopes and gygotopes is based on the edaphic factors, such as soil fertility and humidity. Soil fertility is characterized by trophogenic sequence and denoted by the letters A, B, C, and D. These individual units of trophogenic sequence called trophotopes. Each trophotope is represented by the forest site with equal soil fertility within self boundaries, distinguished from the next by one gradation (Belgard, 1971). Trophotope "A", "bor" or pure pine forest, indicate very poor soil conditions, predominantly with sandy soil, sometimes loamy sands with a short rhizosphere zone; gritty consistency stipulate their poorness. Trophotope "B", "subor" or mixed pine forest is characterized by relatively poor soils. It is represented by loamy sand or sandy soil with thin sandy-loam or loamy layers, or with heavy layers of that at a considerable depth. Trophotope "C", "sugrud" or oak mixed forest has a relatively rich habitat conditions. Soils are represented by sandy-loam, sometimes sand with layers of loam and sandy-loam, or denuded shallow grey forest soil, sometimes brown soil with the little humus horizon, skeletal, derived from volcanic rocks and sandstones. Trophotope "D", "grud" or oak pure forest has the most fertile habitat conditions. The soils are loam with the heavy (greater than 0.8 m) rhizosphere, more rarely sandy and sandy-loam soils with the layers of loam and clay, available to plant roots. Sometimes the sandy and sandy-loam soils occur with shallow horizon of flow "mineralized" groundwater.

Whereas, the trophotopes are divided by six gygrogene sequences: gygotopes with indicators of soil moisture 0 to 5. Gygotopes of 0 range respond to very dry (xerophilic) conditions; 1 – dry (meso-xerophilic); 2 – mesic (mesophilic); 3 – mesic-wet (meso-gygraphilic); 4 – wet (gygraphilic); 5 – swamp (ultragygraphilic).

According to the classification scheme, each site of forest is characterized by two classification units: trophotope and hygotope. In the surveyed forest stands we selected 16 sampling units for Scots pine in existing grades of trophotopes and hygotopes within stands of different ages. The delimitation of sampling units was carried out instrumentally with surface marking and binding to net of rides. The size of sampling units was ranged from 0.25 to 0.5 hectares. Within the sampling units it was determined the total growing stock with the model trees by way of step representation. Model trees were selected for each level of thickness. Work on the sampling units was performed in the following order:

- 1) complete tree enumeration;
- 2) determination of the mean diameter of the stands with usage of average cross-sectional area according to tally sheet, by dividing the sum of basal areas on the total number of trees within the forest stand;
- 3) selection of three medium-size and shape sample trees in the each level of thickness;
- 4) measurement of diameter of the sample tree accurate to 0.1 cm;

- 5) measurement of height of the sample tree accurate to 0.1 m;
- 6) cutting of the sample trees;
- 7) determination of volume of the sample tree by the formula:

$$V_{\text{aver}} = g_{\text{aver}} \times h_{\text{aver}} \times f_{\text{aver}}$$

where:  $g_{\text{aver}}$  – cross-sectional area of the average sample tree;  $h_{\text{aver}}$  – average height of the average sample tree,  $f_{\text{aver}}$  – form factor of the average sample tree;

- 8) determination of stock in the sampling unit according to the formula:

$$M = V_{\text{aver}} \times N$$

where:  $M$  – growing stock,  $V_{\text{aver}}$  – volume of the average sample tree,  $N$  – number of trees within the stand.

Values of the growing stock obtained on the sampling unit for investigated tree species was transferred on 1 ha according to the formula:

$$M = M_{\text{samp.un}} \times F_{\text{conv}}$$

by multiplying it on the conversion factor  $F_{\text{conv}} = 1 \text{ ha}/S_{\text{samp.u.}}$

According to the materials of forest management, there were found the area of Scots pine stands belonging to a particular age class, and the total growing stock of this species within surveyed areas. The age class Scots pine was 10 years. In the stands of the same age, determination of dependence of growing stock formation on the type of trophotope and hygrotrope was determined by usage of the average growing stock by the formula:

$$M_{\text{aver}} = M_{\text{total age cl}} / S_{\text{stand age cl}}$$

where  $M_{\text{total age cl}}$  is the total growing stock in the same age class stands,  $S_{\text{stand age cl}}$  is the area of the stand of given age class.

## RESULTS AND DISCUSSION

According to our silvicultural and forest inventory analysis, the total area of Scots pine plantations within North Dnieper Steppe was 21472.9 ha with a growing stock  $4571 \text{ m}^3 \cdot \text{ha}^{-1}$ . The native pine stands occupy the area of 3693.8 ha (17.2 %), while artificial pine forest stands were located on 17779.1 ha (82.8% respectively) of the pine plantations total area. Age of stands has a great biological and economic importance; it is associated with growth stages of the stands. In forestry, it is known the "normal forest" concept, which is characterized by a uniform distribution of stands by age groups. It is the most favorable from the standpoint of economic benefit. According to actual data, it was established that the Scots pine stands were considerable asymmetric within the age groups. The middle-aged stands were predominated in 58.5 % as well as the young stands occupied 32.7% of the total area (Table 1). The mean age of dominant group was 60 years, and the average young group age was 23 years, with average growing stock 292 and  $92 \text{ m}^3 \cdot \text{ha}^{-1}$  respectively. Amount of the pine stands area in other age groups was not exceeding 10 %.

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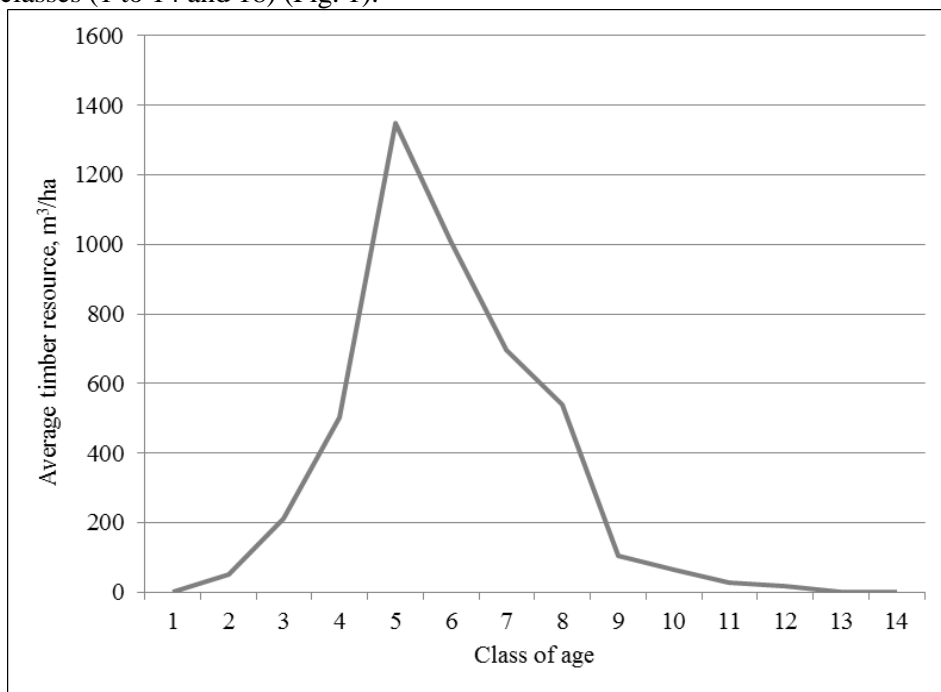


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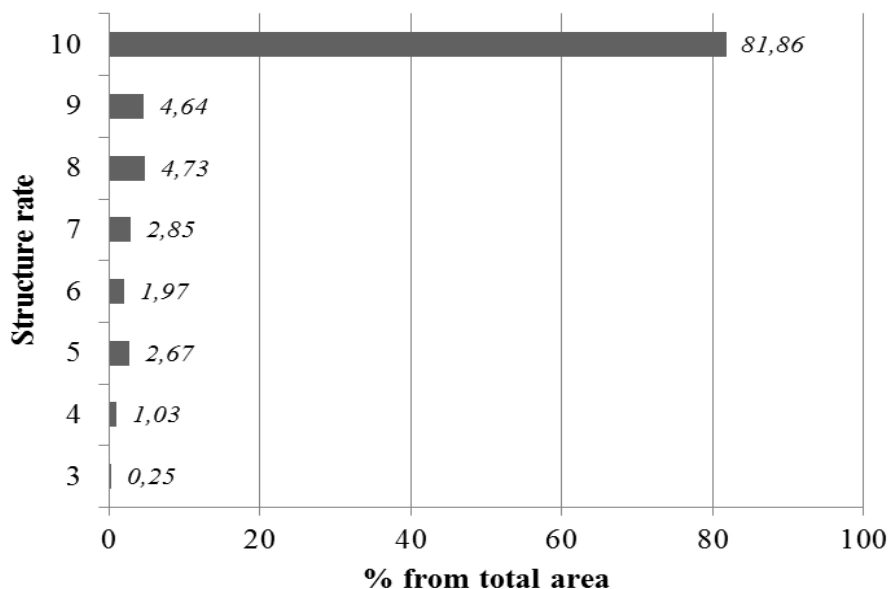


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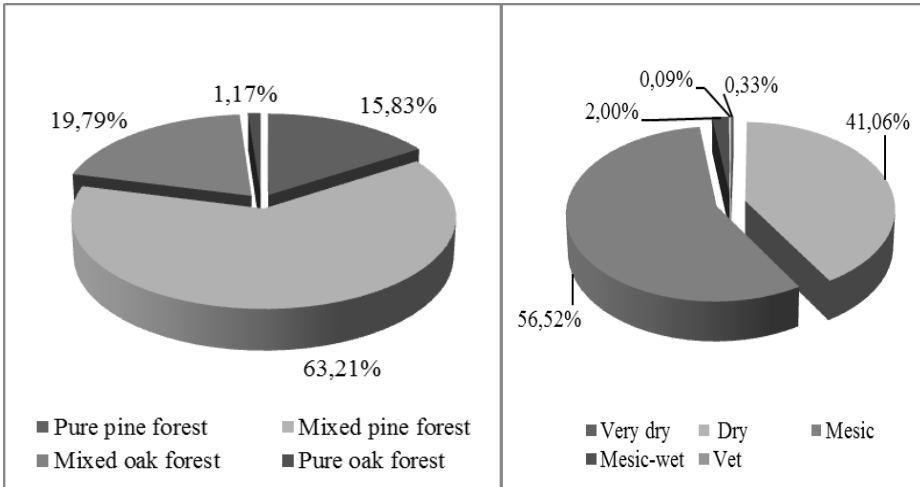


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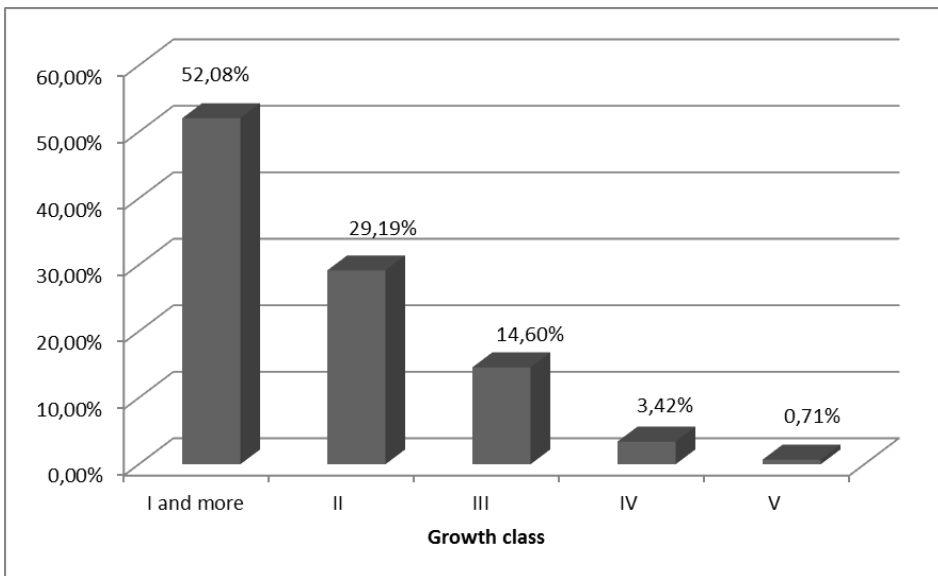


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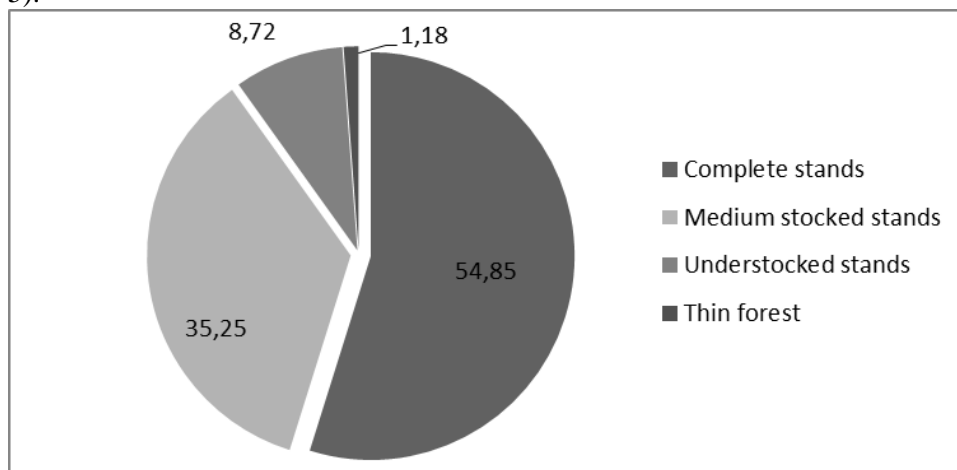


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## **PARTICIPATION OF PHYSICALLY CHALLENGED PEOPLE IN AGRICULTURAL VALUE CHAIN: IMPLICATION ON FOOD SUSTAINABILITY IN NIGERIA**

### **SUMMARY**

This study focuses on assessing participation of physically challenged people (PCP) in agricultural value chain as a means of food sustainability in Nigeria. Specifically, it describes the socio-economic characteristics of the physically challenged people, analyzes PCP perception of agricultural value-chain as a means of income generating activities; determined their training needs as well as identified the major constraints to participation in agriculture. One hundred and five respondents that belong to physically challenged associations were interviewed through the use of structured interview schedule. Data analyses were carried out using frequency counts, percentages, mean, standard deviation and correlation. Results of the study showed that more males were found in this category compared to females, and they were of productive age. Majority was illiterate and relevant pieces of information were sourced from relatives and friends. There was a low level of participation in agricultural value chain due to negative perception to agricultural production, inadequate access to appropriate education and information, inadequate training in the area of agricultural value chain where PCP can be engaged, inappropriate technology, inadequate credit facilities and negative attitude of people to the plight of the PCP. Positive and significant correlation exists between the level of participation of PCP in agricultural value chain at  $p \leq 0.05$  and level of education; source of information; trainings attended and perception towards agricultural production. In conclusion, there is the need to create enabling environment that will encourage the PCP to participate in agricultural production to enhance food security and poverty alleviation.

**Keywords:** Attitude, participation, perception, value-chain, physically challenged people

### **INTRODUCTION**

Physically challenged persons can be described as those certified by a specialist in any field of therapy as having one or more disabilities which might be blindness, partial blindness, emotional disorder, deafness, partial hearing,

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physical handicap, speech defects, learning disability, social maladjustment, exceptional giftedness and mental retardation (Deloitte Access Economics, 2011). Persons with disabilities in rural areas represent the poorest of the poor; they lack access to the most basic social services, including education, health services, access to production resources and opportunities for income generation, and employment. The statistics suggest that unemployment for working age disabled people in developing and industrialised countries is between 80-90% and 50-70%, respectively (Naami et al. 2012). They are often excluded from active participation within their community. This general neglect causes these people to be often not included and their specific needs ignored in agricultural development programmes and policies.

The UN convention on the rights of persons with disabilities that came into force in 2008 marks a paradigm shift in how disability is viewed from people with disabilities as objects of charity or medical intervention, to people with rights and control over their own lives, decisions and futures.

Agricultural sector has been the mainstream of national development in which PCP can also be involved in large scale, if given the opportunities. The United Nations Convention on the Rights of Persons with Disabilities (UNCRPD) (United Nation 2006) provides vast opportunities to increase awareness of disability around the world. The World Food Summit organized by FAO in 1996 acknowledged the fundamental contribution to food security by disabled farmers, noting that a large proportion of the disabled people were farmers with responsibility for the food security of their households (FAO, 2006). Efforts are been made by international organizations and developed countries especially European Union to include physically challenged people in agricultural development programme. The European Union recently adopted disability as a cross cutting issue, giving opportunities to include people with disabilities in regular food security programmes. Numerous successful projects have shown that people with disabilities are able to participate in meaningful agricultural activities. Some go as far as suggesting that people with disabilities are the world's untapped resource and that their inclusion is of paramount importance for global food security (Global Forum on Food Security and Nutrition, 2010). In Nigeria, adequate attention has not been paid to the fact that physically challenged people are the world's untapped resource and that their inclusion is of paramount importance for global food security. Therefore the study assessed the participation of physically challenged people in agricultural value chain for food security and poverty eradication. The specific objectives of the study include describing the socio-economic characteristics of the physically challenged people; exploring areas of interest in agricultural value chains where PCP can be involved in the study area; analyze their perception of agricultural value chains as a means of income generating activities; and identify the major constraints to their participation in agriculture value chains.

## MATERIAL AND METHODS

The study was carried out in three out of six states of Southwestern Nigeria. These are Ekiti, Ondo and Osun States. The states were selected in view of the fact that most of these PCP have associations where they can be easily reached and intervention programme can be extended to them. Ten percent of the local Governments (LGAs) in each state were selected. In all, 7 LGAs were used. Fifteen PCP (physically impaired, visually impaired and hearing impaired) were selected in each LGA, to give a total of 105. Structured interview was used to collect relevant quantitative data. Descriptive statistics such as percentages, mean and standard deviation were used to summarize the data. Pearson correlation coefficient was used to draw inferences from the hypotheses. In order to determine the level of participation of PCP in agriculture value chains, statements of opinion on their level of participation were grouped into three. That is fully participating, partially participating and never participated. These were scored 2, 1 and 0, respectively. Mean  $\pm$  standard deviation was used to categorise statements into high, medium and low level of participation.

## RESULTS AND DISCUSSION

The results from Table 1 show that majority (82.9%) of the PCP were not more than 60 years old. This indicates that majority of the PCP in the study areas were still in their productive age in which they could still be productive and contribute meaningfully to the socio-economic well being of the society. This is in line with Ogunjimi et al. (2012) findings that majority of farmers in southwestern Nigeria were in their productive age. Moreover, majority (61.0%) were male, while 39.0 percent were female. The findings were expected because of involvement of women in domestic activities. It may also be attributed to the tenure system where females right to land ownership is denied. Moreso, farming activities required time and energy which women may not be able to cope with talk of PCP. This finding corroborates previous findings by Tijani (1999) and Ogunjimi (2011) that population of male farmers in Osun and Edo States were higher than females. However, contrary to expectation that majority of the PCP ought to have married, less than average (38.1%) were married while 61.9% were either single, divorced or widowed. This might be as a result of discrimination against PCP where people without disabilities might not be willing to marry them because of their disabilities. Majority (73.3%) of the PCP either had no education or stop at primary level. This might be a result of inadequate provision of schools for disabled people and where available, there were a lot of rigours in getting to schools due to constraints such as inadequate transportation and trained personnel. This finding also corroborated the submission of Beresford (1996) that the unemployment of disabled people is due to lack of education and training. The results of the study further reveals that PCP were rarely visited by extension agents and majority that claimed having extension contact had it less than 5 times in a year.

Major source of information was other rural dwellers (60%), while extension agents who ought to have been the major source of information were either inadequate in number or not well equipped to face the challenges. Results in Table 1 also reveal that majority were living below the poverty level because above average (54%) realized less than 50,000 Naira (294.12 USD) annually. The finding is in line with the study carried out in India and Uganda as reported by Emmel (2012). The report showed that in India, households with people who have disabilities are worse off than the average household. Similarly, research revealed that in Uganda, households headed by an individual with a disability are 38 percent more likely to be poor than households headed by a person without a disability due to low level of income.

Table 1. Distribution of PCP according to socio-economic characteristics  
N=225

Socio-economic characteristics	Frequency	Percentage	Mean/ (STD)
Age (year)			
Below 30	43	41.0	
31 – 60	44	41.9	37.0 (11.3)
61 and above	18	17.1	
Sex			
Male	64	61.0	
Female	41	39.0	
Marital Status			
Single	38	36.2	
Married	40	38.1	
Divorced	23	22.0	
Widowed	4	3.7	
Year of schooling			
1-6	33	31.4	
7-12	21	20.0	
13 and above	7	6.7	
Never	44	41.9	
*Source of information			
Other rural dwellers	63	60.0	
Fadama Facilitators	46	44.0	
Radio and television	38	36.4	
Non-Governmental Organisations	35	33.3	
Extension agents	29	27.6	
Newspaper	21	20.0	
Income /annum			
Less than 50	57	54.3	
51,000-100,000	41	39.0	₦65, 243 (12,352)
Above 100,000	7	6.7	
Extension contact in the last one year			
Never	79	75.2	
1-5	19	20	
6-10	5	4.8	



Results in Table 2 show that almost a half (49.5%) of PCP interviewed had physical impairment (any impairment which limits the physical function of limbs, fine bones, or gross motor ability). Furthermore, 23.9% of PCP interviewed were hearing impaired (hearing impairment or hard of hearing or deafness refers to conditions in which individuals are fully or partially unable to detect or perceive at least some frequencies of sound which can typically be heard by most people). Meanwhile, 14.3% of the PCP were visually impaired. This is loss of vision of a person to such a degree as to qualify as an additional support need through a significant limitation of visual capability resulting from either disease, trauma, or congenital or degenerative conditions that cannot be corrected by conventional means, such as refractive correction, medication, or surgery). Few (12.4%) of the PCP had intellectual retardation (specific learning disability). There is ability in disability. Despite their challenges all are still participating in one agricultural production or the other.

Table 2. Types of disabilities

Types of disabilities	Frequencies	%
Visual impairment (Partial blindness)	15	14.3
Hearing impairment (deafness, partial hearing and speech defect)	25	23.9
Physical impairment	55	49.5
Intellectual (learning disability), mental retardation	13	12.4

Results in Table 3 show major areas of agricultural production in which PCP participated. Majority (58.1%) of PCP were into crop production while others were into livestock production (26.7%), fisheries and aquaculture (6.7%) and beekeeping (5.7%). The implication of this is that despite their disability, they were still involved in agricultural production in which they were able to contribute their quotas to national food sufficiency. This finding corroborated Emmel (2012) report from a work carried out among disabled people in Haraspada village in Puri district, Odisha, India in which majority of disabled people in the village were into agricultural production.

Table 3. Major areas of agricultural production in which PCP participate

Area of agricultural production	Frequencies	%
Crop production	61	58.1
Livestock keeping	31	29.5
Fisheries and aquaculture	7	6.7
Beekeeping	6	5.7

Analysis of the study showed that the total mean score of participation level in agricultural value chain was 2.5 with standard deviation of 0.6. Participation was rated in descending order. Crop production was rated first with mean score of 2.8, while marketing of agricultural products was rated next. Others include livestock production (mean=1.5), processing of agricultural products (mean=1.5), packaging of agricultural products (mean=1.1). Considering the level of participation, crop production had high level of participation while marketing had medium level of participation. For other agricultural production and value chains, participation by pcp was at low level. The implication of this finding is that pcp have low level of participation in other agricultural value chains apart from crop production and marketing of agricultural production which might be as result of inadequate knowledge and skill about other activities. In order to attain high participation in agricultural value chain, factors hindering it must be taken into considerations and necessary actions needed to be taken.

Table 4: Distribution of pcp according to mean score of level of participation in agricultural value-chains

Participation in agricultural production	Production	Rank
Crop production	2.8	1 <sup>st</sup>
Marketing of agricultural products	2.5	2 <sup>nd</sup>
Livestock production	1.5	3 <sup>rd</sup>
Processing of agricultural products	1.5	4 <sup>th</sup>
Packaging of agricultural products	1.1	5 <sup>th</sup>
Fisheries and aquaculture production	1.1	6 <sup>th</sup>
Honey production	0.9	7 <sup>th</sup>

Table 5. Rank–order of statement of opinion on perception of PCP about agricultural production

Statement of opinion	Mean	Rank
Agriculture is worthwhile venture hence PCP should be encouraged to participate in it.	4.1	1 <sup>st</sup>
Agriculture increase the income of farmers hence participating in it is necessary.	3.8	2 <sup>nd</sup>
Market values of agricultural products are commensurate with the cost of production	3.6	3 <sup>rd</sup>
Most of the activities are environmental friendly	3.5	4 <sup>th</sup>
Agricultural value chains required a lot of training hence discouraged participation in them.	2.8	5 <sup>th</sup>
Agricultural activities required a lot of technical skill, which is very difficult to acquire.	2.6	7 <sup>th</sup>
Agricultural production is a waste of time venture hence involvement is not necessary	2.3	8 <sup>th</sup>
Income from other occupations is enough to spend throughout the year hence participation is a waste of time	1.9	9 <sup>th</sup>

Responses from pcp on who market products that they produced in table 5 showed that only 31.4 percent of the pcp marketed their products themselves while a good number (68.6%) claimed that their parents, relatives/friends and other farmers were in charge of marketing their agricultural products for them it could be deduced from pcp responses that they were not directly in charge of what they produced which might be due to constraints such as distance from village to market, transportation problems and other related problems.

Problems encountered by pcp are shown in table 7. They are multi-faceted, which ranges from negative attitude of people to the plight of pcp and discrimination against them (mean=4.4). Other problems encountered were stated in descending order of their severity: inadequate assistive and rehabilitation appropriate for agricultural workers ranked next. This was followed by problems such as inadequate credit facilities, high cost of input, inadequate processing equipment, inadequate skill on improvement practices, inadequate information, distance to rural market, insufficient access to labour and lands. Attention needs to be focussed on all the constraints stated above for livelihood of pcp to be sustainable. The finding is in line with hanko and polman (2002) fao project reports that pcp lack access to the most basic social services, including education, health services, access to production resources and opportunities for income generation, and employment. Moreover, emmel (2002) concluded in a study carried out in puri village in india that absence of savings and credit facilities within villages, corruption and lack of faith in banking institutions by disabled people's ability deny them credit. Furthermore, unavailability of raw materials and limited marketing opportunities are challenges for disabled people in initiating their business.

Table 6. rank–order of statement of opinion on perception of pcp about agricultural production

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Market values of agricultural products are commensurate with the cost of production	3.6	3 <sup>rd</sup>
Most of the activities are environmental friendly	3.5	4 <sup>th</sup>
Agricultural value chains required a lot of training hence discouraged participation in them.	2.8	5 <sup>th</sup>
Agricultural activities required a lot of technical skill, which is very difficult to acquire.	2.6	7 <sup>th</sup>
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Table 7 Constraints to pcp participation in agricultural production value chains

Constraints	Mean	Rank
Discrimination against PCP by people in the society	4.4	1 <sup>st</sup>
Inadequate assistive/ rehabilitation appropriate for agricultural workers	4.1	2 <sup>nd</sup>
Inadequate credit facilities	3.8	3 <sup>rd</sup>
High cost of input	3,5	4 <sup>th</sup>
Inadequate processing equipment	3.4	5 <sup>th</sup>
Inadequate skill on improvement practices	3.2	6 <sup>th</sup>
Inadequate information	3.0	7 <sup>th</sup>
Distance to rural market	2.9	8 <sup>th</sup>
Insufficient access to labour	2.9	9 <sup>th</sup>
Inadequate land	2.7	10 <sup>th</sup>

### *Testing of Hypotheses*

The correlation results show that there exists a positive and significant relationship between PCP perception of agricultural production and level of participation. This indicated that the higher the level of perception the higher the level of participation. Furthermore, there were significant relationship at  $P \leq 0.5$  between participation in agricultural production and some socio-economic characteristics of the respondents such as educational status ( $r=0.412$ ), extension contact ( $r=0.378$ ) and income realized from agricultural production ( $r=0.317$ ) while age ( $r=-0.008$ ) was not significantly correlated. The implication of the finding is that high educational level, extension contact, income and good source

of information have positive effect on the participation of PCP in agricultural production.

Table 8. Correlation analysis between level of participation of pcp and personal, socio-economic characteristics variables and type of disabilities.

Variables	Correlation ®	Co-efficient of determination ( $r^2$ )
Perception of PCP about agriculture	0.547**	0.229
Level of education	0.412**	0.170
Extension contact	0.378**	0.143
Income realized from agricultural production	0.317**	0.101
Type of disabilities	-0.251**	0.063
Age	0.056	0.003

### CONCLUSIONS

The overall conclusion is that majority of the physically challenged people participated in agriculture at low level, which were due to the constraints ranging from discrimination, inadequate assistive and rehabilitation appropriate for agricultural workers. Moreover, PCP had high perception towards agricultural production and other related value chains such as processing, parking and marketing. However, high level of perception of PCP about agricultural production did not translate to high level of participation which might be as result of challenges encountered by PCP in the course of their participation in agricultural production.

There is need to arouse the interest of physically challenged people through training on the relevant agricultural value chains from production to consumption by the extension agents and other relevant agencies. Government at all levels and Non-Governmental Organisations should integrate disabled people into sustainable agriculture and rural development policies and programmes meant for them. Provision of appropriate technologies and credit facilities through public and private partnership will facilitate maximum participation in agricultural production. The future implication for food security and poverty alleviation is that, if the capability of physically challenged persons is enhanced through training by extension agents and necessary materials are provided, they can produce their own healthy foods and make meaningful contribution to agriculture and community development.

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## **SEARCHING FOR RESISTANCE SOURCES TO WHEAT COMMON BUNT DISEASE AND EFFICIENCY OF BT GENES AGAINST *TILLETIA TRITICI* AND *T. LAEVIS* POPULATIONS**

### **SUMMARY**

Common bunt disease incited by *Tilletia tritici* and *T. laevis*, is one of the most important disease of wheat in Iraqi Kurdistan region. The disease can cause severe yield losses when the susceptible cultivars are grown without chemical treatment. This study was carried out to search for resistance sources to common bunt disease in wheat and also to determine the efficiency of Bt genes against the pathogen to be introduced in the future breeding program to improve disease resistance. Seeds of different wheat genotypes including the released cultivars and the differential varieties set were artificially inoculated with a mixture of the pathogen teliospores and planted at Bakrajo, Sulaimania for three successive generations. Disease scoring for each genotype was conducted at maturity stage. Results revealed that most of the tested wheat cultivars showed susceptible and intermediate reaction to the disease while the local wheat cultivars Ashor, Acsad, Farris1, Hasad, Waha, Simmetto and the promising advance lines Shaho, Hamada and Charmo2 showed high resistance level to the disease. The international resistance sources Nadro, carbidit, Togano, tillet and Firsal were highly resistant to the local pathogen population. The known resistant genes Bt<sub>1</sub>, Bt<sub>3</sub>, Bt<sub>5</sub>, Bt<sub>9</sub>, Bt<sub>11</sub> and Bt<sub>12</sub> were highly effective against the races of *Tilletia tritici* and *T. laevis* under Sulaimania conditions.

**Keywords:** Cover smut, *Triticum aestivum*, Resistant genes, Host response, Iraq

### **INTRODUCTION**

Fungal diseases are the most important biotic constraints for wheat production in Iraq. Several diseases, particularly rust and smuts, have drastically decreases grain yield and quality of wheat. Among the smuts, common bunt incited by *Tilletia tritici* and *T. laevis* is the most important disease. Occurrence and distribution of the disease was formerly limited in Iraqi Kurdistan region and the northern parts of Iraq. Yield losses reached up to 70% when the susceptible cultivars grown without chemical treatment (2, 4). Recently the disease was observed for the first time in the central and southern parts of Iraq. High disease

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incidences were detected in most wheat fields in the region during the last decade, which causes potential hazard to wheat production in the country (6).

Chemicals treatment of seed is regularly used to control the disease. Organic mercury compounds were widely used in the past (26). Historically, many poisoning problems were reported due to the direct or indirect consumption of treated seeds by farmers. Application of ethyl mercury (Cerasan) was the principle cause of human and livestock poisoning in 1956 and 1960 in Iraq, while seed treatment with methyl mercury compounds (Panogen and Methyl Mercury Acetate) caused severe poisoning in 1972, where 459 people dead and other 6530 were poisoned in Iraq (9). Later on Diathane M-45 was widely used to protect wheat from the infection (5). Other previous studies confirmed efficacy of some other chemical compounds and fungicides (1, 13, 20). Recently Al-Maaroof *et al.*, (2004, 2011) indicates the high efficiency of Mancozeb, Diathane, Dividend and Lamardor in wheat protection from bunt diseases (5, 8). Chemical seed treatment is not safe and not allowed in organic agriculture and several active ingredients will be banned from the Phytosanitary register in near future (32). The deployment of genetic resistance is one promising option to efficiently control the disease in an environmentally friendly manner. Specific gene for resistance to common bunt are tabulated by McIntosh, 1983 (33), while the occurrence of bunt races with specific virulence that can overcome of the specific resistance of the host greatly increase the difficulty of attaining long term effective control (22, 34). Major breeding efforts have been made to develop multiple gene resistance. Plant breeders mainly depend on the cultivars Martins containing resistant gene Bt1, Turkey (Bt4) and Redit (Bt13) as a source for resistance to improve disease resistance against cover bunt and dwarf bunt diseases (28). The resistant source PII78383 contain resistance genes Bt8, Bt9, Bt10 confer durable resistance for more than 20 years in USA, this was due to the lack of virulence against Bt8 in the pathogen races, while virulence's against this gene was found in European races (16, 31). Many studies were conducted in Iraq to improve wheat resistance to the disease using different breeding program. Ibrahim *et al.* (1988), able to get 42 resistant mutants by irradiation Saberbeg and it hybrid with Maxipak by different doses of gama rays and fast neutrons (24), two mutant cultivars with moderate resistance to the disease were also developed (23). Al-Maaroof *et al.*, (1993) obtained 15 variants resistant to covered smut for four successive generations (M4-M7) by irradiating Saberbeg hybrids with Maxipak, Ajeeba, Araz and Abu Ghraib with different doses of fast neutrons (3). Furthermore, two mutant wheat cultivars resistant to covered smut and Septorial leaf blotch were developed by mutation teqniuques (4). Many Advance resistant lines of wheat against common bunt, leaf rust and yellow rust diseases were developed from the resistant sources and their hybridization with the local cultivars (7, 10). The new resistant cultivars Farris, Hamada, Alaa, Hsad and



Maarroof were recently registered by the Ministry of agriculture and released to the farmers as new sources for resistant (34).

The current study conducted to search for resistance sources to common bunt disease in the international wheat sources for disease resistance and the local wheat cultivars. Also, to determine the efficiency of Bt genes against the bunt pathogen population under Iraqi environmental conditions to introduce them in the commercial susceptible wheat cultivars to improve bunt disease resistance program and grain quality of wheat in Iraq.

### MATERIAL AND METHODS

Three separate experiments were conducted at Faculty of Agricultural Sciences Field, Sulaimani University (N 35°32'351'', E 45°21'978''), about 15 km north west of Sulaimania province in the Iraqi Kurdistan region. The first experiment include 36 registered and released wheat cultivars, 30 bread wheat and 6 durum wheat cultivars obtained from the state board for seed testing and certification, Iraqi Ministry of Agriculture, The second experiment include 22 resistant wheat cultivars to common bunt disease from the international wheat collections introduced from the European Tilletia Ringtest (ETR). It has been initiated involving several international breeding and research institutes including Sulaimani University based on a Memorandum of Understanding (MoU). The exchange of material was governed either by MTAs (material transfer agreement) or by the breeders' privilege according to the UPOV convention. The third experiments include differential variety set for common bunt disease including 15 genotype that contains resistant genes Bt1 to Bt15 for identification of *T. tritici* and *T. laevis* races. The winter wheat Red Bobs were used as universally susceptible lines (designated in previous work as Bt0) in field nurseries to indicate disease pressure in the experiment. Seed of common bunt differential set was obtained from Dr. Blair Goates (USDA-ARS, Aberdeen, Idaho, USA). Seeds of each wheat genotype from the above mentioned experiments were artificially inoculated with a bulk population of *T. tritici*, *T. laevis* and *T. intermedia* teliospores collected from different locations in the previous season at a rate of 0.5 g / 100g seeds. Inoculated seeds were mechanically mixed for 15 minutes by shaker at 80 rpm/minute (19). Each genotype was planted by hand at a depth of 5 cm in two 1.5 m rows (5 gm seed/line) with 30 cm apart between lines and 60 cm between treatments. Planting were done by the mid of December for two successive season (2011-12 and 2012-13) when the soil temperatures were 5-10°C. Plots were arranged in Randomized Complete Block Design (RCBD) with three replicates and two meters space between blocks. Field management and all the agricultural practices were conducted according to Ministry of Agriculture recommendations. Infection percent of each genotype from the first two experiments was calculated at dough stage by counting number of healthy and infected spike per each meter according to Dodoff and Todorova, 1974 modified method (15) where R= Resistant (Infection percent 0-10%), I= Intermediate resistant (Infection Percent 11-30%), S= Susceptible (Infection Percent 31-50%),

HS= Highly Susceptible (Infection Percent 51-100%). Data were statistically analyzed by using ANOVA table at L.S.D. 0.05.

Data from each differential line were averaged to determine a virulent-resistant (0-10% spike infection) or virulent-susceptible (11-100% spike infection) in accordance with Hoffman and Metzger method (21).

## RESULTS AND DISCUSSION

Table 1 shows that there are a wide range of differences in the response of the tested wheat cultivars against *T. tritici* and *T. laevis* population under artificial inoculation condition in Sulaimania. The registered and released wheat cultivars can be divided into four groups according to their average resistance to the disease. The first group includes the cultivars (Farris 1, Ashoor, Hasad, Hamada, Charmo 2, Cimmeto and Acsad), which are characterized by their resistance to the disease and significantly surpassed the other groups during both seasons. The mean infection percent of this group was between 2.36% in cv. Acsad to 9.73% in cv. Waha. The second group included cultivars (Iratom, Tamuz 2, Tamuz 3, Furat, Al-Iraq, Sham 6, Alaa, Maarof, Shahoo, Azmar, Azad, Um Rabia and IPA'99) characterized by their intermediate resistance to the disease ranging from 12.7% in cv. IPA'99 to 27.5% in cv. Maarof, while the third group included the susceptible cultivars to the disease (AbuGhraib, Araz, Rabia, Babel, Rasheed, Latifia, Sali, Hashmia, Tahedi, Fateh, IPA'95 and Al-Ize with mean infection rate of 31.98% in cv. Rasheed to 44.10% in cv. Sali. The fourth group was represented by the highly susceptible wheat cultivar SaberBeg and Al-R.V23 with means infection percent of 57.38 and 50.55 % respectively.

Combined analyses results revealed that the mean infection percent of the cultivars with common bunt disease in 2011-12 significantly surpassed the mean infection percent in 2012-13. This is significantly reflected on the response of Cvs SaberBeg, Babel, Tamuz 3, Furat, Sali, Hashmia, Fateh, IPA'95, Sham6, Al-Ize and Rasheed in 2011-12 season. The infection percent was higher between 23% in cv. IPA'95 to 68% in cv. Tamuz 3 in the first season comparing with the second season. The high infection incidence in the first season may be attributed mainly to the favorable environmental conditions for infection and disease development during 2011-12 particularly temperature, humidity and light. This also led to significant variation in host response of some genotypes "Babel, Tamuz 3, Al-Ize, Maarof, Azad and Whaha" to the disease between seasons. Results of the study revealed change in resistance categories of some wheat cultivars in comparing with the previous studies results. This was clear in response of cv. Tamuz 2 and cv. Iratom which changed from intermediate resistant to resistant and from intermediate resistance to susceptible in cv. Al-Ize (38). Resistant changes of this cultivars may be attributed to the appearance of new

ances in the pathogen population as it is also was confirmed in previous studies (6, 7).

The high level of resistance in Durum wheat cultivars may be correlated to the inoculum component of the pathogen species used in the artificial inoculation in the study. Frequency incidence of *T. tritici* was more prevalent than *T. laevis* and *T. intermedia* in the mixed population inoculum, which is more virulent on bread wheat cultivars than durum wheat cultivars (38).

Results of Table 2 revealed that most of the introduced international resistant sources showed resistant to moderately resistant reaction to the local *T. tritici* and *T. laevis* populations under artificially inoculation conditions. The genotypes Nadro, Carbidit, Tillet, Togano, Casan, Frisal, Skagen, Torrild, Tirone, Tommi, Rehti, Ridit and Rio showed resistant reaction to common bunt disease. The mean infection percent of the resistant genotypes varied from 0.0% in the genotypes Casan, Frisal, Tommi and Rio to 7.75% in the resistant genotype Tillet which was significantly less than all other intermediat resistance and susceptible genotypes. The genotypes Fiorina, Lorenzo, Surita and Urho showed intermediat resistant to the disease. The mean infection percent in this group were from 16.91% in Surita to 29.20% in Urho, which were significantly less than the infection percent of the susceptible group except Urho. The susceptible group include the genotypes, Grenia, Sertori, Lona 59 and Segor. No significant differences were found in the infection percent of this group with the local wheat cultivars. On the other hand, the genotype Runal showed highly susceptible reaction to the disease with infection percent of 62.53%, which were significantly, surpassed all other genotypes including the local cultivar Araz. No significant differences were found between the seasons although infection percent was higher in the first season. The high resistance level in the resistant sources Casan, Frisal, Tommi and Rio encourage their introduce in breeding program for common bunt disease improvement in Iraq.

Host parasite interaction of common bunt resistant genes (Bt genes) with the prevalent populations of *T. tritici* and *T. laevis* under artificial inoculation conditions in Sulaimania are presented in Table 3. Results revealed that Bt1, Bt2, Bt4, Bt7, Bt10, Bt113, Bt14 and Bt15 genes showed high infection type to the pathogen population, while Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 genes showed low infection type to the pathogen population at both seasons. Therefore, it turns out the possibility of exploiting Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 in breeding programs for improving common bunt disease resistance in Iraq.

Table 1. Host reaction of registered and released wheat cultivars with common bunt disease under artificial inoculation conditions during 2012-2013 seasons at Bakrajo, Iraq

Cultivar	Wheat Type <sup>1</sup>	2011/12 season		2012/13 season		Mean	
		Infection % <sup>2</sup>	Infection Type <sup>3</sup>	Infection%	Infection Type	Infection%	Infection Type
SaberBeg	BW	67.42	HS	47.33	S	57.38	HS
AbuGhraib	BW	36.76	S	43.07	S	39.92	S
Araz	BW	43.29	S	39.02	S	41.12	S
Rabia'	BW	32.30	S	45.40	S	38.85	S
Iratom	BW	16.20	I	13.12	I	14.66	I
Babel	BW	47.40	S	22.03	I	34.72	S
Tamuz2	BW	17.65	I	22.93	I	20.29	I
Tamuz3	BW	23.35	I	07.33	R	15.34	I
Furat	BW	25.60	I	15.03	I	20.32	I
Al-Iraq	BW	15.30	I	13.87	I	14.59	I
Farris1	BW	5.10	R	1.57	R	3.34	R
Rasheed	BW	39.13	S	24.83	I	31.98	S
Latifia	BW	35.77	S	29.60	I	32.67	S
Sali	BW	53.80	HS	34.40	S	44.10	S
Hashmia	BW	47.40	S	35.47	S	41.44	S
Tahedi	BW	37.80	S	32.50	S	35.15	S
Ashoor	BW	9.10	R	3.10	R	6.10	R
Fateh	BW	43.33	S	31.40	S	37.37	S
IPA'95	BW	47.66	S	36.93	S	42.30	S
Sham6	BW	26.78	I	17.33	I	22.06	I
Al-Ize	BW	48.40	S	27.17	I	37.79	S
Alaa'	BW	26.09	I	18.20	I	22.15	I
Maarroof	BW	31.33	S	23.66	I	27.50	I
Hsad	BW	9.66	R	5.30	R	7.48	R
Hamada	BW	4.86	R	9.20	R	7.03	R
Shaho	BW	20.60	I	14.8	I	17.70	I
Azmar	BW	14.96	I	10.50	I	12.73	I
Charmo 2	BW	7.40	R	8.90	R	8.15	R
ALR.V23	BW	60.40	HS	40.70	S	50.55	HS
Azad	BW	36.6	S	13.2	I	24.9	I
Um Rabee'	DW	26.78	I	14.8	I	20.79	I
Cimmetto	DW	3.71	R	5.30	R	4.51	R
IPA'99	DW	22.26	I	14.96	I	18.61	I
Acsad	DW	2.02	R	2.70	R	2.36	R
Sawa	DW	43.66	S	38.33	S	40.99	S
Waha	DW	2.66	R	16.80	I	9.73	R
Mean		28.68		21.69		25.18	
L.S.D 0.05 Cultivars Seasons Cult. * Seas.		9.73	-	8.55		9.03 6.75 9.35	

Table 2. Mean infection percentage of different wheat genotypes with common bunt disease under artificial inoculation conditions during 2012-2013 at Bakrajo, Iraq.

Genotype/ Cultivar	2011/12		2012/13		Mean	
	Infection 1 %	Infection 2 Type	Infection %	Infection Type	Infection %	Infection Type
Greina	40.73	S	36.57	S	38.65	S
Runal	65.06	HS	60.00	HS	62.53	HS
Nadro	0.00	R	6.20	R	3.10	R
Carbedit	1.64	R	6.50	R	4.07	R
Fiorina	27.19	I	23.9	I	25.54	I
Sertori	39.90	S	34.13	S	37.02	S
Lona 59	24.60	I	35.70	S	30.15	S
Tillet	13.10	I	2.40	R	7.75	R
Lorenzo	23.46	I	20.36	I	21.91	I
Togano	0.00	R	11.20	I	5.60	R
Surita	18.93	I	14.90	I	16.91	I
Casan	0.00	R	0.00	R	0.00	R
Segor	43.10	S	30.80	S	36.95	S
Frisal	0.00	R	0.00	R	0.00	R
Skagen	0.00	R	3.20	R	1.60	R
Torrild	0.00	R	5.40	R	2.70	R
Tirone	33.7	S	31.0	S	2.35	R
Tommi	0.00	R	0.00	R	0.00	R
Rehti	0.00	R	3.60	R	1.80	R
Urho	37.10	S	21.30	I	29.20	I
Ridit	3.60	R	1.90	R	2.75	R
Rio	0.00	R	0.00	R	0.00	R
Araz	45.35	S	43.66	S	44.51	S
Mean	18.15		17.07		16.31	
L.S.D 0.05 genotypes Seasons Genot.*Seas	13.10		11.35		11.66 n.s 12.31	

Table 3. Host parasite interaction of common bunt differential varieties (Bt genes) with a mix population of the pathogens under artificial inoculation conditions at faculty of agricultural sciences field, bakrajo, sulaimania, Iraq.

Entry No.	Differential Variety *	Resistant genes	Infection Type **	
			2012	2013
1	M84-504 to 510, Red Bobs	Bt0	H	H
2	M84-512 to 520, RB/WF 8	Bt1	L	H
3	M84-522 to 530, RB/SEL 1403	Bt2	H	H
4	M84-532 to 538, RB/RDT.	Bt3	L	L
5	M82-542 to 550, RB/TK 3055	Bt4	H	H
6	M82-34, Promose	Bt5	L	L
7	M84-552 to 560, RDT.	Bt6	L	L
8	M82-562 to 570, RB/TK 3055	Bt7	H	H
9	M78-9496, RB/PI 178210	Bt8	L	L
10	M84-597 to 605, RB/CI 7090	Bt9	L	L
11	M84-625, SEL M83-162	Bt10	H	H
12	M82-2123	Bt11	L	L
13	P.I. 119333(M82-2141), BW	Bt12	L	L
14	Thule III; P.I. 181463, BW Bt 13	Bt13	H	H
15	Doubi, DW	Bt14	H	L
16	Carlton, DW	Bt15	H	H
Number of virulence's			7	7

\*. Source of differential varieties seeds is Dr. B. Goates (USDA-ARS), Aberdeen, Idaho, USA.

\*\* . Infection Type: L= Infection less than 10%, H= Infection more than 10% according to Hoffman and Metzger, 1974

Results of this study confirms efficiency of the known resistance genes Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 in resistance of most prevalent *T. tritici* and *T. leavis* races in most of wheat fields in Iraq according to what has been referred

by many other researchers in previous studies in the world (12, 26, 28, 37 and 39). The high level of resistance in the known resistant genes Bt9, Bt11 and Bt12 against the pathogen population at both seasons encourage their implementation in the breeding programs for common bunt disease resistance in wheat through their use as parents in hybridization with the high yielding susceptible commercial wheat cultivars, particularly most of Iraqi wheat cultivars are susceptible to the disease (6 and 24). Mamluk and Nachit (1994 and 1999), (28, 29) confirmed the effectiveness of the resistance genes Bt5, Bt8, Bt9, Bt10 and Bt11 in differential varieties in Syria. On the other hand, some previous studies referred that the pathogen races overcome resistance of Bt1, Bt2, B3, Bt4, Bt6 and Bt7 in Syria and resistance genes Bt1, Bt2 and Bt7 in Lebanon (25 and 29). In Turkey pathogen isolates has possessed virulence against the resistance genes Bt1, Bt2, Bt3, Bt4 and Bt7, while the Iranian isolates showed virulence against resistance genes Bt4, Bt7 and BtP (14, 17, 36). It was found that virulence against the resistance genes Bt2, Bt5, Bt7, Bt8, Bt9 and Bt10 were prevalent in pathogen communities in India (12). The resistant genes Bt9 and Bt10 showed high efficiency in resistance of pathogen races in Europe followed by genes Bt5, Bt6 and Bt8 (11, 16, 30 and 37). Breeding for common bunt disease resistance in the USA depend mainly on the genotype PI 178383 of Turkish origin which possesses resistance genes Bt8, Bt9 programs, Bt10 and one of undefined resistance genes. These genes also adopted in breeding programs for disease resistance in both former Russia and Australia (18).

## CONCLUSIONS

The current study results refer to changes in resistance categories of some commercial wheat cultivars due to appearance of new pathogen races, While wheat Cvs Farris 1, Ashoor, Hasad, Hamada, Charmo 2, Cimmeto and Acsad are characterized by their resistance to the disease. It can be also concluded that the high levels of resistance in the international resistant sources Casan, Frisal, Tommi and Rio and the known resistance genes Bt3, Bt5, Bt6, Bt9, Bt11 and Bt12 turns out the possibility of exploiting them in breeding programs for improving common bunt disease resistance in Iraq. In conclusion, there are urgent needs to use all the technological possibilities to control bunt disease resistance and improve grain quality of wheat.

1. BW= Bread Wheat, DW= Durum Wheat,
2. Each number is representing the mean of three replicates,
3. R= Resistant (Infection percent 0-10%),  
I= Intermediate resistant (Infection Percent 11-30%),  
S= Susceptible (Infection Percent 31-50%),

HS= Highly Susceptible (Infection Percent 51-100%) according to Dodoff and Todorova, 1974 modified method.

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## INSECTICIDES RESIDUAL HAZARD TO BEES

### SUMMARY

The objective of the study was to determine the insecticides residual effect duration to honey bee, alfalfa leaf-cutting bee and bumble bee. The bees can be ranged by the increase of susceptibility to insecticides as follows: bumble bee, honey bee and alfalfa leaf-cutting bee. The most ecologically safe chemicals to bees were pyrethroids. Their residues on plant surface were non hazardous to bumble bee. Non hazardous to honey bee were the residues of Fastac, Sumicidin, Mavrik; low hazardous - Decis, Ambush, Cymbush, Sumi-alfa and Talstar. To leaf-cutting bee the residues of only Mavrik were non-toxic. Detoxication of other pyrethroids took 0.5 – 5.0 days. More hazardous to pollinators were organophosphorus insecticides. Bazudin, Fosfamid, Dursban applied at minimal application rates were hazardous, at maximal rates – highly hazardous during 6.0 – 7.5 days to honey bee and 8.0 -10.0 days to leaf-cutting bee. To bumble bee the residues of Carbofos and Hostaquick were not hazardous at minimal rates and low hazardous at maximal ones. Zolone and Actellic were not hazardous at all rates. These insecticides were low hazardous to honey bee, but hazardous to leaf-cutting bee at all rates. Karate took the intermediate position to these 2 kinds of bees. Zolone was slightly toxic to honey bee at maximal rate. Of tested neonicotinoid insecticides the residues of Pondus were practically not hazardous to bees, the residues of Confidor were hazardous to honey bee for 2 days and to alfalfa leaf-cutting bee – 4 days, but not hazardous to bumble bee.

**Keywords:** insecticides, residues, hazard, bees.

### INTRODUCTION

The danger of bees poisoning is becoming one of the most important factors of sustainable agricultural system's functioning, particularly for those crops that need cross-pollination by bees (UNEP, 2010; Cane and Tepedino, 2001). The main objective of pesticides use on these crops – the prevention of a negative effect of pest on a yield, contradicts the purposes of entomophilic crop cultivation because pollinating activity of bees primarily determines the potential crop productivity.

Even prohibition of pesticide treatments of entomophilic crops during their bloom can not fully prevent the possibility of pollinators contacts with pesticides,

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because bees usually explore larger spectrum of flowering plants than target pollinated crop (Dobrynin, 1998).

The need to solve this dilemma forces to search for special approaches in chemical plant protection based on the study of ecological factors determining the possibility of intoxication of pollinators by pesticides, on knowledge of toxicity and rate of pesticides hazard to pollinators.

The studies of pesticide effect on pollinators were mostly conducted on one bee species – honey bee, much more seldom on artificially reared solitary bees and some bumble bee species. In most of the works it was pointed out that the effect of chemicals in field conditions was much less than in laboratory ones and one of the main causes of bee poisoning at chemical treatments of plants is a violation of pesticide application regulations (Atkins, 1993).

The probability of appearance, the course and consequences of the process of intoxication greatly depends upon biological features of pollinators and pollinated crops, abiotic environmental features, pesticide properties, methods and objects of their application (Dobrynin, 2013).

Three biological groups of pollinators: wild solitary bees, bumble bees and honey bees differ both in their anatomic-morphological and bio-ecological features, life cycle and the extent of management by man. It became clear that these differences significantly influence the probability of a contact of every pollinator group with pesticides and that a trophic factor plays a leading role since foraging activity of pollinators presumes obligatory contact with pollinated plant which, when treated with pesticide, can perform as a mediator between toxicant and pollinators (Dobrynin, 2000). In turn, foraging activity of pollinators is significantly influenced by abiotic environmental factors (mostly the weather ones). Different combinations of these factors determine the intensity of pollinator flight on entomophilic crops and hence probability of pollinator-pesticide contact in case of chemical treatment of plants (Dobrynin, 2000). The possibility and manifestation of pesticide toxic effect on pollinators also depend on the properties of a pesticide itself. Its composition, target direction, character of action on pest objects, ways of penetration into insect body, time of application and other properties greatly influence the probability of contacts with pollinators and the rise of intoxication process in them (Dobrynin, 2000). On the whole, the analysis of ecological factors influencing pesticide-pollinator relationships showed that application of selective pesticides and activation of ecological mechanisms preventing or reducing the realization of pesticide toxic effect to insect pollinators can serve as a basis of bee safe pesticide application techniques in the system of chemical crop protection (Dobrynin, 2013), where the first rule is avoiding of direct impact of pesticide on bees by treating the plants out of pollinators' day flight time. Once insecticide is applied out of the time of pollinators day activity, the major factor determining the hazard of a chemical to bees is its residual effect duration, the determination of which was the main objective of the present study.

## MATERIAL AND METHODS

The study was conducted on 3 most commercially used species of bee pollinators: honey bee (*Apis mellifera* L.), alfalfa leaf-cutting bee (*Megachile rotundata* F.) and bumble bee (*Bombus terrestris* L.). The degree of insecticides hazard to bees was determined by the length of the time period necessary for reducing of the amount of an active ingredient of a chemical on treated plants to the level non-hazardous to pollinators. It was assumed that an average lethal doze (LD50) to be such level, because when determining LD50 in laboratory conditions the tested insects kept in cages have to be in contact with pesticide treated plants 24 hours a day during the experiment, whereas in field conditions the daily activity of pollinators lasts not more than 10 - 12 hours and a considerable part of this time between flights they spend in their nests, having no contacts with treated plants.

LD50 for three commercially used species of bees (honey bee *Apis mellifera* L., alfalfa leaf-cutting bee *Megachile rotundata* F. and bumble bee *Bombus terrestris* L.) were determined earlier in laboratory conditions by the method of bees contact with insecticide treated surface (Illarionov, Dobrynin, 1995), since the main cause of pollinator intoxication under field conditions is most often a contact toxicity of insecticide residues on visited plants.

Determination of pesticides LD50 is mainly a method of preliminary evaluation and comparison of different chemicals toxic activity and the susceptibility of different objects. It cannot fully characterize toxic parameters of pesticides to pollinators because it provides no information for practice about the duration of the period of insecticide toxic activity in the field. The degree of pesticide hazard under field conditions depends both on composition, rate, time of pesticide application and also on environmental factors influencing conservation of pesticide toxic activity on plants and pollinators behavior.

To predict the rate of pesticide hazard and to prove the waiting period for using the bees on pollination, we studied the duration of the period of detoxication of the most used insecticides applied on plants of alfalfa (*Medicago sativa* L.) in recommended dosages to the level safe to bees under field conditions. Samples of alfalfa plants were taken periodically (every 12 hours) for analysis of insecticides residues until they reach the LD50 level. Experiments were conducted under optimum or close to weather conditions for pollinator flight activity. The amount of each tested insecticide on alfalfa samples was determined using a standard method of gas chromatography.

## RESULTS AND DISCUSSION

The results of the study are presented in the following table 1. The data of the table show that different kinds of bees can be ranged by the increase of susceptibility to insecticides as follows: bumble bee, honey bee and alfalfa leaf-cutting bee.

From the point of view of pollinators protection the most ecologically safe chemicals to bees were pyrethroid compounds. Their residues on plant surface

(when these chemicals applied at the recommended rates) were below levels of susceptibility of bumble bee *B. terrestris*. Non toxic to honey bee were the residues of Fastac, Sumicidin, Mavrik,; and the residues of Decis, Ambush, Cymbush, Sumi-alfa and Talstar with not prolonged (0.5 – 2.5 days) period of toxic activity were low hazardous to the bee.

At the same time, to alfalfa leaf-cutting bee only the application of Mavrik did not leave toxic residues on plants. Detoxication of other tested pyrethroids to the safe level to the bee took 0.5 – 5.0 days after treatment, depending on the rate of application.

More hazardous to pollinators were organophosphorus insecticides. Long residual toxicity of Bazudin, Fosfamid, Dursban applied even at minimal rates is the reason to characterize them as hazardous, and at maximal rates – as highly hazardous with long toxic activity (6.0 – 7.5 days to honey bee and 8.0 -10.0 - to leaf-cutting bee). To bumble bee the residues of Carbofos and Hostaquick were not hazardous at minimal or close to minimal rates of application and also of Zolone at all the dozes.

Low hazardous to bumble bee were only the residues of Carbofos and Hostaquick at maximal dozes, and of Actellic - at all the dozes. These insecticides can also be related as low hazardous to honey bee, but hazardous to leaf-cutting bee at all the rates. Pyrethroid Karate took the intermediate position by its residual action on these two kinds of bees. Slightly toxic action of Zolone on honey bee was observed only at maximal rate of application.

Neonicotinoid insecticides (Pondus and Confidor) differed by their effect on bees, that can probably be explained by the specifics of their structure. While the residues of cyanogen substituted chemical (Pondus) were practically not hazardous to honey and bumble bees and if applied the night before - to alfalfa leaf-cutting bee either; the residues of nitro substituted chemical (Confidor) were hazardous to honey bee during 2 days and to alfalfa leaf-cutting bee – 4 days, but not hazardous to bumble bee. The experiments also showed that upon precipitation more than 5 mm after pesticide treatment or at the air temperature higher than 25 0 C the length of hazardous period for bees was reduced by 1-2 days, and at the temperature below 16 0 C the hazardous period for bees increased by 1 day. The data of the table can help agriculturists to make reasonable decision both for purchase and usage of pesticides, which, on the one hand, could reliably protect plants from pests and, on the other hand, be non- or low hazardous to insect-pollinators.

Moreover, the data of the table can help the utilization of different techniques for differentiation of bees and toxicants in time and space allowing to the maximum extent to avoid contacts with pesticides. These techniques can be accomplished in two principle ways: the first – by changing the place of foraging, and the second – by isolation of bees.

The first way is the most complicated, requiring great labor and resource expenses and is used if it is necessary to separate bees and the plants treated with toxicant for a long period.

Table 1. The duration of insecticide residual hazard to different kinds of pollinators

№ by the or- der	Insecticides, (their active ingredients) concentration and preparative form	Rate of application, l/ha	Period of detoxication (days) of insecticides on plants to safety level for		
			honey bee	alfalfa leaf- cutting bee	bumble bee
1.	Actellic (Pirimiphos-methyl), 50 % EC	0.5 1.0	2.5 3.0	3.5 4.0	0.5 1.5
2.	Ambush (Permethrin), 25% EC	0.3 0.4	1.0 1.5	3.0 3.0	0 0
3.	Bazudin (Diazinon), 60 % EC	1.0 2.0 3.0	4.0 5.0 6.0	6 7.0 8.0	0.5 2.5 2.5
4.	Decis (Deltamethrin), 2.5 % EC	0.1 0.5	0 2.0	2.0 3.5	0 0
5.	Dursban (Chlorpyrifos-methyl), 40.8 % EC	0.8 1.5	5.0 5.5	7.5 8.0	1.5 2.5
6.	Zolone (Phosalone), 35 % EC	0.5 1.0 2.0 3.0	0 0 0 0.5	1.0 1.5 2.0 -	0 0 0 0
7.	Carbofos (Malathion), 50 % EC	0.2 0.5 1.0 2.0	1.0 2.0 2.5 3.0	2.0 3.0 4.0 4.5	0 0 0 0.5
8.	Karate (Lambda-cyhalothrin), 5% EC	0.15 0.2 0.5	2.0 2.5 3.5	2.5 3.0 -	0 0 0
9.	Mavrik (Fluvalinate), 2E, 25% EC	0.1 0.3	0 0	0 0	0 0
10.	Sumi-alfa (Esfenvalerate), 5% EC	0.3 0.5	0 0.5	1.5 2.0	0 0
11.	Sumicidin (Fenvalerate), 20% EC	0.3 0.6	0 0	2.0 3.0	0 0
12.	Talstar (Bifethrin), 10% EC	0.4 0.6	2.0 2.5	4.0 4.5	0 0
13.	Fastac (Alfa-cypermethrine), 10% EC	0.15 0.2	0 0.5	0.5 1.0	0 0
14.	Fosfamid (Dimethoate), 40% EC	0.5 1.0	5.0 6.5	8.0 9.0	3.5 4.5

15.	Hostaquick (Heptenophos), 50% EC	0.3	2.0	3.5	0
		1.0	3.0	4.0	1.0
		1.8	3.5	5.0	2.0
16.	Cymbush (Cypermethrin), 25% EC	0.1	2.5	-	0
		0.24	3.0	5.0	0
17.	Pondus (Thiacloprid), 48% SC	0.18	0	0.5	0
18.	Confidor (Imidacloprid), 20% WSC	0.5	2.0	4.0	0

The second way presumes isolation of bees, the length of which depends on the duration of the pesticide residual action on treated plants.

Practice showed that the most technologically suitable and effective way is the isolation of bees in hives directly in the field for the period of pesticide application and detoxication. From this point of view, the most promising were insecticides and the rates of their application (see the table) which period of hazard did not exceed 2.5 days to leaf-cutting bee – the average longevity of female's life without feeding in isolated conditions in a field (Dobrynin, 1998), and 3 days - for honey bee provided with enough forage and water (Dobrynin, Illarionov, 1996). Bumble bees do not likely need any particular measures of protection from the most of the tested insecticides. So it is possible to choose the insecticide non- hazardous to bumble bees and at the same time having the period of hazard not more than 2.5 days to leaf-cutting and honey bees. As for the protection of other bee species, it is possible to suppose with high rate of probability that if leaf-cutting bee is the most susceptible among the main bee-pollinators, the pesticides non- or low hazardous to the bee, will not be more hazardous to other wild bee species.

### CONCLUSIONS

Different kinds of bees can be ranged by the increase of susceptibility to insecticides as follows: bumble bee, honey bee and alfalfa leaf-cutting bee. The most ecologically safe chemicals were pyrethroids. More hazardous to pollinators tested were the organophosphorus insecticides. Neonicotinoid insecticides differed by their effect on bees: Pondus was practically not hazardous to bees, whereas Confidor was hazardous to honey and alfalfa leaf-cutting bee, but not hazardous to bumble bee. The data obtained can help agriculturists to make reasonable decision both for purchase and usage of pesticides, which, on the one hand, could reliably protect plants from pests and, on the other hand, be non- or low hazardous to insect-pollinators.

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## SOIL TYPES IN MANAGEMENT UNIT RISTOVAČA IN PUBLIC ENTERPRISE VOJVODINAŠUME (SERBIA)

### SUMMARY

This paper analyses the possibility of soil mapping perspectives and recent management options on local level and future perspectives. The dominant soil type in management unit Ristovaca was meadow black soil. The total area of this soil type was 268.82 ha. Meadow black soils are a subtype of chernozem. This type of soils situated in highest position with oscillation of ground water from 1 to 2 meter. Meadow black soils in the riparian zones of rivers are very similar to alluvial semigleys. The main soil processes represented there are humification and humization. This type of soil is clayly loams texture with a thick humus-accumulative horizon. The CaCO<sub>3</sub> concentration increases most frequently with the depth. The most common tree species is pedunculate oak. The second dominant soil type in management unit Ristovaca is salic chernozem. The most important species on meadow black soil in management unit are pedunculate oak on the area of 176.62 ha with volume of 56,291.4 m<sup>3</sup>. The highest volumes are determined in age of 81 -100 years (53.298 m<sup>3</sup>). The most endangered tree species in Serbia regarding to climate change scenarios are pedunculate oak.

**Keywords:** soil types, soil mapping, management unit

### INTRODUCTION

Digital soil mapping involves research and operational applications to infer on patterns of soils across various spatial and temporal scales (Grunwald, 2010). In forestry, it is necessary to know distribution of soil and tree species, as well as their interaction in space. The study area is situated in defended part of alluvial plain of Danube near Plavna. Soils in this area are unfavorable for agricultural production due to one or more properties. This is the reason for using it for forestry. Similar areas are located along the defended part of the alluvial plain of Danube (Simić, 1987; Galić, 2011). At this site, limiting soil properties are affecting the potential production of this type of soils. It is essential to determine the distribution of each soil unit in the studied area.

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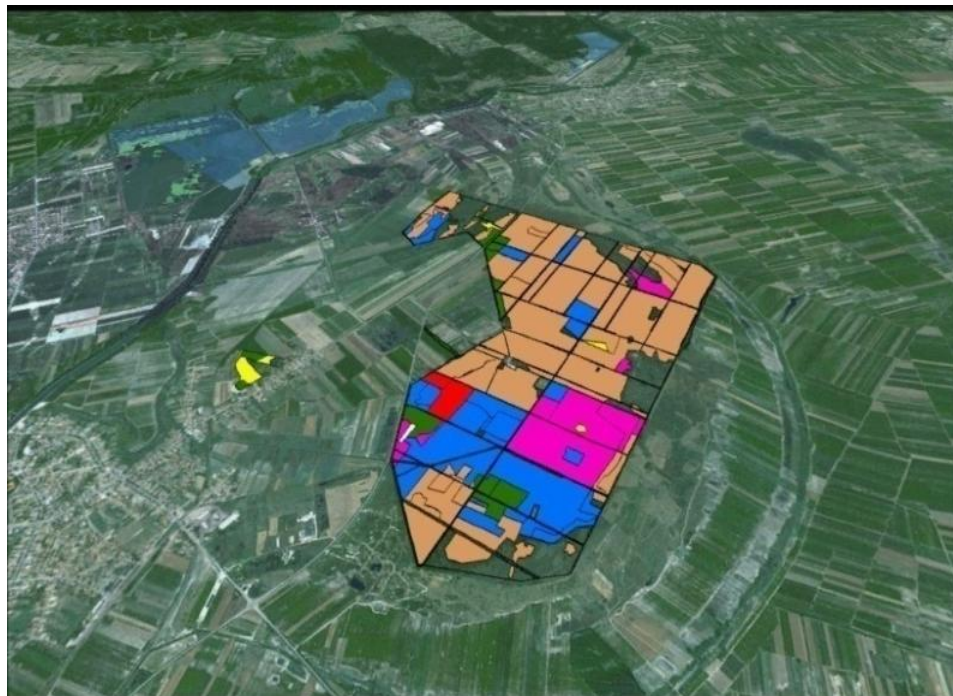
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The second goal of the research is to determine the suitability of soil unit for different tree species growing. For this purpose, analyzed were the productivity of different tree species, their interaction in space and indicators of tree species productivity on different soil unit are shown.

The aim of the study was to analyze the production characteristics depending on the soil types in management unit Ristovača.

### MATERIAL AND METHODS

The study area is situated in defended part of alluvial plain of Danube near Plavna.



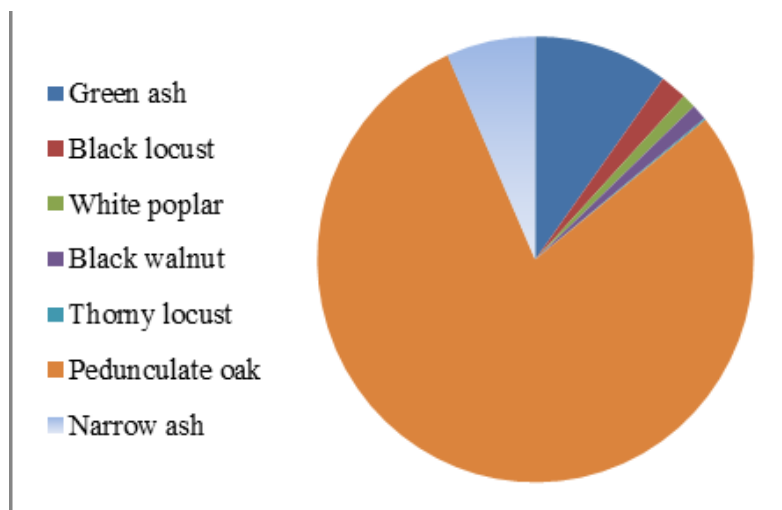
Depending on the micro-relief conditions, soil profiles were opened for the study of soil morphology and soil samples were taken for the laboratory analysis. Particle size composition (%) was determined by the international B-pipette method with the preparation in sodium pyrophosphate (Bosnjak et al., 1997). Soil particle classification in the particle size composition was based on Atterberg's classification.

Chemical characteristics were determined by the following methods (Hadžić et al., 2004); humus (%) by Turin's method, modification by Simakov 1957;  $\text{CaCO}_3$  (%) volumetric method, by Scheibler unit calcimeter; pH in  $\text{H}_2\text{O}$  electrometric method with combined electrode on Radiometer pH meter. Production characteristics are based on a data for forest management unit Ristovača (Forest Management plan for period 2008-2017).

Spatial analysis of soil and species distribution was analyzed using the software package ArcMap 10.1.

## RESULTS AND DISCUSSION

The total volume in management unit Ristovaca was 106.605 m<sup>3</sup> (graph 1).

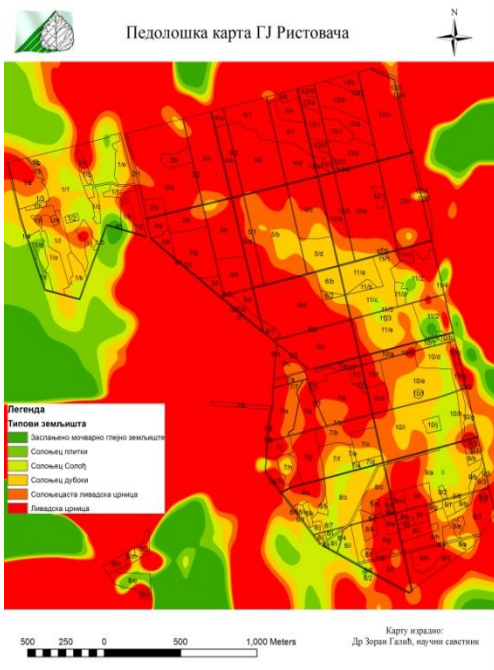


Graph 1. Total volume in management unit Ristovaca

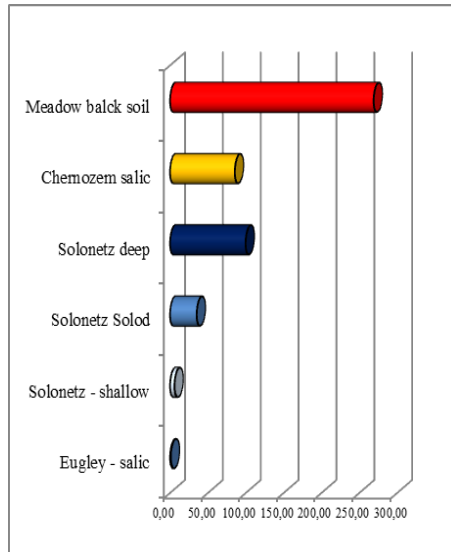
The dominant tree species was oak with a share in the total volume of 79.19 % or 84,424 m<sup>3</sup> (graph 1.). Those characteristics confirm the fact that the forests in the protected part of the alluvial plain of Danube incurred as a result of the needs of the rural population of firewood (Simić, 1987; Galić, 2011). Participation of green ash in the total (*Fraxinus americana*) was 9.95 %, while the share of narrow ash was 6.58 %. Other species (black locust, black walnut, white poplar and thorny locust) have a share in the total of 4.28% or 4,561 m<sup>3</sup>. In the above analysis it is observed a high share of green ash (invasive species) in the total volume.

The most common soil unit in management unit Ristovaca was meadow black soil (picture 1, graph 2). This soil unit covered 268.82 ha or 54.23 %. The second most common soil type was solonetz. This soil unit covered nearly 20.5% area of management unit Ristovaca. Chernozem-salic covered 85.42 ha or 17.23 % of this area. The most unfavorable soil units (solonetz - shallow and deugley - salic) covered only 1.35% or 6.68 ha.

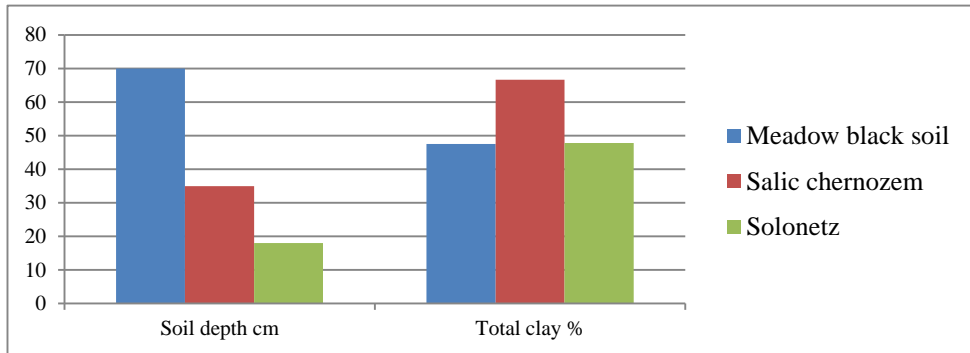
Meadow black soils are a subtype of chernozem. This type of soils situated in highest position with oscillation of ground water from 1 to 2 meter. Meadow black soils in the riparian zones of rivers are very similar to alluvial semigleys. The main soil processes are humification and humization. This type of soil is clayly loams texture with a thick humus-accumulative horizon. The CaCO<sub>3</sub> concentration increase the most frequently with the depth.



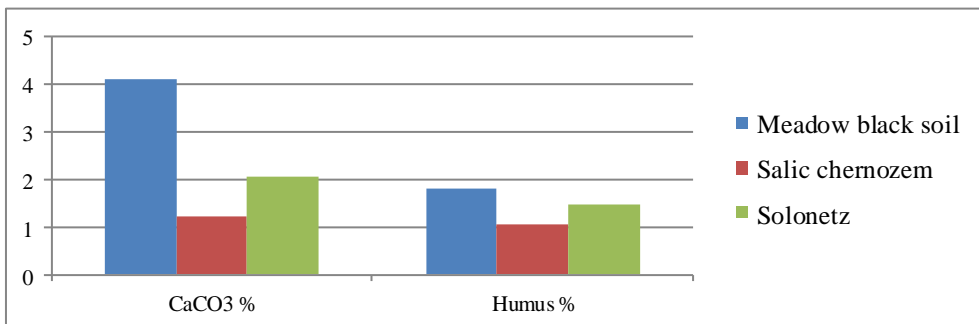
The second dominant soil type in management unit Ristovaca is salic chernozem.



Graph 2. Soil Characteristics

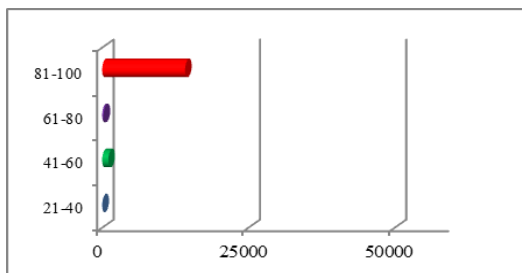


Graph 3. Soil depth and total clay contents

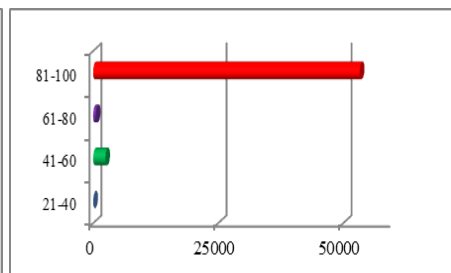


Graph 4. Soil CaCO3 and humus contents

The most important species on meadow black soil in management unit is pedunculate oak on the area of 176.65 ha with total volume of 56,291.4 m<sup>3</sup> (graph 4). The highest total volume are determined in age of 81 -100 (53,298 m<sup>3</sup>) on meadow black soil and chernozem salic (graph 5 and 6).



Graph 4. Total volume on chernozem - salic



Graph 5. Total volume on meadow black soil

## CONCLUSIONS

The dominant soil type in management unit Ristovaca was meadow black soil. The total area of this soil type was 268.82 ha.

The most important species on meadow black soil in management unit are pedunculate oak on the area of 176.62 ha with volume of 56.291,4 m<sup>3</sup>.

The highest volume are determined in age of 81 -100 (53.298 m<sup>3</sup>).

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2008-2017: Forest Management Plan for Management Unit Ristovača



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## USING E-EXTENSION SYSTEM FOR RURAL TOURISM DEVELOPMENT

### SUMMARY

Article is devoted to perspectives of use of electronic e-Extension system for the development of rural tourism in Ukraine. The concepts of rural tourism and problems of its formation in Ukraine are examined. There are different kinds of rural tourism representative in Ukraine such as sustainable tourism, agrotourism, ecological tourism ecoagrotourism, apitourism etc. The importance of advisory service with its e-Extension as a driving force for its development is determined. There were used methods: abstract, logical, system analysis, dynamic series, grouping, graphical, target-oriented. E-Extension as a thematic agrarian website of Ukraine concentrates much diverse agricultural information by Communities of specialists. The possibility (efficiency) of the use of e-Extension system and Community of specialists created on its base for consulting support for rural tourism in Ukraine is proven. E-Extension is a new tool for agricultural producers and to other people who are interesting in knowledge and information.

**Keywords:** e-Extension system, rural tourism, agriculture, development, consulting, market, interactive information system, environment.

### INTRODUCTION

Rural tourism development needs to pay attention to extremely important advisory service which was organized in Ukraine by the Law «Developing Agricultural Advisory Activities», adopted in 2004.

The green rural tourism is a specific form of recreation in private households in rural areas. The green rural tourism involves the use of demesne and human resources, holding and farming, natural recreational and cultural features of places, historical and ethnographic heritage of the region. In world nowadays the concept of three «S» («Sun-Sea-Sand») are changed by the concept three «L» («Landscape-Lore-Leisure») - landscapes, traditions and entertainment. The travels to rural areas are becoming more popular and where tourists are received exotic: agricultural and rural landscapes, traditional rural and peasant lifestyle, livestock, organic products and more. Today tourism

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industry in Ukraine is not a major part of budget revenues. But its share should be increased in accordance with such objective conditions: favorable geopolitical location of Ukraine in the center of Europe, the presence of significant tourist and recreational potential, favorable climate, rich flora and fauna, numerical cultural and historical monuments of world value. These kinds of rural green tourism are developing in Ukraine now: sustainable tourism, agrotourism, ecological tourism (green tourism, natural tourism) ecoagrotourism, apitourism etc. (Figure 1.) (Kalna-Dubinyuk T., 2008; Malska M., Hudo V., 2012; Byrkovych V., 2008).

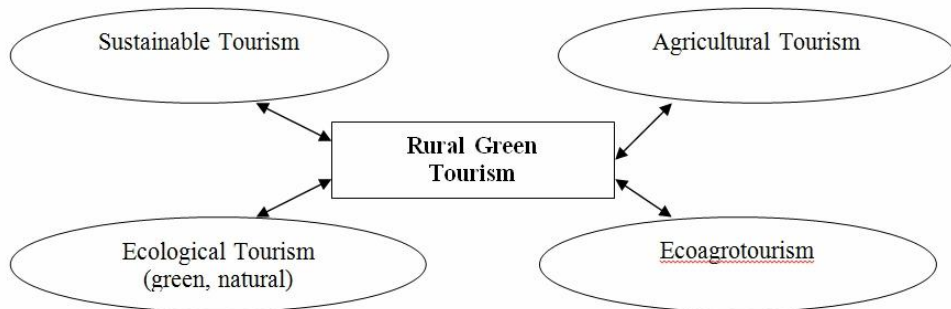


Figure 1. Kinds of rural green tourism in Ukraine

**Sustainable tourism** is a modern concept of tourism development that based on the global sustainable development principles. Sustainable development is a process for meeting human development goals while sustaining the ability of natural systems to continue to provide the natural resources and ecosystem services upon which the economy and society depend. Such tourism provides all human needs today and guarantees the similar opportunities of future generations.

Sustainable tourism is based on the following principles:

- 1) Environmental protection, minimizing ecological damage in the tourism, environmental surveillance tourism development activities;
- 2) The controlled using of technology tourist services, especially transport, energy, drinking water etc.
- 3) Social justice for local communities, the income and other benefits from tourism must be distributed on the local population interests;
- 4) Aesthetic harmony of natural resources using in tourism, the tourism activity and infrastructure must harmonically integrate into the unique history of each location.

The specific features of agricultural tourism region are: clean environment; low level of urbanization and industrialization, low population density, limited intensity of agricultural and forest products;

favorable agricultural structure; harmonious agricultural landscape; low income people; free home resources.

The rural tourism is often identified with the agricultural tourism but agrotourism is a form of rural green tourism.

**Agricultural tourism (agrotourism)** is a recreation type of tourism and concentrated in rural areas. Agrotourism uses farming for recreation, education or some activities in traditional forms of home keeping.

The rural green tourism is close in meaning to the ecological tourism. They often have the same kind of leisure.

**Ecological tourism** (ecotourism) is a cognitive and recreation type of tourism that focuses on nature (little modified by human) territories. Ecotourism includes classes in different forms of active recreation in natural landscapes without causing environmental damage. There are different forms of ecotourism as active (hiking, cycling, water, horse, gathering, fishing, hunting); faunal and floral tours (ornithological trips, photography, themed trips); cultural and ethnographic trip.

The difference features between traditional and ecological tourism are:

- 1) Natural advantage, not cultural tourism destinations;
- 2) Sustainable natural resources using;
- 3) Slight using of resource and energy;
- 4) Personal participation in socio-economic development of territory;
- 5) Environmental education of tourists.

Today the ecotourism is most dynamically developing in areas with natural value (national and landscape parks). Ecotourism is aimed at protecting the natural and cultural environment of the region which are visited by tourists. Ecotourism predicts the high environmental consciousness of participants in these trips.

**Ecoagrotourism** involves the use of guest houses without their owners or houses that located within or on the sides of biosphere reserves and national parks. Such proposes the ecological agriculture and a wide range of environmental and sport tourist classes (hunting, fishing, etc.).

Ukraine is rich in various natural tourism resources: climatic, biological, hydrological and landscape resources, mineral waters, mud and others. It is an important prerequisite for the development of green rural tourism. The total suitable land area for recreation and tourism using occupies 9.4 million ha (15.6 % of the total territory of Ukraine).

The important natural tourism resources of country are forests. The total area of Ukrainian forest fond of is 10 million ha (15 % of the

country). The mineral water sources are in all regions of Ukraine. Large reserves of therapeutic muds are concentrated in the south of Ukraine.

The various environmental objects locate throughout Ukraine: national and landscape parks, natural monuments and more. There are many rivers, lakes, some cliffs, canyons and the territory with wonderful views.

Using advisory service with its e-Extension gives wide possibilities for development of rural tourism in Ukraine and Worldwide.

E-Extension is developing on a base of the National University of Life and Environmental Sciences of Ukraine. It shows success when multiple actors such as government agencies, universities, nongovernmental organizations, international agencies and donors, as well as producers, workers, consumers, and private business work together. Associations (communities) of specialists by activity are established for this, and rural green tourism is one of them. E-Extension promotes coordination and consistency of green tourism's hosts efforts and possible actions in their local level to help navigate people to have a good rest in the rural area.

## **MATERIAL AND METHODS**

### **Survey and fungal isolation**

Theoretical and methodological basis of the study is the dialectical method of understanding of economic and consulting processes in their interconnection and development, fundamental scientific works and elaborations of domestic and foreign economists on problems of extension and rural tourism.

To realize the objectives of the research were used the following methods: abstract, logical, system analysis – to summarize theoretical and methodological provisions defining the essence of economic concepts and categories, substantiation of conclusions; dynamic series, grouping, graphical, – to study the current state and trends in rural tourism and extension; target-oriented – to develop of practical recommendations on ways to improve using of electronic extension.

The world practice of organization of information and consultation providing for agricultural producers shows the effectiveness of such activity, the need for its development in market conditions. Advisory services must be certified according to international standards (Kalna-Dubinyuk T., 2009).

However, the question of application of information and consultation methods and technologies in the extension needs further improvement to their innovative providing. An important problem of the national consulting system in Ukraine is a need for prompt and efficient access of the general public to various sources of information and services which is possible in conditions of preferential use of information systems based on Internet for collecting, recording, processing, distribution (cataloging) of information and consulting. Its solution is possible on a base of interactive information systems. Today there is a

need for practical implementation of electronic system of extension service in Ukraine. As part of an integrated information environment, it is possible a transition from simple informing to providing quality services to users based on technologies that can be implemented, particularly in the form of Web services and provide mobile access to information resources.

The main goal of the development of information technologies for agricultural consulting based on knowledge is the creation of modern information technology for support of processes of consultation and decision-making for agricultural extension services. The current paradigm of information technology in agriculture - is support on various agricultural issues at any time and in any place, by any means and in any applied agrarian sphere (Chaplinskiy U., 2003).

Hence the importance of e-Extension will be effective for the development of green tourism in Ukraine. Particular attention in this belongs to the development of interactive information and consultation support for the organization and development rural houses and its service.

Developed interactive consulting system for green tourism indicates its great advantages on quality and fast recommendations.

Consulting algorithms for interactive consulting systems provides dialogue between users and computer step by step; as a result, the display shows the necessary information for the user. In this case the following essential requirements for the formation of interactive solutions are provided: simplicity and ease of use; availability of consistency and continuity; completeness of forming solution, that is in a set of software functions should not be significant omissions; resistance to user errors; productivity and efficiency; economy, that is software should not be expensive (Rutynskyj M., Zinko Y., 2008).

Interactive e-Extension system and created on its base associations (communities) of specialists by activities where the Green Tourism is one of them should provide: accurate operative information on socio-economic development of agricultural production, agricultural market and ecology of rural areas; generating information and knowledge based on deeper integration of intellectual and information resources of agricultural research institutions, educational institutions, advisory services, and other members of the agrarian sector of Ukraine; tools and means for spreading of knowledge; participation of skilled consultants and expert- consultants; individual consultation; effective information search and comprehensive answers to requests; conditions for free access to information and knowledge at any time and in any place

## **RESULTS AND DISCUSSION**

For the priority development of green rural tourism in Ukraine and bringing to EU standards is considered appropriate:

- 1) Creation of the Law "About Green Rural Tourism" and the regulation acts according to the standardization of its activities;
- 2) Development of national and regional programs of green rural tourism;
- 3) Monitoring of developing and implementing these programs;

- 4) To complete the reform of the state statistics system for green rural tourism;
- 5) To create an infrastructure of state financial support of rural green tourism;
- 6) To organize the study market needs services of green tourism specialists;
- 7) To create the educational system for preparing specialist and retraining courses for the tourism sphere;
- 8) To create the Community of Green Tourism for information and analytical support of green rural tourism as part of the e-Extension information system. Users of the system are all agricultural producers and the public, the government of Ukraine, International Association for the extension and others.

Scientists and specialists of the National University of Life and Environmental Sciences of Ukraine develop e-Extension system ([www.edorada.org](http://www.edorada.org)) as a universal resource to meet the information needs of the agricultural areas. By this time e-Extension as a thematic agrarian website of Ukraine concentrate much diverse agricultural information by Communities of specialists. The portal is developing. It will contains a large amount of information by different communities such as on livestock, of Agricultural, Education and Science, Economics materials from agriculture, its legal security, fundamentals of business, analytical and statistical information and more. A growing number of users show the great interest in the existence of such information resource and the need for its development. Innovations in this regard will be to develop an interactive system for consulting assistance advisers and their clients on the site

## CONCLUSIONS

One of the most characteristic features of the present stage of our society is the growing influence of information and consulting technology to support innovative development of agricultural production, improving competitiveness of agricultural enterprises.

Objective need for innovation extension service requires a new interactive approach to spreading of knowledge and information in Ukraine through the creation of electronic educational and scientific advisory system for information support of agricultural producers, population and rural development.

The development of information and consultation technologies gives us a possibility to make decision in short terms at any time and at any place to increase sustainability in the agricultural sector. Electronic extension (e-Extension) service with interactive consulting system plays a main role in this. E-Extension is a new tool for agricultural producers and to other people who are interesting in knowledge and information. Today the rural tourism became popular in Ukraine as well as throughout the world. Different forms and service of this tourism needs to be improved through the use of best practices.

So the use of e-Extension as information and consulting system to the development of innovative consulting in rural green tourism will facilitate the development of green tourism in Ukraine and increase its competitiveness in the international tourism market.

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## **INFLUENCE OF SOME APPLE ROOTSTOCKS GRAFTED BY GOLDEN DELICIOUS ON N, P, AND K MINERAL UPTAKE UNDER RAINFED CONDITIONS**

### **SUMMARY**

The study was carried out at the agricultural scientific research center in Sweida- GCSAR, to evaluate the influence of apple rootstocks on N, P and K minerals uptake in the vegetative parts during growth season. Two apple rootstocks; vegetative rootstock MM106 and seedling rootstock *Malus domestica* Borkh. Which grafted by Golden delicious cultivar were studied. The results showed that MM106 rootstock revealed highest significant accumulation of Nitrogen in shoots in May (1.69%), while the seedling rootstock revealed the highest accumulation of nitrogen in leaves in May (2.81%). The highest fruit concentration of N was in June for the two rootstocks. Likewise, phosphorous content in shoots was the highest in June for the two rootstock (0.214% and 0.213% for MM106 and seedling rootstock, respectively), and the Phosphorous concentration of leaves was the highest in May on the seedling rootstock than MM106 rootstock (0.23 and 0.22%, respectively), fruits significantly revealed the highest P content in August for the two rootstocks which was 0.15% in seedling rootstock and 0.18% in MM106. The K content was the highest for shoots and leaves in May for the two rootstocks, and seedling rootstock revealed higher concentration than MM106. Likewise, fruits significantly revealed the highest K content in August in trees grafted on seedling rootstock (1.15%). Consequently, this investigation showed different responses of rootstocks for uptake and accumulation of N, P and K mineral elements during growth season which lead to establish the correct strategy for fertilization management according to the planted rootstock under rainfed conditions.

**Keywords:** MM106, seedling rootstock, apple, N, P and K.

### **INTRODUCTION**

Apple rootstocks show different responses to mineral uptake, which considered as the main factor affecting tree growth. Kennedy et al. (1980) reported that leaf mineral concentration was more influenced by environmental factors than by rootstock genotype. On the other hand, rootstocks effect flower

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development, yield and fruit quality of apple cultivars (Gao et al., 1992; Fallahi et al., 1994; Hirst and Ferree 1995). The influence of apple rootstock on tree size reflects the effect of rootstock on nutrient requirements (Fallahi, 2002). MM106 which classified as semi vigorous vegetative rootstock revealed higher content of K than M9 dwarf rootstock (Lockard, 1976). Fallahi et al.(2002) stated that the M9 rootstock revealed the lowest concentration of k in the comparison with M7 and M26. Cong et al.(2014) evaluated the efficiency of various apple (*Malus domestica* Borkh) rootstocks in their K uptake , they found that *M. sieversii* and *M. rockii* are K-inefficient genotypes; *M. prunifolia* is K-efficient genotype; *M. hupehensis* and *M. robusta* have moderate levels of potassium efficiency. Sotiropoulos (2008) studied the influence of five rootstocks grafted with Imperial Double Red Delicious on leaf mineral content, he found that the concentration of N and K in leaves were significantly lower in M7 and MM106 in comparison with seedling rootstock, while MM106 revealed the highest concentration of P. Likewise, Amiri et al. (2014) found that Golden delicious which grafted on seedling rootstock revealed high efficiency in K uptake in comparison with vegetative rootstocks, and showed the highest accumulation of N content on M9 rootstock, While revealed high efficiency in P uptake on MM106 rootstock.

Apple tree considered as the main fruit tree in Syria, and most of apple cultures are rainfed and depends on seedling rootstocks, in addition to start new planting of the semi vigorous rootstock MM106.Thus, the current study aimed to evaluate the response of the two rootstocks to uptake mineral nutrients under local conditions and to establish a guideline to estimate tree requirements of (N, P and K) nutrients which lead to an effective fertilization management.

## MATERIAL AND METHODS

The present investigation was carried out at the Pome and grapevine division –GCSAR in Sweida province, which is located 1525m altitude at the south of Syria. The soil is clay with low content of organic material and nitrogen, high content of phosphorous and moderate content of potassium, pH 6.5 to 6.8, the rainfall 525mm.

### Plant material

-Seedling rootstock *Malus domestica* Borkh: Vigorous rootstock with deep roots has good tolerance to frost and drought conditions can grow in different soil types.

-MM106 rootstock: semi vigorous vegetative rootstock, derived from the hybridization between M1 and Northern spy, resistant to wooly apple aphid, has moderate tolerance for frost and drought.

-Golden delicious cultivar: Produced by seed selection in America, vigorous cultivar, fruits are globose- conical in shape with good flavor, maturity time during October under the conditions of target area.

### Soil analysis

It was achieved two times, the first analysis was at autumn before adding the nutritional elements and the second one was achieved after adding the

nutritional elements and organic material at the beginning of growth season to determine the soil content of organic material, N, P, and K according to (Jackson, 1962) N, P and K contents in the vegetative parts (Shoots, leaves and fruits) 15 years old apple trees Golden delicious cultivar which grafted on seedling and MM106 rootstocks were studied under rainfed conditions monthly during growth season:

-Shoots and leaves: 5 shoots and 30 leaves from each tree in each replicate were collected, washed with distilled water, dried on 68°C, and powdery grinded for chemical analysis.

-Fruits: 5 fruits from each tree in each replicate were collected monthly from June until October, Washed with distilled water and dried, then prepared as slices and dried on 68°C until weight stability and powdery grinded for chemical analysis.

-Nitrogen content: Kjeldahl method was used to determine the nitrogen content by humid digestion according to (Van Schouwenberg and Walinge, 1973).

-Phosphorous content: Total phosphorous was measured by colorimetric method after humid digestion. (Jones et al., 2001)

-Potassium content: Samples were prepared by humid digestion, the potassium content was determined using flame photometer apparatus (Benton, 2001).

### **Statistical analysis**

Completely randomized block design to compare the two rootstocks. The analysis of variance was done using two way ANOVA to compare means of measured parameters by LSD test ( $p < 0.05$ ).

## **RESULTS AND DISCUSSION**

### **Soil analysis**

Table 1, showed that the organic material was raised in the spring but still low than the normal content which was less than 2%, (Nelson and Sommers, 1982) stated that the poor soils contain less than 2% organic material, moderate soils contain 2-3%, while rich soils contain more than 3%. Phosphorous was in high concentration for the two treatments in the two times, depending on Olsen et al. (1954) the soil content of phosphorous is very high if reached more than 20 ppm. Potassium content was very high in MM106 rootstock treatment, while it was moderate in seedling rootstock treatment. Thomas (1982) stated that the soil content of potassium is moderate when it was 240-320 ppm, high from 320 to 400 ppm and very high (more than 400 ppm).

N, P and K contents in the vegetative parts mineral analysis based on whole tree is an effective method to determine tree nutrient requirements (Weinbaum et al. 2001). In general, vegetative parts content of N, P, and K decreased along the apple tree vegetative cycle (Nachtigall and Dechen, 2006).

Table 1. Soil content of organic material, Nitrogen (N), Phosphorous (P) and Potassium (K)

Time	Treatment	Organic material%	(ppm) N	(ppm) P	(ppm) K
Autumn	Seedling rootstock	0.96	240	60	118
Autumn	MM106 rootstock	0.92	230	59.7	245
Spring	Seedling rootstock	1.508	372	88.5	260
Spring	MM106 rootstock	1.856	464	74	500

### Nitrogen (N) Content

The results showed that the shoots of Golden delicious cultivar grafted on MM106 significantly has higher accumulation of N than the seedling rootstock, the highest significant concentration of N in the two rootstocks was in May, then continuously decreased until July to arise again in August, after that the seedling rootstock showed stability of N content until October to arise again in November, while MM 106 rootstock revealed continuously decreasing of N accumulation until October, to arise again in November with high accumulation than the seedling rootstock. The high accumulation of N in shoots during November may due to the mobility of N contents from leaves to the shoots. Leaves, also, revealed the highest significant accumulation of N in May for the two rootstocks, and the seedling rootstock significantly revealed high accumulation than MM106 (2.81% and 2.71%, respectively), the two rootstocks showed different behaviors of N accumulation during the growth season (Table 2). N content continuously decreased in seedling rootstock until September to arise again in October, while MM106 showed an increasing of N accumulation in August in significant variance with seedling rootstock (2.21% and 2.07%, respectively) then decrease in September to arise again in October with no significant variation between the two rootstocks. Kucukyumuk and Erdal. (2011) stated that MM106 rootstock revealed the highest accumulation of N in leaves during July, then decreased in August. Fruits also, significantly revealed the highest concentration of N in June for the two rootstocks, seedling rootstock showed higher content of N than MM106 with no significant variance (0.76% and 0.74%, respectively). The two rootstocks showed the same track by continuously decreased until August, to arise in September, then decreased again in October with no significant variance between the two rootstocks. Thomas and Drake (1997) showed that fruit N contents was negatively related to N content in leaves. Likewise, Casero et al., (2005) stated that there is negative correlation between leaf N content and fruit N content at harvesting time.

### **Phosphorous (P) Content**

P requirements in apple trees are small relative to other nutrients (Jackson, 2003). P is the factor in energy transfer and is a constituent of nucleic acids (Salisbury and Ross, 1992), and always required at stages of meristematic activity (Nielsen and Nielsen, 2003). The highest significant accumulation of P in shoots of Golden delicious cultivar grafted on MM106 rootstock was in May and June, while the seedling rootstock revealed the highest significant accumulation of P in June in the comparison with the other growth months. MM106 revealed higher accumulation of P than the Seedling rootstock except in October and November with significant variance. Somehow, the rate of P uptake by the two rootstocks was variable during the growth season; In seedling rootstock, the P content was continuously decreased until August which significantly revealed the lowest content (0.13%), to arise in September and October to decrease again in November, in MM 106 rootstock, the P content still decreased until July, then arise in August and September to decrease again in October and November which showed low concentration than seedling rootstock with significant variance in November (Table 2). In leaves, Seedling rootstock showed High significant concentration of P in May than MM106, also, the two rootstocks significantly revealed the highest concentration of P in May in the comparison with other growth months, then continuously decreased until July, which was in agreement with Nachtigall and Dechen (2006). P content increased again in August for MM106, and then decreased in September and October, while the seedling rootstock continued decreasing of P content until September to arise again in October. Sotiropoulos (2008) found that the concentration of P in apple leaves during July was higher in MM106 than seedling rootstocks which was disagree with our results. Fruits revealed the highest P content in August for the two rootstocks which was 0.15% in seedling rootstock and 0.18% in MM106 with significant variance. The two rootstocks showed the same behavior for P uptake during all growth months and the seedling rootstock revealed high accumulation of P in October than MM106 (0.07 and 0.06, respectively). Nachtigall and Dechen, (2006) obtained significant positive linear regressions in Golden Delicious' for total P in the fruits, and the total P nutrient quantities removed by fresh fruits at harvest time of Golden Delicious cultivar was 0.005% which was in agreement with our results.

### **Potassium (K) content**

Potassium is the most abundant cation in the cytoplasm and plays an important role in pH stabilization, osmoregulation, enzyme activation, protein synthesis, stomatal movement, photosynthesis and cell extension (Faust, 1989). Potassium deficiency is harmful to the physiological functions as well as fruit development (Stassen et al., 1999). As in N and P the results showed that the two rootstocks revealed the highest significant accumulation of K in shoots during May in the comparison with all other growth months, while there was no significant variation between the two rootstocks. In the seedling rootstock the amount of K continuously decreased until September to arise again

until November which revealed higher accumulation than MM106 (0.58% and 0.56%, respectively). MM 106 revealed the same track as seedling rootstock until July to arise in August then decreased in September to arise again in October and decreased in November. Stassen and Stadler (1982) indicated two stages of K uptake, the first one three weeks after bud break and the second at leaves drop. Leaves, also showed the highest amount of K in May for the two rootstocks, and seedling rootstock significantly revealed high accumulation of K during the vegetative growth cycle in the comparison with MM106.

**Table 2.** N, P and K contents in the vegetative parts of Golden Delicious trees grafted on seedling and MM106 rootstocks

Month	Rootstock	N%			P%			K%		
		shoots	leaves	fruits	shoots	leaves	fruits	shoots	leaves	fruits
May	seedling	1.55b	2.81a	-	0.19ab	0.29a	-	0.91a	1.96a	-
	MM106	1.70 a	2.71 b	-	0.21 a	0.24 b	-	0.86 a	1.64 c	-
June	seedling	0.81 f	2.37a	0.76a	0.21a	0.19c	0.13c	0.53bc	1.94a	0.97 b
	MM106	0.88 e	2.53 c	0.74a	0.21a	0.19c	0.13c	0.55b	1.46d	0.87c
July	seedling	0.68g	2.19 ef	0.31b	0.13e	0.19c	0.10 d	0.44cd	1.88b	0.70 e
	MM106	0.79f	2.14f	0.33b	0.16cd	0.18c	0.10 d	0.35de	1.44d	0.75 d
August	seedling	0.79f	2.07g	0.21f	0.13e	0.15d	0.15 b	0.37de	1.14e	1.15 a
	MM106	1.00d	2.21e	0.23ef	0.18b	0.18c	0.18 a	0.39de	0.86f	0.86c
September	seedling	0.80f	1.66k	0.27c	0.17bc	0.10g	0.11 d	0.32e	0.89f	0.47 g
	MM106	0.95d	1.87i	0.27c	0.18b	0.13e	0.13c	0.32e	0.69 g	0.51f
October	seedling	0.80f	1.91h	0.24de	0.17bc	0.12 f	0.07 e	0.53b	0.87f	0.49 f
	MM106	0.89e	1.93h	0.2cd	0.17bc	0.12f	0.06 e	0.58b	0.68g	0.49 fg
November	seedling	0.84 ef	-	-	0.16cd	-	-	0.56b	-	-
	MM106	1.16c	-	-	0.14de	-	-	0.54b	-	-
LSD5%		0.05	0.05	0.02	0.02	0.01	0.01	0.09	0.05	0.02

The accumulation rate was identical for the two rootstocks; The K concentration still decreased from May until October. Our results were in agreement with Nachtigall and Dechen (2006), they found that the leaf K decreased along the vegetative growth cycle. Mengel and Kirkby (2001) explained that the mineral concentration is depending on the leaf status; young leaf tissues usually present lower water content and higher N, P and K concentrations, meanwhile older tissues are rich in Ca, Mn, Fe and B mainly. Fruits revealed high concentration of K in June for the two rootstocks, which decreased in July to show another stage of K accumulation in August which was 0.86% in MM106 while the seedling rootstock significantly revealed the highest value (1.15%) of all growth months, to decreases in September and arises again in October (Table 2). Likewise, MM106 showed continuously decreasing until October. Potassium was the nutrient present in highest quantities in apple tree fruits and thus, the most removed from the soil (Nachtigall and Dechen, 2006).

Faust (1989) stated that the most of K nutrient is accumulated in fruits, and in the absence of fruits it is stored in the leaves, and after harvest the total tree showed a reduction in the K content due to the fruit removal, which is in agreement with our results.

## CONCLUSION

As a result, Golden delicious cultivar which grafted on seedling rootstock and MM106 significantly revealed high concentration of N, P and K in May for shoots and leaves, and in June for fruits except K in seedling rootstock. Seedling rootstock showed higher concentration of K in leaves and fruits than MM106, while MM106 revealed higher concentration of N and P in shoots. On the other hand, each rootstock revealed another peak for each mineral nutrition especially shoots but in different times, also, the apple tree fruits removed higher quantities of potassium as compared to the other nutrients. That leads to establish the correct strategy for fertilization management according to the planted rootstock and the type of soils and irrigation. Moreover, these results might be useful as standard reference values of different apple tree phenological stages for the leaf analysis interpretation of current laboratory data.

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## SOYBEAN OIL YIELD AS AFFECTED BY THE GROWING LOCALITY IN AGRO-CLIMATIC DIVERGENT YEARS

### SUMMARY

The subject of this study are two-year results of the oil yield of six NS soybean genotypes, 0 and I maturity group (MG) at two growing localities (Rimski Sancevi and Sombor in Serbia). Sombor had higher oil yield than Rimski Sancevi (by 119 kg ha<sup>-1</sup>, i.e. 15.97%). In the locality of Sombor, in 2010, of the oil yields were statistically significantly higher (1.088 kg ha<sup>-1</sup>) compared to 2009 (640 kg ha<sup>-1</sup>), which is higher by 448 kg ha<sup>-1</sup> or 70% of the average oil yield in 2009. The average oil yield, for all tested genotypes at both locations was 805 kg ha<sup>-1</sup>, and ranged from 745 kg ha<sup>-1</sup> (Rimski Sancevi) to 864 kg ha<sup>-1</sup> (Sombor). At both sites significantly higher oil yield was recorded in 2010 compared to 2009. The highest average oil yield at both sites was achieved growing genotype Sava (840 kg ha<sup>-1</sup>). Sava had highest oil yield (887 kg ha<sup>-1</sup>) and Balkan (902 kg ha<sup>-1</sup>) in locality of Sombor. On average for both genotypes and growing localities I MG had higher average oil yield of 29 kg ha<sup>-1</sup> (3.67%) than 0 MG genotypes. Realizing the potential for soybean productivity depends on genetic factors, the cultural practice implemented, meteorological conditions and the growing localities.

**Keywords:** soybean, maturity group - MG, genotype, meteorological conditions, locality, oil yield

### INTRODUCTION

Global importance of soybean (*Glycine max.* (L.) Merr.) is continually growing, with soybean planted areas reaching almost 113 million ha in 2013 (FAO). Areas and yields have had a growing tendency (and hence higher production) in recent years, in our country and abroad. For the production of soybean in addition to high yield, technological quality of grain is also essential (Popovic, 2010; Miladinovic et al., 2008; Popovic et al., 2014; Glamoclija et al., 2015).

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According to these indicators, soybean is the most important industrial plant worldwide, both as a basic source of protein nutrients, and as the most important source of plant oil, Miladinovic et al., 2008. Both protein and oil contents are in part determined by additive gene action, with heritability values ranging from medium to high (Rodrigues et al, 2014). The oil fraction of soybean represents 20% of the seed dry mass and is primarily (95%) used for edible oils. The remaining soybean fractions are used to create a variety of industrial products, such as fatty acids, soaps and biodiesel ([http://www.soyatech.com/soy\\_facts.htm](http://www.soyatech.com/soy_facts.htm)). Soybean oil contents approximately 11, 4, 23, 54, and 8 % palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acid, respectively. The amounts and relative proportions of each fatty acid are important factors, as they affect the flavour, stability, and nutritional value of the oil (Katan et al., 1995). For example, saturated acids have been shown to increase low density lipoprotein (LDL) cholesterol levels as well as the risk for coronary heart disease (Wilson, 2004), and high levels of polyunsaturated fatty acids can cause rancidity and undesirable odours. In particular, oleic acid is less susceptible to oxidation during storage and frying. Therefore, decreasing the levels of saturated (16:0 and 18:0) and polyunsaturated fatty acids (18:2 and 18:3) and increasing the levels of monounsaturated acids (18:1) has been the goal of many studies aimed at improving edible soybean oils (Priolli et al, 2015).

In plant populations, variation in the expression of a quantitative trait is due to both genetic and environmental variability and an interaction between the two. Variation due to genotype by environmental interaction (G x E) that stems from differences in ranking of genotypes among environments reduces heritability and makes it difficult to obtain good estimates of genotypic breeding value. Given that such interactions occur, the plant breeder is faced with decision: which environments should be used for testing and how many are necessary for adequate genotypic evaluation. The two questions are linked because often the number of necessary environments is dependent upon the kind of environments chosen. A related approach to this problem is to study genotype response to environment and in so doing characterize genotypes according to their performance under a given set of environmental conditions (Miladinovic et al, 2008; Popovic et al., 2015).

Environmental variation can be considered as a continuum from predictable to unpredictable (Allard and Bradshaw, 1964). Predictable variation results from those conditions which are controlled in some way (greenhouse, irrigated). Unpredictable variation is usually weather related (Miladinovic et al, 2008; Popovic et al, 2014).

The aim of this study was to determine the productivity soybean oil yield of varieties in the regions of Sombor and Rimski Sancevi-Novi Sad (Serbia), in agro-climatic divergent years.

## MATERIAL AND METHODS

Examination of six genotype productivity soybean oil yield during divergent examination years was on the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, at the Rimski Šančevi and Agriculture Technical Service Sombor, experimental field Toplana; in Serbia, where experiments were performed during 2009 and 2010 on the chernozem meadow soil type. Four soybean varieties from the 0 maturity group (Galina, Valjevka, Becejka and Proteinka) were used as material, as well as two varieties of the I maturity group (Balkan and Sava), which are at the same time the current assortment (Popovic et al., 2012, 2013) in Republic of Serbia, tab.1.

Fields trials were designed as a randomized complete block design (Rimski Sancevi and Sombor) with 3 replications using plots of 10 m<sup>2</sup> (Popovic et al., 2012, 2013). Sowing was carried in the first half of April, with micro-experiments planter on 50 cm row spacing. Microbiological preparation NS Nitragin was applied during sowing. Crop density was 500,000 plants per hectare for the 0 maturity group and 450,000 plants per hectare for the I maturity group. Crops were harvested mechanically on September 2009 and 2010 at localities of Rimski Sancevi and Sombor. Yield was measured after harvest and average samples were taken from each trial replicate to determine oil content in grain. Total oil content in grain (Popovic et al., 2012, 2013) was determined by infrared spectroscopy technique on the apparatus PERTEN DA 7000, NIR/VIS Spectrophotometer, employing non-destructive method.

Table 1. Examined varieties\*

Ordinal No	Variety	Years of recognition	MG	Flower colors	Hilum colors
1.	Galina	2006	0	White	Colorless
2.	Valjevka	2003	0	Violet	Brown
3.	Becejka	2004	0	Violet	Yellow
4.	Proteinka	2001	0	Violet	Brown
5.	Balkan	1994	I	White	Yellow brown
6.	Sava	2004	I	Violet	Yellow

\*The cultivars is developed at the Institute of Field and Vegetable Crops in Novi Sad, Serbia

The data used for the calculation of the oil yield was total grain yield with accounting plot per replication and oil content percentage. Experimental data were analyzed by analytical statistics, using the statistics software package Statistica 12 for Windows. The significance of differences among the mean values of different factors studied in the paper was tested by adapted two-way ANOVA. All evaluations of significance were made on the basis of the LSD test at 5% and 1% significance levels.

## RESULTS AND DISCUSSION

### Weather conditions

The data from Rimski Sancevi (Novi Sad) meteorological station was used for the analysis of weather conditions. The total amount of precipitation for the studied period was 478 mm and ranged from 271 mm (2009) to 684 mm (2010), Fig. 1. During 2009 average air temperature was 18.41°C, which was 0.51°C higher (Popovic et al., 2012) than the average temperature in 2010, Fig. 1.

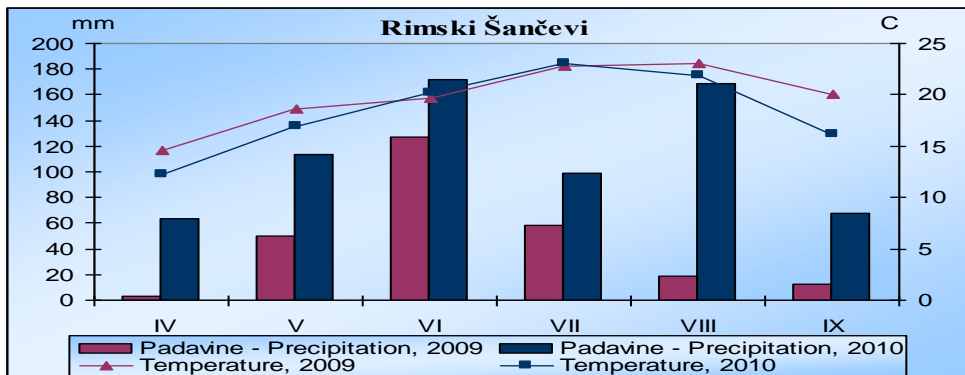


Fig. 1. Average temperature and precipitation, Rimski Sancevi-Novı Sad, Serbia

The data from Sombor meteorological station was used for the analysis of weather conditions. The total amount of precipitation for the studied period was 476 mm and ranged from 258 mm (2009) to 694 mm (2010), Fig. 2. During 2009 average air temperature was 19.45°C, which was 1.4°C higher (Popovic et al., 2013) than the average temperature in 2010, Fig. 2.

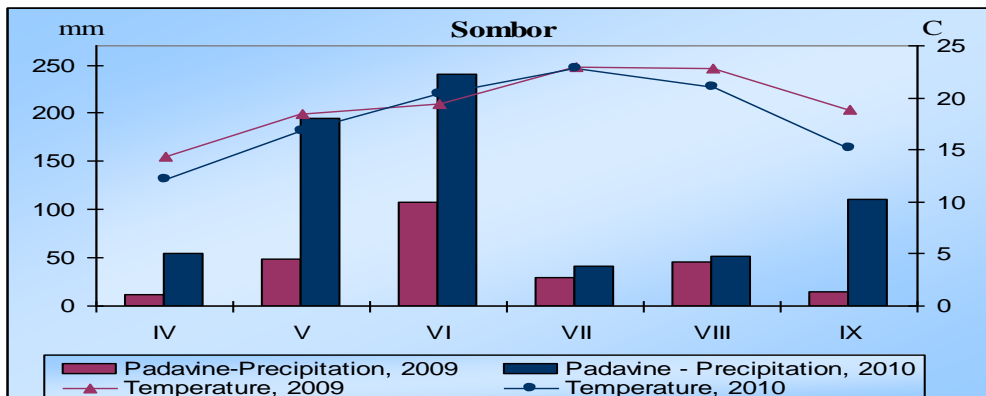


Fig. 2. Average temperature and precipitation, Sombor, Serbia

In contrast to 2009, monthly precipitation distribution during the humid 2010 was more favorable and it reflected on soybean plants up growth and contributed to the achievement of higher oil yields.

### Oil yield in soybean grain

Year and genotype had a statistically significant effect on the oil yield ( $p < 0.01$ ) at locality Rimski Sancevi. The oil yield average of all genotypes of soybean in 2009-2010, amounted to  $745 \text{ kg ha}^{-1}$ . The highest average of oil yield had genotype Sava ( $792 \text{ kg ha}^{-1}$ ), followed by genotypes Valjevka ( $780 \text{ kg ha}^{-1}$ ) and Becejka ( $753 \text{ kg ha}^{-1}$ ), Tab. 2.

Tab. 2. Oil yield in soybean grain ( $\text{kg ha}^{-1}$ ), Rimski Sancevi and Sombor, Serbia

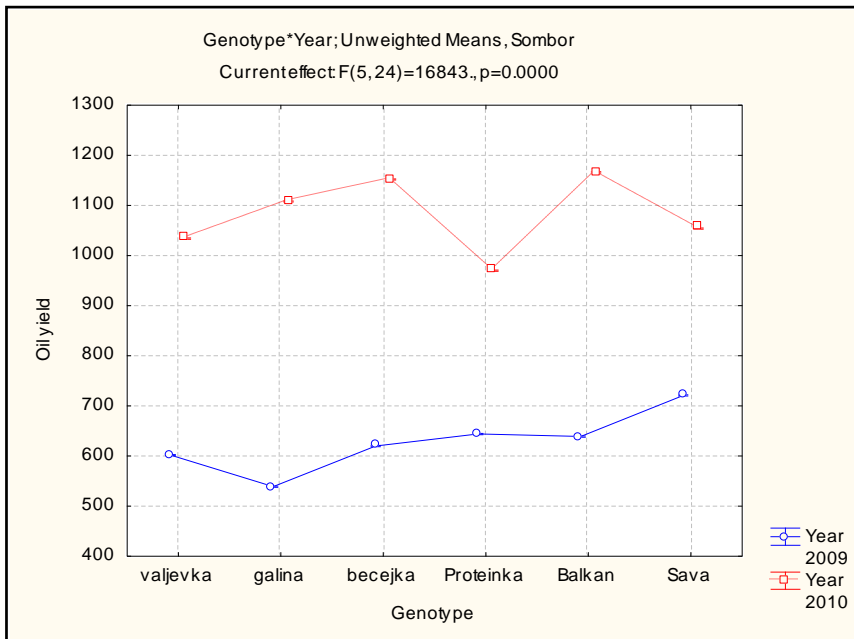
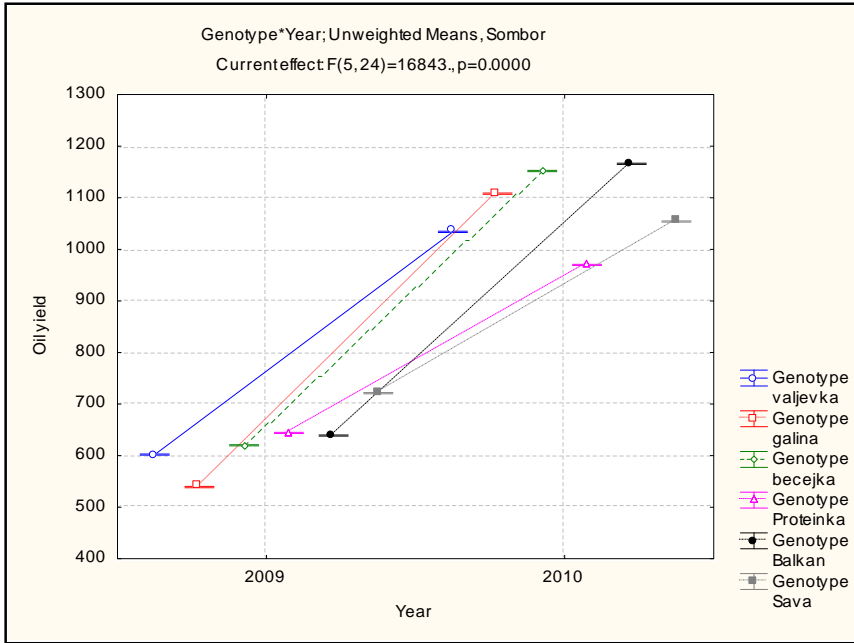
Genotype	MG	Rimski Sancevi, Novi Sad			Sombor		
		2009	2010	$\bar{X}$	2009	2010	$\bar{X}$
Galina	0	667	792	730	539	1.108	823
Valjevka	0	748	811	780	602	1.034	818
Becejka	0	730	777	753	620	1.152	886
Proteinka	0	681	769	725	644	970	807
Average 0 MG		707	788	748	601	1.066	833
Balkan	I	608	784	696	639	1.166	902
Sava	I	749	834	792	721	1.054	887
Average I MG		678	809	744	680	1.110	895
Average		697	794	745	640	1.088	864

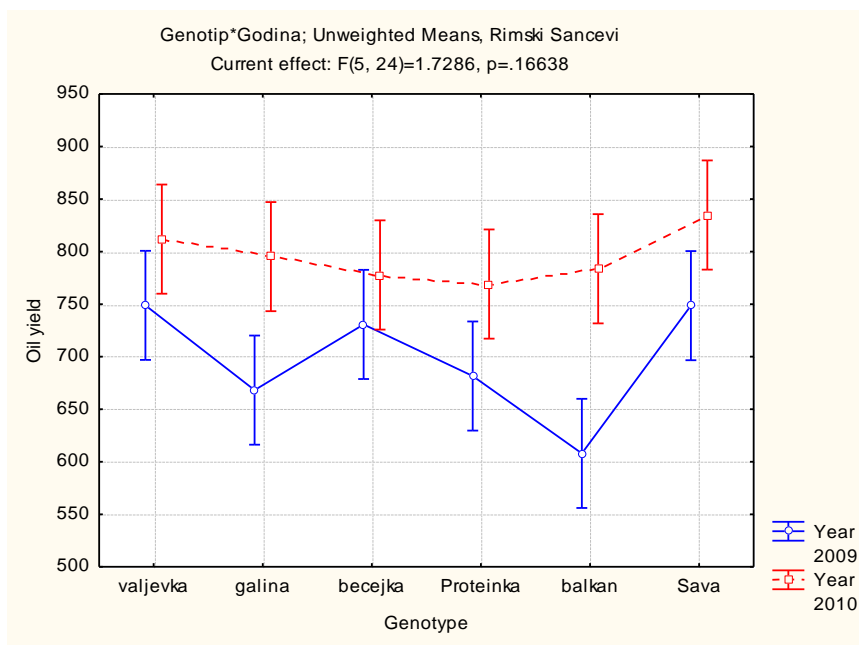
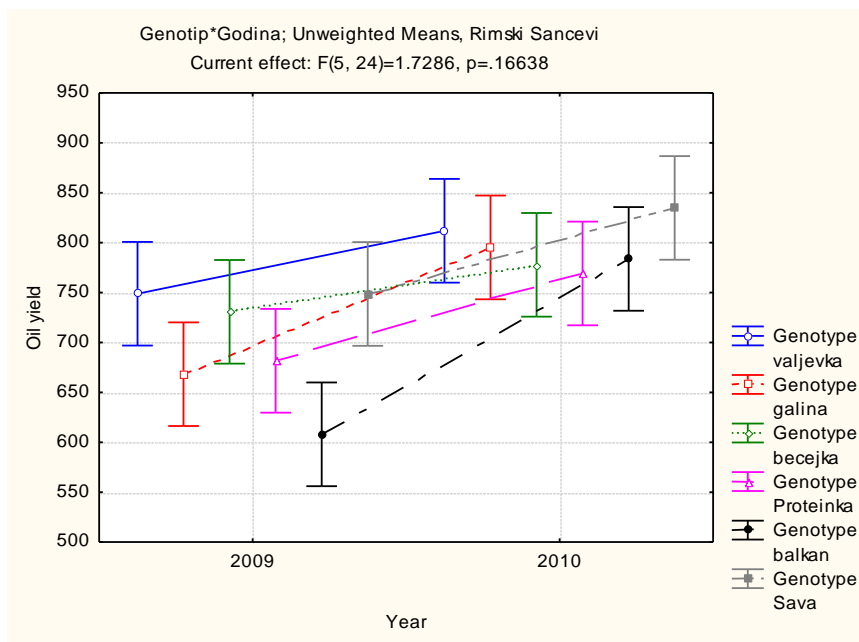
Parameter	Locality	Rimski Sancevi, Novi Sad		Sombor	
LSD test		5%	1%	5%	1%
Year		30	40	69	94
Genotype		52	70	120	163
Interaction		73	99	170	230

Genotype Sava, at locality Rimski Sancevi, had significantly higher oil yield, statistically, compared to genotypes Galina, Proteinka and Balkan. Statistically significantly higher oil yield, had genotype Valjevka compared to genotypes Proteinka and the Balkan as well as genotype Becejka in relation to genotype Balkan. In 2010, of the oil yields were statistically very significantly higher ( $794 \text{ kg ha}^{-1}$ ) compared to 2009 ( $697 \text{ kg ha}^{-1}$ ), which is higher by  $97 \text{ kg ha}^{-1}$  or 13.92% compared to 2009, Tab. 2.

In the locality Sombor, year had statistically significant effect on the oil yield ( $p < 0.01$ ). In 2010, of the oil yields were statistically significantly higher ( $1.088 \text{ kg ha}^{-1}$ ) compared to 2009 ( $0.640 \text{ kg ha}^{-1}$ ), which is higher by  $448 \text{ kg ha}^{-1}$  or 70% of the average oil yield in 2009. The oil yield average for all soybean genotypes in 2009-2010 amounted to  $864 \text{ kg ha}^{-1}$ . The highest average oil yield genotype Balkan ( $902 \text{ kg ha}^{-1}$ ) followed by genotype Sava ( $887 \text{ kg ha}^{-1}$ ) and Becejka ( $886 \text{ kg ha}^{-1}$ ), Table 2, Fig. 3. In the locality of Sombor genotype and interaction genotype x years had statistically significant effect on the oil yield, Table 2, graph. 3.

Figure 3-6. Year and genotype effects in oil yield in soybean (kg ha<sup>-1</sup>), Sombor and Rimski Sancevi





If we compare locations of growing, it is evident that the location of Sombor the oil yield was higher for 119 kg ha<sup>-1</sup>, or 15.98% compared to location of Rimski Sancevi, Novi Sad, Table 2, Graph. 3.

At both locations the highest average oil yield had is genotype Sava (840 kg ha<sup>-1</sup>). Genotype Sava had highest average oil yield at locality of Sombor, while highest average of oil yield had genotype Balkan (902 kg ha<sup>-1</sup>) at the locality Sombor, Table 2, Graph. 3.

On average for both localities, genotypes of the maturity group I had higher average oil yield of 29 kg ha<sup>-1</sup> respectively to 3.80% compared to genotype maturity group 0, Table 2.

The soybean production involves more factors that individually and in interaction, cause the success of the production of soybean cultivars. In addition to genetic factors, a large impact on the variability of the studied traits of soybean cultivars were and agro-ecological factors and locality. Highly significant year x genotype interaction shows that there are differences in the expression of the traits studied in soybean. Similar results were reviews Vidic et al. (2010) and the Popovic et al., 2012, 2014 and 2015.

### CONCLUSIONS

Based on two-year examination of the oil yield for six NS soybean genotypes on two growing localities may be the following conclusions:

- Year and genotype had a statistically significant effect on the oil yield at locality Rimski Sancevi. The average oil yield of all genotypes of soybean in 2009-2010. amounted to 745 kg ha<sup>-1</sup>. The highest average oil yield had genotype Sava (792 kg ha<sup>-1</sup>). In 2010, of the oil yields (794 kg ha<sup>-1</sup>) were statistically significantly higher compared to 2009 (697 kg ha<sup>-1</sup>), which is higher by 97 kg ha<sup>-1</sup> or 13.92% compared to 2009.

- In the locality of Sombor, year had statistically significant effect on the oil yield. The average oil yield of all genotypes of soybean in 2009-2010. amounted to 864 kg ha<sup>-1</sup>. The highest average oil yield had genotype Balkan (902 kg ha<sup>-1</sup>), followed by genotype Sava (887 kg ha<sup>-1</sup>) and Becejka (886 kg ha<sup>-1</sup>). In 2010, of the oil yields were statistically significantly higher (1.088 kg ha<sup>-1</sup>) compared to 2009 (640 kg ha<sup>-1</sup>), which is higher by 448 kg ha<sup>-1</sup> or 70% of the average oil yield in 2009.

- If we compare the locations, it is evident that the location of Sombor the oil yield was higher for 119 kg ha<sup>-1</sup>, or 15.97% compared to location of Rimski Sancevi, Novi Sad.

- The soybean production involves more factors that individually and in interactions, cause success of the production of soybean cultivars. In addition to genetic factors, a large impact on the variability of the studied trait of soybean cultivars had agro-ecological factors and the growing locality.

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## ADVANCED CROPPING TECHNOLOGY OF MAIZE (*Zea mays* L.) IN SERBIA

### SUMMARY

Studies on maize growing practices have been performed in the Maize Research Institute, Zemun Polje since its establishment. Numerous studies realised in accordance with contemporary global trends indicated the direction of development of maize growing practices with the aim to use the genetic potential of newly derived hybrids. Although, the genetic potential of the yield of maize hybrids grown in Serbia is 10 to 15 t/ha, the recorded average yields are significantly lower. The experimental trials with the application of standard growing practices conducted during the fifteen growing seasons showed that the maize yield varied from 10.46 to 11.38 t/ha. The obtained results indicated that, for the region of central Serbia, contemporary maize growing systems should include a correctly applied crop rotation with legumes (soybean), conventional tillage with deep autumn ploughing, precisely determined plant density and the time of sowing. Another important factor is the application of fertilisers the content and formulation of which are adjusted to the requirements of the cultivated plant and irrigation tuned to the requirements of crops and climate in terms of norms and frequency of the application.

**Keywords:** growing, maize, crop rotation, soil tillage, yield

### INTRODUCTION

Sustainability of agricultural production is important not only for the protection of the environment, but also for the reduction in costs and other inputs. Although a complete replacement of all components of the production is not possible, e.g., replacement of mineral fertilisers with alternative sources, it is very important to pay more attention to agro-ecological measures (Council for the Environment and Infrastructure, 2013). The reduction of yield losses caused by pests, pathogens and weeds are major challenges to agricultural production. Globally, approximately 35 % of potential yield is lost to pre-harvest pests. In addition to the pre-harvest losses, transport, storage, marketing, etc. losses also occur.

With reference to the above-stated, the question that arises is whether intensive but not sustainable production in relation to environmental protection

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could contribute more to the reduction in losses than ecological agriculture, the efficient application of which is based on innovative and creative knowledge and research (Popp et al., 2014).

Maize and winter wheat, in terms of area, requirements and profit, are the two most important crops in Serbia, and on average, 50 % of arable land is cultivated with these two crops. Although the genetic potential of the yield of maize hybrids grown in Serbia is 10 to 15 t/ha, the recorded average yields are significantly lower, mainly due to frequent droughts, unfavourable soil properties in certain regions, insufficient application of mineral fertilisers, obsolete machinery, small farms, etc. However, experimental studies performed in the period from 1998 to 2012 showed that average maize yields, when standard growing practices were applied, amounted to 10.46, 10.39 and 11.38 t/ha for hybrids of FAO maturity groups 300–400, 500 and 600–700, respectively (Videnović et al., 2013a). The most important limiting factor for successful maize production is meteorological and irrigation and they have to perform very important measure within a cropping technology (Dragičević et al., 2015). A more significant improvement of maize production requires a long-term strategy programme that would elaborate the following: defragmentation of holdings, the construction of irrigation systems on areas as large as possible and intensification of maize growing practices. Studies on maize growing practices have been performed in the Maize Research Institute, Zemun Polje since it was founded. Numerous studies performed in accordance with contemporary global trends indicated the direction of development of maize growing practices with the aim to use, to the greatest extent, the genetic potential of newly derived hybrids. The most important maize growing practices are crop rotation, tillage, density and time of sowing, fertilisation and irrigation. These studies are aimed at better understanding of the long-term effects of these practices to soil and crop and optimisation of crop growing practices.

## MATERIAL AND METHODS

All experimental results were collected from multi-year field trials conducted in the experimental field of Maize Research Institute Zemun Polje, in the vicinity of Belgrade (44°52'N 20°20'E). The soil was slightly calcareous chernozem with 47 % clay and silt and 53 % sand. The 0–30-cm layer had 3.3 % organic matter, 0.21 % total N, 1.9 % organic C, 14 and 31 mg per 100 g soil of available P and extractable K, respectively, 9.7 % total CaCO<sub>3</sub> and pH 7.8. The crop was hand sown on different dates of April and May in each year, according to the experimental design and goal. All planned experimental treatments and practices were applied on time. The maize was harvested and the yield was measured and calculated with 14 % moisture at the end of the season

## RESULTS AND DISCUSSION

### The importance of crop rotation

Crop rotation has direct and preventive effects not only on plant production but also on the protection of the agro-ecosystem. This is also an economically very efficient measure, because no additional investments are necessary, and advantages over continuous cropping during the shorter or a longer period are numerous and are reflected in the improvement of chemical, physical and biological properties of soil, and more efficient and secure protection of crops against weeds, diseases and pests (Videnović et al., 2007). The most important advantage of crop rotation is significant yield increases in relation to crops in continuous cropping systems (Spasojević et al., 2013). Depending on weather conditions, the yield increases achieved in maize grown in a crop rotation system can be greater by 10 % and in some cases even by 30 % in relation to crops grown in continuous cropping systems. The rotation of three or four crops is the optimum system. The crop rotation systems should not include cultivated plants that belong to the same family, but it is necessary to include small grains (wheat, barley, oats, rye), maize or sorghum, legumes (soybean, French bean), as well as industrial plants (sunflower, etc.). This allows the growing of plant species with different root architectures, various nutrient and water requirements, no common pathogens causing diseases in plants, etc.

Although the use of the crop rotation should be an integral part of current maize growing practices, there is a great problem in Serbia regarding the use of an appropriate crop rotation, since maize is sown on much larger areas than other field crops. According to the Statistical Yearbook of Serbia, the annual area cultivated with maize in Serbia ranges from 1,200,000 to 1,250,000 ha, while wheat, as the second most distributed crop, is sown on an area of 450,000 to 500,000 ha. The distribution of maize over large areas is the result of its great demand on the market, first as fodder and then as food, as well as the needs of the processing industry. Due to such a sowing structure, maize is grown in continuous cropping on a certain percentage of areas. If crop rotation is used, then the well known "Balkan crop rotation" (maize–winter wheat) is applied. The advantage of this growing system over maize continuous cropping lies in the easier suppression of weeds. Wheat is a crop of a dense stand that begins to develop in spring much earlier than weeds and therefore prevents their normal growth and development. Even in a two crop rotation, wheat contributes to efficient reduction of weediness if it is the preceding crop to maize, especially the control of perennial weeds such as Johnson grass - *Sorghum halepense* L. and to a lesser extent bindweed - *Convolvulus arvensis* L., (Spasojević et al., 2014b).

Soybean is a nitrogen-fixing rotation crop that can provide significant amounts of available nitrogen for the succeeding crop and as a result, the input of mineral fertilisers could be reduced. The obtained results showed that the average maize yield was higher by 11.4 % in soybean–maize rotation, despite the amount

of applied nitrogen fertiliser being reduced by 50 % (Videnović et al., 2013b). Moreover, the maize yield recorded in the three-crop rotation with wheat and soybean was higher by 32.4 % than the one obtained in the two-crop rotation (maize–wheat). The lowest average maize yield (5.37 t/ha) was recorded in continuous cropping (CR1).

Three-crop rotations: soybean–wheat–maize (wheat is the preceding crop to maize) and wheat–soybean–maize (soybean is the preceding crop to maize) are the most often used variants of crop rotations. These two types of three-crop rotation are identical in relation to the crops included, but due to the sequence of crops (alternation of crops), there are significant differences, before all in their effects on the reduction of weed infestation (Spasojević, 2014b). Under Serbian agro-ecological conditions, the soya bean–wheat–maize (wheat is the preceding crop to maize) rotation very efficiently affected a reduction in the total weed infestation, even when reduced amounts of herbicides were applied, which is very important from the aspect of agro-ecosystem protection (Simić et al., 2015). In dependence on the level of herbicide application, the maize-soybean-wheat three-crop rotation had the greatest effect on the reduction of maize weeds, especially perennials species, after only one rotation of crops (Spasojević, 2012).

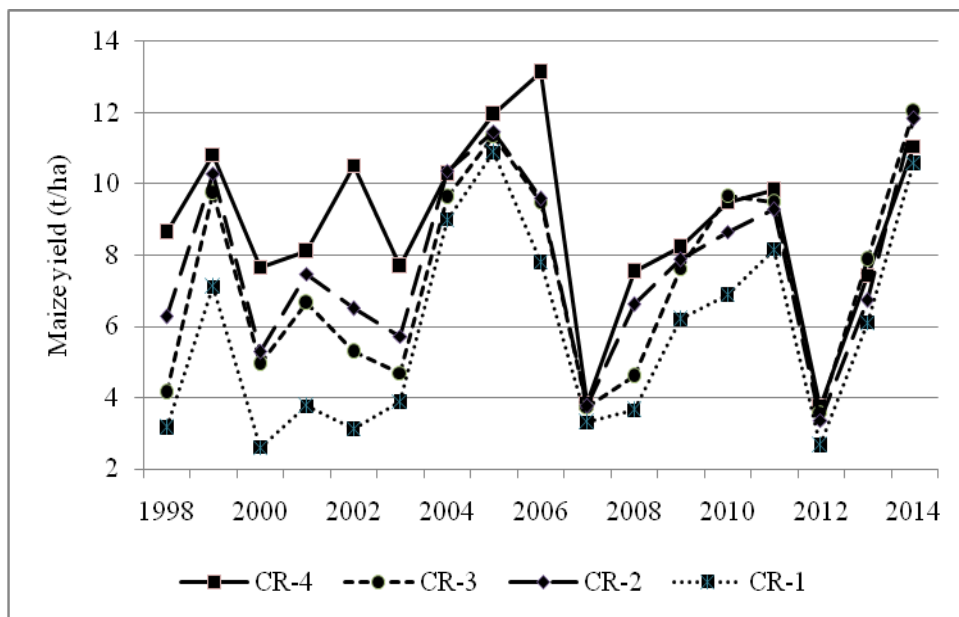


Figure 1. Significance of growing system for maize yield: CR1 – maize continuous cropping; CR2 – two-crop rotation: maize–soybean; CR3 – two-crop rotation: maize–winter wheat; CR4 – three-crop rotation: maize– winter wheat–soybean (Videnović et al., 2013b)

The most recent studies on crop rotation performed in the maize research institute, zemun polje, showed that the use of crop rotation combined with other

maize growing practices might provide optimum conditions for the maximum growth of crops and efficiency of photosynthesis. When combined with herbicide application or hoeing up, crop rotation positively affected maize yields by increasing the leaf area index, content of chlorophyll and carotenoids and reduction of free energy (spasojević et al., 2014a). The effects of crop rotation are even more pronounced in years with unfavourable conditions for maize production (videnović et al., 2013a).

### Soil tillage systems

Correct tillage affects the formation and renovation of a favourable soil structure, i.e., of a loosened soil layer and moisture accumulation. This improves the air–thermal soil regime, provides incorporation of organic residues of previous crops and fertilisers, and a better distribution of the herbicides applied to the soil. Under irrigation conditions, tillage has a significant role in increasing maize yield, because it enhances the effect of beneficial microorganisms, reduces erosion, and provides timely harvest and application of other cropping practices (kresović, 2003).

Considering biological and ecological relationships and environmental conservation, it is necessary to apply effective tillage practices in the maize growing systems. Conservation and reduced tillage systems have gained widespread acceptance in many countries over the past 25 years due to savings in time and economic input and reductions in environmental pollution and soil degradation (özaslan and gürsoy, 2015). With respect to this, in the recent decades, certain operations in soil tillage have been omitted (reduced tillage) or specially designed maize planters have been used to sow directly into non-tilled soil (direct sowing). Direct sowing is generally defined as sowing of crops into non-tilled soils that contain a minimum of 30 % of harvest residues. tillage applied in maize production in Serbia requires a greater number of different methods of primary tillage and pre-sowing land preparation and contributes to the achievement of the highest grain yields (Videnović et al., 2011a), Table 1. In the 2004–2008 period, the average maize grain yield in the variant with the conventional tillage, tillage with a cultivator (reduced tillage) and in the variant with direct sowing amounted to 10.6 t/ha, 9.0 t/ha (lower by 1.6 t/ha) and 6.9 t/ha (lower by 3.7 t/ha), respectively.

Table 1. Maize yield (t/ha) depending on the tillage system

Tillage method	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Average
Direct sowing	6.4	3.0	6.0	8.5	6.0	11.5	8.9	8.0	6.4	3.9	6.9
Reduced tillage	10.6	5.2	8.0	8.8	7.5	12.8	12.4	10.8	7.6	6.3	9.0
Conventional tillage	11.4	8.8	9.0	10.3	8.8	14.3	13.9	12.1	8.3	9.6	10.6
Average	9.5	5.3	7.7	9.1	7.4	12.9	11.7	10.3	7.4	6.6	8.8

Under the agro-ecological conditions of zemun polje, if reduced tillage and direct sowing are applied, greater amounts of herbicides are necessary for the suppression of weeds, particularly perennial species (simić et al., 2012a). The highest number of weed plants per species was recorded in variants with reduced tillage, which is in accordance with the significant distribution of perennial weed species that have very developed root systems and pronounced vegetative propagation.

### Sowing time and density

The size and shape of the growing space in which each plant will obtain the necessary area to grow and develop are defined by sowing. Maize, belonging to the late-maturing crops germinating in spring, requires a lot of heat and is very susceptible to low temperatures. Maize sowing encompasses several operations - selection of the hybrid, preparation of the seeds for sowing, time of sowing, depth of sowing, density of sowing, i.e., the number of plants per hectare and the plant arrangement in the plot. In the recent decades, due to climatic changes, the springs have frequently been dry. Therefore, in order to ensure better use of the moisture necessary for germination and emergence, the optimum time of sowing is very often the second decade of april. The long-term results obtained in trials conducted on the slightly calcareous chernozem in zemun polje showed that the highest yields were recorded when the sowing was performed in the second decade of april, table 2 (videnović et al., 2011b). On average, the highest yield of 11.2 t/ha (100.0 %) was achieved by sowing on april 15. The yield was lower at all remaining times of sowing: april 5, 98.5 % and april 25, 97.9 %. Sowing realised in may resulted in significantly lower maize yields in comparison with sowing performed on april 15: may 5, 93.9 %; may 15, 93.3 % and may 25, 86.6 %.

Table 2. Effects of sowing time (t) on average yields (t/ha1) of zp maize hybrids (h) in the period 2003–2008 (videnović et al., 2011b).

	H1	H2	H3	H4	H5	Average	%
April 5	10.6	11.3	10.9	10.9	11.5	11.0	98.5
April 15	10.9	11.0	11.6	10.7	11.9	11.2	100.0
April 25	10.7	11.0	11.0	10.8	11.4	11.0	97.9
May 5	10.0	10.8	10.7	10.2	11.1	10.5	93.9
May 15	9.9	10.9	10.1	10.2	11.2	10.5	93.3
May 25	9.7	9.9	9.5	9.4	10.0	9.7	86.6
Average	10.3	10.8	10.6	10.4	11.2	10.7	–
%	92.3	97.0	95.3	92.8	100.0	–	–
LSD <sub>0,01</sub>	(T)	0.485	(H)	0.505	(T×H)	0.765	



Minimum temperature of 10–12 °C at a 10-cm depth of the sowing layer usually provide seedling emergence within 10–15 days if the moisture is favourable. On the other hand, with favourable moisture and a temperature of 20 °C, maize seedling will emerge in 6–7 days. In the majority of years, maize sowing was performed between April 10 and April 30. The sowing depth affects not only germination and emergence, but also the development of the root system and plant productivity. An increase in the sowing depth leads to inhibition and delay of emergence. In order to mitigate adverse effects of low temperatures on the emergence rate, it is necessary to sow maize to a depth of 6–8 cm and 3–5 cm in coarse textured soils and heavy soils, respectively.

The sowing density is achieved by timely sowing of the optimum number of plants for the given conditions of the habitat and the hybrid. The greater the crop density is, the higher is the total yield of the above ground weight. The increased crop density also increases grain yield, but only to a certain extent and further increases in the crop density result in the yield reduction (videnović et al., 2007; kresović et al., 2011), table 3.

Table 3. Average yield (t/ha) of hybrid zp 684 in variants with different sowing densities (Kresović et al., 2011)

Density	2006	2007	2008	2009	Average
40816	11.9	11.9	11.4	10.8	11.5
50125	12.8	12.4	13.6	12.0	12.7
59524	14.6	12.2	14.6	12.2	13.4
69686	14.2	11.1	15.9	12.7	13.5
79365	14.6	11.9	15.9	12.6	13.7
89286	13.8	12.4	16.2	12.6	13.7
98522	10.4	11.7	15.8	12.6	13.5
Average	13.7	11.9	14.8	12.2	13.2
	Year	Density	Year x	Density	
LSD <sub>0.05</sub>	1.290	0.595	1.087		
LSD <sub>0.01</sub>	1.808	0.722	1.443		

New Generations Of Maize Hybrids Are Characterized By A Better Ability Of Plants To Be Grown In Denser Stand, As They Were Selected Under Such Conditions. The Higher Density Results In The Appearance Modification Of The Maize Genotype Plant. Newer Generations Of Maize Hybrids Selected In Higher Densities (60-100,000 Plants/Ha), Have Less Robust Plants, Ears Are Placed More Lower, While The Angle Of Top Leaves In Relation To The Stalk Is Smaller (Simić Et Al., 2009). The Sowing Density Has To Be Adjusted To The Genotype, I.E., The Fao Maturity Group Of The Appropriate Hybrid, And Soil Quality, Amount Of Nutrients And Water Available To The Plants During The Growing Season. Early Maturing Hybrids (Fao 300–400) Require 70,000–80,000 Plants Per Hectare, The Hybrids Fao 500–600 Require 60,000–70,000 Plants Per Hectare, While Late Maturing Hybrids (Fao 700–800) Require 60,000 Plants Per Hectare, Which Is Significant Increase In The Number Of Plants In

Comparison To The Number In The Previous Period (Videnović Et Al., 2007). Yields Of Hybrids Characterised By A Great Height And A Powerful Habitat Are Higher At Lower Densities, While Yields Of Early Maturing And Hybrids With Shorter Plants Are Higher At Larger Densities. Newly Developed Hybrids Can Give High Yields Only When The Necessary Number Of Plants Per Unit Area Is Achieved (Simić And Stefanović, 2007). The Maize Density Directly Affects Good Crop Coverage And Consequently Increases Its Competitive Ability Against Weeds (Simić Et Al., 2009). Some Maize Genotypes May Considerably Differ From Each Other In Their Morphology And Competitive Ability Against Weeds (Simić Et Al., 2012b), Table 4.

Table 4. Effects of growing density and hybrids on yields and shelling percentage of sweet maize in the period 2008–2009 (simić et al., 2012b)

Hybrid	Ear yield (t/ha)					Shelling percentage (%)				
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	Average	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	Average
ZP 424su	9.85	10.43	11.48	12.24	11.00a	66.89	67.66	66.51	66.08	66.79ab
ZP 462su	9.72	10.75	12.11	11.73	11.07a	66.22	65.27	65.00	63.85	65.08b
ZP 504su	8.41	9.38	8.67	10.63	9.27c	72.11	69.02	64.97	68.50	68.65a
ZP 521su	9.21	9.43	10.73	11.21	10.14b	65.93	67.76	66.47	66.48	66.66ab
Average	9.30d	10.00c	10.74b	11.45a		67.79a	67.43ab	65.74b	66.23ab	
	LSD <sub>0.05</sub> = 0.49					LSD <sub>0.05</sub> = 2.00				

D<sub>1</sub>- 40,000 plants/ha; D<sub>2</sub>-50,000 plants/ha; D<sub>3</sub>-60,000 plants/ha and D<sub>4</sub>-70,000 plants/ha

### Fertilisation

The application of organic and mineral fertilisers, as well as certain land improvement practices (calcification, phosphatisation, humisation), significantly contribute to an improvement of unfavourable soil characteristics. It is changing chemical properties of soil, such as neutralisation of altered soil acidity, an increase of the availability of some nutrients, an increase in microbiological activities and the establishment of a favourable air and water regime in the soil. Nitrogen fertilisers can positively, but also negatively, affect soil, which indicates that fertilisation with nitrogen is very complex (Dragičević et al., 2012a). The content of available nutrients depends firstly on soil fertility (soil texture, chemical properties, organic mater content, etc.), preceding crops, weather conditions, irrigation, application of organic fertilisers, amounts of harvest residues in the soil, etc.

Previous studies conducted in Zemun Polje indicated that chernozem, as a naturally fertile soil with easily available nutrients, was less responsive to fertilisation than other soil types, especially when crop rotations with soybean were used. Such soils do not need more than 120 kg of nitrogen, 90 kg of phosphorus and 60 kg of potassium fertilisers (Videnović et al., 2007). Harvest residues of the preceding crop were crushed and ploughed down and 30–50

kgN/ha applied in autumn for easier microbiological decomposition of the organic matter. The highest yield of a late maturing hybrid in the experimental plot with long-term continuous cropping (over 35 years) was achieved when manure was applied, harvest residues ploughed down completely and N fertiliser was applied in spring at the beginning of the growing season (Simić et al., 2013). However, the highest yield of maize was not achieved when the highest amount of mineral fertiliser had been applied, and regardless of the high yielding potential of the late maturing hybrid, the yield recorded in 2011 was not greater than 10.4 t/ha. This suggests that maize production may be efficient only if the whole system of practices (crop rotation) is applied. The integrated effect of this system contributes to the achievement of record yields.

The highest maize yield in a long-term trial with three-crop rotation (maize rotated with wheat and soya bean) and the application of mineral fertilisers was achieved with 270 kgNPK/ha (Table 5). In maize continuous cropping and the maize–soybean rotation, 180 and 270 kgNPK/ha, respectively, had the best effects on yields, while higher yields in the maize–wheat rotation were obtained with greater amounts of fertilisers.

Table 5. Long-term analysis of maize yields (t/ha) in dependence on the amounts of applied mineral fertilisers and the crop sequence in the crop rotation (Zemun Polje, 1998–2009)

	Continu ous cropping	Maize– Wheat	Maize– Legume	Maize– Wheat– Legume	Average	
Without fertilisation	3.64	6.53	5.85	8.52	6.14	
NPK	180 t ha <sup>-1</sup>	5.81	8.07	6.96	9.20	7.51
	270 t ha <sup>-1</sup>	6.10	7.99	7.27	9.18	7.63
	360 t ha <sup>-1</sup>	5.95	7.81	7.21	9.23	7.55
Average	5.37	7.60	6.82	9.03	7.21	

The higher the maize yield is in the crop rotation, the higher is the outtake of nutrients. Hence the greatest outtake of nitrogen was recorded in the part of the plot where legume was the crop preceding maize in both rotations, with two crops (maize–legume + 270 tNPK/ha) and three crops (maize–winter wheat–legume + 360 tNPK/ha), Table 6.

The n content increased by fertilization level but has decreased during vegetation, down to harvesting phase and in higher degree in irrigation compared to rainfed cropping as well as in no-till treatment compared to conventional and reduced cropping (Dragičević et al., 2012 a).

Table 6. Effects of crop rotation on nitrogen outtake (kg/ha) with maize yield (Zemun Polje, 2013)

	Continuo us cropping	Maize– Wheat	Maize– Legume	Maize– Wheat– Legume	Average	
Without fertilisation	47.82	96.16	117.00	115.14	94.03	
NPK	180 t ha <sup>-1</sup>	88.35	94.36	136.38	128.08	111.79
	270 t ha <sup>-1</sup>	92.49	93.21	141.78	131.07	114.64
	360 t ha <sup>-1</sup>	98.07	89.26	129.41	144.67	115.35
Average	81.68	93.25	131.14	129.74	108.95	

With the aim of protecting the agro-ecosystem, it is possible to use foliar fertilisers in maize crops, mainly for side dressing, especially if the fertiliser formulations provide its use together with plant protection products (brankov et al., 2011). Foliar fertilisers provide macro-elements and other physiologically active substances (amino acids, phytohormones, growth stimulators, etc.) Necessary for plants to overcome stress conditions more easily and to have higher yields (brankov et al., 2012; dragičević et al., 2012b). In addition to increasing the plant height and leaf area, foliar fertilisers, particularly amino acid ones, affected an increase in grain yield of maize inbred lines, table 7.

Table 7. Effects of amino acid (F1) and phosphorus (F2) foliar fertilisers on grain yield and the harvest index (Zemun Polje, average for the period 2010–2011)

	Grain yield (t/ha)						Harvest index (%)					
	L1	L2	L3	L4	L5	Average	L1	L2	L3	L4	L5	Average
K	1.86	3.11	6.09	5.6	2.81	3.89	27.2	29.4	39.3	47.6	64.1	41.5
F1	2.57	3.26	6.28	6.43	4.39	4.59	39.8	53.2	47.5	47.7	55.8	48.8
F2	1.97	3.19	6.21	5.6	5.03	4.4	36.4	58.4	52.6	50.7	51.6	49.9

L1, L2, L3, L4 and L5 – different inbred lines of zp maize hybrids

### Irrigation

The achievement of high yields of maize under conditions in serbia is limited by two factors: the uneven distribution and occasional deficit in precipitation. The critical period begins 15–20 days prior to tasselling and lasts until the beginning of the milk stage of maize. When the years are average in terms of weather conditions, the maize production under irrigation conditions will results in yields higher by 15–30 % or even by 50 % in seed maize. Irrigation provides the optimum water supply, activates soil microorganisms and nutrient reserves and contributes to a better utilisation of incorporated fertilisers. Grain yields of some zp maize hybrids grown under irrigation conditions showed a dependence on the amount of water that reached the soil surface (kresović et al., 2013).

Irrigation norms depend on climatic conditions, type of soil, and the type and specificities of hybrids. Water requirements of plants during the growing season under agro-ecological conditions of serbia vary from 450 to 600 mm. Guideline monthly values are as follows: 35 mm (april), 90 mm (may), 95 mm (june), 120 mm (july), 75 mm (august) and 40 mm (september). The differences between the stated amounts and amounts of effective rainfall should be compensated by irrigation. According to the obtained results, different available precipitation sums ( $f = 801.9051$ ,  $p < 0.01$ ,  $cv = 1.95\%$ ) affected yields in a way that the highest yield of  $15.08 \text{ t ha}^{-1}$  was achieved in the variant with the greatest precipitation sum, i.e., in the variant with a pre-irrigation soil moisture of 80–85 % of field water capacity - fwc (table 8). In relation to this variant, yields were statistically very significantly lower in the other variants:  $13.55 \text{ t/ha}$  (70–75 % fwc),  $12.54 \text{ t/ha}$  (60–65 % fwc) and  $10.20 \text{ t/ha}$  (rain-fed regime).

Table 8. Average yields (t/ha) of maize in dependence on irrigation norms (Zemun Polje, 2006–2008)

Year	80–85 % FWC	70–75 % FWC	60–65 % FWC	Rain-fed regime	Average
2006	14.59	12.46	11.41	11.14	12.40
2007	16.33	14.54	13.51	10.74	13.78
2008	14.31	13.65	12.69	8.73	12.35
Average	15.08	13.55	12.54	10.20	12.84
	Year	Norm	Year x Norm		
<i>LSD</i> <sub>0.05</sub>	0.236	0.210	0.364		
<i>LSD</i> <sub>0.01</sub>	0.339	0.284	0.492		

Irrigation showed the best effects when it was applied together with other maize growing practices, first, with fertilising. The studies performed on slightly calcareous chernozem in the dry year of 2008 showed that the highest maize yield was obtained when conventional tillage was performed (Table 9).

The obtained yield was two-fold higher than the one obtained in the no-till variant. Irrigation increased yields, on average, by approximately 21 % in relation to those obtained under the rain-fed regime. Moreover, irrigation settled soil moisture to the similar level between tillage practices (Dragičević et al., 2012a).

The obtained results indicate the significance and contribution of certain cropping practices applied under the experimental conditions with the aim of increasing the yield of maize. Improvements of maize growing practices are possible by the correct combination and application of several practices as a system of measures adjusted to the crop-growing region and applied over a longer period. The effects of crop rotation, tillage and fertilisation on maize yields would be even greater if inputs in the application of all necessary practices regardless of the size of estates

would be permanent. Therefore, an agricultural development strategy is necessary in order to regulate the defragmentation of holdings, construction of irrigation systems and an increase in the funding of improvements of maize growing practices.

Table 9. Effects of different types of tillage and fertilisation (F1, F2 and F3) on maize yields

		Irrigation					Rain-fed regime				
		2005	2006	2007	2008	$\bar{X}$	2005	2006	2007	2008	$\bar{X}$
Conventional	F1	13.18	10.54	10.86	10.69	11.32	13.13	10.08	5.72	9.64	9.64
	F2	14.53	13.51	11.88	12.20	13.03	13.80	13.51	8.57	10.27	11.54
	F3	15.68	15.06	13.00	12.99	14.18	13.92	12.72	9.10	8.78	11.13
	$\bar{X}$	14.46	13.04	11.91	11.96	12.84	13.61	12.10	7.80	9.57	10.77
Reduced	F1	10.51	11.74	11.11	7.88	10.31	10.87	9.29	5.85	3.41	7.35
	F2	12.20	12.68	12.75	9.33	11.74	12.35	10.84	9.20	8.37	10.19
	F3	12.62	13.23	11.91	11.61	12.34	13.95	12.15	8.23	7.17	10.38
	$\bar{X}$	11.78	12.55	11.92	9.60	11.46	12.39	10.76	7.76	6.32	9.31
No-till	F1	6.69	8.98	7.70	3.55	6.73	5.90	5.49	6.48	2.25	5.03
	F2	12.41	12.16	11.15	6.57	10.57	6.93	7.76	7.22	4.54	6.61
	F3	12.86	13.56	11.97	8.47	11.72	14.70	10.67	6.22	5.01	9.15
	$\bar{X}$	10.65	11.57	10.27	6.20	9.67	9.18	7.97	6.64	3.94	6.93
LSD 5 %	Year	Tillage	Fertilising			Year	Tillage	Fertilising			
	3.58	3.46	2.83			6.71	2.42	3.32			

## CONCLUSION

The obtained results indicate the significance and contribution of certain cropping practices applied under the experimental conditions with the aim of increasing the yield of maize. Improvements of maize growing practices are possible by the correct combination and application of several practices as a system of measures adjusted to the crop-growing region and applied over a longer period. The effects of crop rotation, tillage and fertilisation on maize yields would be even greater if inputs in the application of all necessary practices regardless of the size of estates would be permanent. Therefore, an agricultural development strategy is necessary in order to regulate the defragmentation of holdings, construction of irrigation systems and an increase in the funding of improvements of maize growing practices.

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## **SERICULTURE IN PROVINCES OF BURSA, AMASYA AND MUGLA (TURKEY)**

### **SUMMARY**

Bursa was the important city where silkworm egg and cocoon were produced; silk and velvet were woven by hand and exported to the Europe in the period of Ottoman Empire. Since silkworm was brought to Anatolia, Bursa sustains its importance in this sector. Sericulture has begun at the same period in Amasya that is one of the Ottoman cities. Silks came from Iran were woven in this city because of being on Iran's trade route. The fabrics woven in Amasya were preferred by Ottoman Palace and also exported to the other countries. Sericulture and cocoon cultivating were made in Mugla the city has eligible climate condition to cultivate mulberry. Silk was woven by using simple bench as a family work. Bursa became rapidly an industrial city on silk texture because of having raw material by beginning of republic period. Silk texture has subsisted as a traditional Turkish handcraft at Amasya and Mugla.

**Keywords:** Bombyx mori, native breed, genetic resource, Silk Road, cocoon

### **INTRODUCTION**

Some animal species and breeds had important place in history of some countries such as Merino sheep in Spain, Angora Goat in Turkey and sericulture in China (Ertugrul et al., 2010). Sericulture in the World is generally made in Asian countries. In the World production percentages of China and India are 80% and 15% respectively (Kaya and Tutkun, 2012). The production of fresh cocoon is about 134 in 2012 (Table 1). Silk has some unique characteristics in kinds of fabric. It is shiny, soft, strong, and has a fabric can be dyed. It is so sensitive, therefore it is effected by noise, smell, wind, temperature change, even carers hygiene (Imer, 2005). Generally four kinds of silkworm which are Mulberry, Eri, Tasar and Muga are reared in the World. Mulberry silkworm (*Bombyx mori*) (Table 2) has the majority in them which percentage is about 95% (Akbay,

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1981). In Turkish society silkworm seem cute. A survey study was realized in primary school students. According to study between 9 and 40% of students in different levels rear insects. Students rear silkworm, ant, ladybug and grasshopper which showed that silkworm was one the loveable insect in insect groups (Tezcan et al., 2010).

Table 1. Production of apiculture and sericulture (Anon 2010, 2013).

Year	Number of Villages Engaged in Sericulture	Number of Families Engaged in Sericulture	Number of Egg Boxes Produced	Production of Fresh Cocoons (tonnes)
1936	2.201	49.338	56.278	2.135
1940	2.422	63.498	73.045	3.014
1950	3.013	69.354	62.927	2.501
1960	2.530	60.370	50.865	2.444
1970	1.559	43.589	64.340	1.461
1980	1.601	43.025	66.042	1.707
1990	1.916	44.541	80.544	2.171
2000	230	2.210	3.147	60
2012	342	2.572	5.576	134

Table 2. Scientific classification of the silkworm (Akbay, 1981).

Phyluym	Artropoda
Class	Insecta/Hexapoda
Ordo	Lepidoptera
Subordo	Macro Lepidoptera-Heterocera
Super Family	Bomycoiden
Family	Bomycidae
Genus	Bombyx
Species	B. mori

### Sericulture in Bursa

Importance of Bursa in sericulture happened in 14th century during Ottoman Empire (Ergenc, 2013; Ersevinc, 2013; Inalcik, 2013, Kirayoglu, 2013a; Peker, 2013) but before there was a weaving industry before Bursa conquered in 1326 by Ottomans (Tas, 2013). The second important bazaar of floss silk trade was in Aleppo apart from Bursa. Persian merchants brought floss silk to Bursa and bought woollen from Europe, pearl from Persian Gulf, sugar from Egypt and Cyprus, and even spices from India. Even in 16th century Persian merchants used to sell floss silk in Bursa and bought tin, woollen and spices. Florence silk market used to decide prices depending on Bursa silk market (Ersevinc, 2013; Gunay, 2013; Inalcik, 2013). Schiltberger, Clavijo, Pero Tafur, and B. De le Broquiere reported that Bursa was one the most important floss silk bazaar in the World.

Table 3. Travellers, writer, scientist and tourist visitors to Bursa and arrival dates (Ceyhan 2013)

Name	Year of arrival	Name	Year of arrival
Ibni Batuta	1333	George Keppel	1830
Johann Hans Schiltberger	1397	Charles Texier	1833
Bertrandon de La Broquiere	1432	Richard Burgess	1834
Pero Tafur	1437	Duc de Raguse	1834
Benedetto Dei	1470	Charles Greenstreet Addison	1835
Bonsignore Bonsignore	1498	Aucher-Eloy	1835
Bernardo Michelozzi	1498	William J. Hamilton	1935
Arnold von Harf	1496	Julia Pardoe	1836
Maringhi of Medici	Early 16 <sup>th</sup> century	Robert Walsh	1836
Pierre Belon	1546	Edmund Spencer	1836
Hans Dernschwam	1555	M. Baptistin Poujoulat	1837
Stephan Gerlach	1576	Eliza C. A. Schneider	1830-1840
John Newberie	1581	Serafeddin Magmumi	1894
George Chritoph Fedrenberger	1588	Hayrullah İbni Abdulhak	1844, 1851, 1863
Reinhold Lubenau	1588	G. W. Frederick Howard	1853
Vincent Stochove	1630	Lean Henry Abdolone Ubicini	1855
Evliya Celebi	1640	Kevork Keresteciyan	1855
Jean-Baptiste Tavernier	Several times in his life	Sandison	1855
Thevenot	1666	Charles James Monk	1855
Spon	1675	Cyrus Hamlin	1855
Covel	1675	Georges Perrot	1856, 1857
Wheler	1675	Journal de Constantinople	issue of Nov. 1863
Smith	1683	Sir Hubert E. H. Jerningham	1870
Edmund Chishull	at the end of 17 <sup>th</sup> century	Maling	betw. 1869-1872
Aubry de La Motraye	1701	Georgina Adelaide Muller	1873
Joseph Pitton de Tournefort	1701	Henry C. Barkley	1878
Paul Lucas	1702 and 1705	Nikola Nachov	1879
Richard Pococke	1738	Edmond Duteuple	1880
Carsten Niebuhr	1767	Marie de Launay	1880
Dominique Sestini	1779	Omer Suphi	1889
Andre-Joseph Lafitte-Clave	1786	Ibnulcemal Abdul Tefvik	1890s
Le Chevalier	1786	Clement Imbalt Huart	1891
William Hunter	1792	Mehmet Ziya	1892-1893
Guillaume Antoine Olivier	1790	Fatma Fahrunnisa	1895
Von Ignatz von Brenner	1793	Paul Lindau	1897
James Dallaway	1794	Vasil Kinchev	1899
Antonie Galland	at the beginnig of 18 <sup>th</sup> century	Osmanzade Huseyin Vassaf	1901
William George Browne	1802	Regis Delbauf	1905
Joseph von Hammer-Purgstall	1804	Richard Davey	1906
Lady Hester Stanhope	1811	Hasan Taib	1907
Christophe Aubin	1812	Paul Fesch	1907
John MacDonald Kinneir	1813 and 1814	P. N. Daskalov	1909
John Fuller	1818	Kethy Brown	1911
Charles MacFarlene	1820s	Andre Gide	1914
Victor Fontainer	1821	Ewald Banse	1918
William Martin Leake	1824	Grace M. Ellison	1924
Joseph-Marie Jouannin	1825	Clare Consuelo Sheridan	1924-1925

After floss silk was imported to Bursa, it was drawn in filature factories and woven textile factories, after than it was exported to Europe (Ergenc, 2013). In history of Bursa lots of travellers, writer, scientist and tourist visited the city and mention about sericulture in Bursa (Table 3). For example Heat Lowry mentioned in his book of 'Seyyahların Gozuyla Bursa' (Bursa from Travellers Eyes) about 60 travellers who visited Bursa. There were some special profession on this sector. 'Hamcılar' used to draw silk fibres from cocoon by using tool of 'mancinik' and after than silk fibres are made silk thread by using tool of 'dolap' (Karaarslan, 2013). Silk thread was dyed by 'boyacılar' and later than dyed silk thread was woven by 'dokumacılar'. (Ergenc, 2013; Oguzoglu, 2013). By depending on their work experience, dokumacılar were into three groups called as 'cirak', 'kalfa', and 'usta'. Cirak which meant apprentice was a beginner. If a cirak works 1001 days in this work, they are promoted as kalfawhich meant headworker. After a kalfa worked several years, he was tested by a commission and became an usta which meant 'master' (Tas, 2013). Women generally used to work in filature factories rather than weaving factories. Girls commonly used to work until they marry to gain dowry money. After girls marry, they leave the job (Akkus, 2013, Soysaldi and Ozdemir, 2013).

The various kinds of woven fabric were named in different names such as 'arsin, dip, seraser, dose melik, carsaflik, cekme, sestari, hakim, keyfiye, ipekli abani, ipek hayten, sacak, serit, oya, puskul, kemha, atlas, kutnu, futa, kadife (velvet), tafta, cifte tafta, yigit tafta, and vale (Oguzoglu, 2013; Turkoz, 2013). Bursa Olgunlasma Enstitüsü (Maturation Institute of Bursa) is established in Bursa in 2007 to educate girls on native cultural items. In the school girls are educated on lots of kinds of woven silk fabric mentioned above (Goral, 2013; Kemankas, 2013) Florentiner Maringhi reported that quality of silk fabric definitely much more better than silk fabric made in China (Turkoz, 2013). In order to obtain different kinds of colours, silk fabric was dyed. There were used plenty of plants to dye fabric. Yellow colour was obtained from 'Altın agac, Katirtirnagi (Spartium - Spartium junceum), sumak (sumac), gence, and safran (saffron)'. Brown colour was obtained from 'mazi' (thuja), 'mese' (oak), 'ceviz ve yapragi' (walnut and walnut leaves). Red was obtained from 'pine bark' and 'kokboya' (madder), green colour from 'yabani nane' (wild mint), blue colour from 'Hint bitkisi', gray colour from sutlegen (spurge, Euphorbia)', black colour from combination of 'karpuz otu' and 'kara dal otu' (Oguzoglu, 2013).

After steam engine invented in Lyon in 1824, a French family, Glaizal Family, built a filature factory in Bursa in 1837 but after for a while this factory went bankrupt (Basaran, 2013; Ciftci, 2013). Austrian consul of Falkheisen bought the factory. He reopened the factory in 1945 together with Tasciyan who was an Ottoman Empire citizen and work in British consulate as a translator. After this filature factory, sericulture industry developed in Bursa region. (Dortok-Abaci, 2006; Cakici, 2013; Ciftci, 2013). For example in 19th century there were 130.000 families who reared silkworm. This number increased to 150.000 families in 1900s. Also about 19.000 employee worked in filature sector and

2.000 employee in textile sector (Altun 2013). Steam engine filature factories used to need high amount of firewood. This firewood was brought from Ulu Mountain which was the nearest mountain in Bursa region via Nilufer River. In late 19th century although firewood requirement was about 15.000 tonnes for filature factories, it was just about 5.000 tonnes for whole Bursa people at homes (Ciftci, 2013).

In Bursa there were lots of specific bazaars or markets depending on goods sold such as Bakircilar Carsisi (for coppersmiths), Oduncular Pazari (for firewood), Yemeniciler Carsisi (for shoes), (Pirinc Han (for rice), Tahil Han (for grains), and Tuz Han (for salt). Ipek Han (for floss silk), and Koza Han (for cocoon) were two of those specific places (Kirayoglu, 2013a,b)

### **Sericulture in Amasya**

Apart from Bursa, Amasya region is one the most important sericulture centres in Turkey (Kivrim and Elmaci, 2011; Yucekaya, 2013). Amasya is also hometown of Strabon (B.C. 64/63 - A.D. 24) who was a famous historian, geographer and philosopher (Anon 2014). Climate conditions of Amasya is very eligible for sericulture and looks like Bursa climate. On the other hand Amasya had a strategic position on way of Anatolian roads in Ancient time and still has. Amasya was on way of the Silk Road from Iran to Bursa (Gunay, 2013; Kivrim and Elmaci, 2011). There is a village named as Ipek Koy (Silk Village in English) which is 9 km far from Amasya city centre (Kivrim and Elmaci, 2011). It can be said that history of sericulture in Amasya is old as history of sericulture in Ottoman Empire (Yucekaya, 2013). During Ottoman King of Selim I there was a war between Ottomans and Iran. The King Selim I put into action of embargo for silk trade between Ottomans and Iran. During this embargo Amasya was one of the most important sericulture centre for export silk material in Bursa silk market. In different times Amasya sent some sericulture experts to other cities. For example Bor county demanded 8-10 families who were expert on sericulture and Amasya approved this wish. Ispir county demanded some experts for sericulture (Kivrim and Elmaci, 2011). In Amasya there was established a Sericulture Station in 1921 (Colak, 2013).

### **Sericulture in Mugla**

The Province of Mugla is located at southeast of Anatolia. It has a mild climate and eligible for mulberry cultivation and sericulture. Textile industry was based on sericulture and it had important for economy of Mugla. There are made famous 'duven' fabric in Yesilyurt town and Mugla city centre. Sericulture in Mugla is a traditional family business. Mugla region used to be a closed economy because of some deficiencies about transport and geographic conditions. Sericulture is generally made by women, so they invest to buy gold jewelry for theirself income of sericulture. After 1950s, economy of Mugla opened to outer bazaars. People started to deal with different kinds of business, so then sericulture business decreased. In 1970s tobacco cultivation started to be supported by governments and people cut mulberry trees and started to cultivate tobacco like Bursa immigrants who came from Greece in 1924 (Colak, 2013).

## CONCLUSIONS

Bursa is regarded as one of big centers in that silk trade an industry in history (Inalcık 2013). Anatolia, especially Busa was an very important station silk road in 15th and 16th century. Silk trade between Europe and Asia was made through Bursa. On that period Bursa became an entrepot city where the silk that came from Iran was weighted, stored and taxed (Sahan 2013). Sericulture in Bursa had big changes in 19th century. Modern catapult texture industry was begun to use instead of classical hand-made texture. Amount of silk product increased significantly by using mechanical industry. By the changes of wold textile industry, mechanized mass production was begun to use instead of hand-made and fine workmanship production. Then, cotton fabrics especially English cotton was begun to use rather than silky fabrics (Altun 2007). On the contrary Bursa was efficient on silk industry in that period when a lot of factory were established, a lot of people studied at new schools and specialized in sericulturing. Bursa has significant role on worldwide textile industry because of having big economical potential of silk texture industry (Altun 2007). However silk texture exists as a Turkish traditional handcraft in Amasya and Mugla.

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## **A MODEL OF NATURAL RENEWAL OF PINE TREES IN TAIGA ZONE**

### **SUMMARY**

The dynamic of the natural pine trees renewal process in Taiga zone based on mathematical modelling is examined. Dynamic modelling is used for prediction of natural renewal success depending on seeds' yield and bumper crop years' recurrence. In current study mean bumper crop recurrence is assumed to be once per 5 years, this means that a bumper crop year may occur in 4 years as well as in 6 years. The analysis of the state of natural renewal depending on maternal stand among main forest types is made. The bearing age of 30 years is assumed, with the maximum seed productivity age of 110-130 years, depending on the forest type. The data on trunk quantity distribution depending on age was obtained as a result of a simple sum. The algorithm of the sum has been implemented in Interpol procedure contained in the initial text file gav4.cpp. The initial distribution of trunk quantity depending on age was taken from the growth progress tables. The change of trunk quantity by decades was determined using extrapolation of known values according to power function  $N = 424316,4 \times A^{-1,3533}$ . The value of approximation certainty factor equals  $R^2 = 0.9857$ . The age of stands changes from 20 to 190 years. The amount of bearing trees aged 31-40 years old was assumed as 5-10%, whereas at the maximum bearing age (110-130 years old) the share of bearing trees totals 70-90% depending on the forest type. The presented model is discrete, dynamic, stochastic and descriptive. Stochasticity of the process is caused by the uncertainty in the quantity of bearing trees depending on age, uncertainty in the quantity of seeds from a single tree, uncertainty in the quantity of sprouts and in the occurrence of normal and bumper crops. To account for randomness a function  $ravnom(a,b)$  has been introduced, which generates uniformly distributed random numbers from  $a$  to  $b$ . Implementation of this function is possible using C++ language. Due to the stochasticity of the natural renewal process more implementations of the program are needed for a more reliable prediction.

**Keywords:** natural renewal, pine tree, logging, tree stand, live groundcover.

### **INTRODUCTION**

Dynamic models are an innovative tool for evaluating possible changes in the structure and state of forest phytocenoses [1, 2, 3, 4, 5]. Mathematical

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modeling is widely used in Russia's forestry complex [9, 10]. Dynamic modeling can also be used for predicting success of natural renewal depending on main factors which define the process' success: forest environment, maternal stand age, seed yield, recurrence of bumper crop years. Due to that it is necessary to draw attention of native researchers to modeling of natural processes. The first step towards that is development of existing models by means of their calibration and validation for use in certain forest environments. The goal of this study is the development of NAFOR model as the means of a long-term prediction of forest ecosystems renewal potential in north-western Russia.

## MATERIAL AND METHODS

### 1. Pine tree seed productivity

Seed bearing years—once per 5-6 years;

Bearing start age- 30 years;

Quantity of bearing trees aged 31-40 years old is 5-10%; at the maximum bearing age (130-160 years old) the share of bearing trees must be 70-90% (in following years the share of bearing trees is decreased each decade, down to 5-10% at 300-400 years old age);

number of seeds from a single tree is 400 in the first 10 years;

each following decade 100 seeds are added (10 each year, or differentially: 1-2 in the first year, 2-4 in the second year, 3-6 in the third year etc.) up till the age of 110 in an oxalis type forest, of 120 in a bilberry type, of 130 in a long moss type;

the peak of bearing is in the age of 110 in the oxalis type forest, 120 in the bilberry type, 130 in the long moss type;

further, the quantity of seeds is reduced by 100 in each following decade until the end of the life (by 10 each year, or differentially: 1-2 in the first year, 2-4 in the second year, 3-6 in the third year etc.);

each year 1 to 3% of seeds become sprouts; each year 50 to 95% of sprouts die off;

1 to 10% of trees live up to bearing age.

### 2. Prediction of trunk quantity (by age stages)

The pine starts bearing at the age of 30. The prediction must be made for 200 years. Therefore,  $A_{min}=30$ ,  $A_{max}=200$ . The initial distribution of trunk quantity depending on age is obtained from tables of growth progress [8]. Change of trunk quantity by decades was determined by extrapolating known values according to power function  $N= 424316,4x^A-1,3533$ . Age variable  $A$  varies from 20 to 190 years old. Approximation certainty factor equals  $R^2= 0,9857$  which is the evidence of the model's adequacy. The approximating function's appearance and its parameters have been obtained by programmatic means. Graphical results of approximation are presented on Figure 1. Curve 1 corresponds with initial data about trunk quantity by age classes, whereas curve 2 corresponds to modeling results.

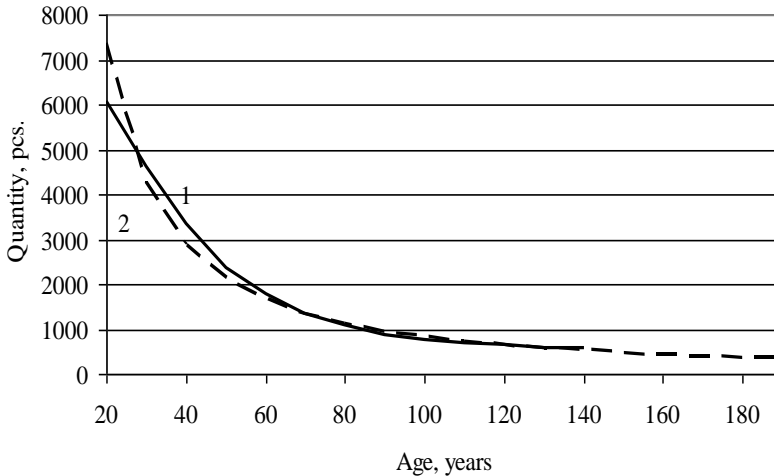


Figure 1. Approximation results (quantity of trunks)

The second stage which is tied to conversion of initial data is the decrease of the tree age interval to one year. For this purpose we will study the age change interval from  $A$  to  $A+10$  years, where  $A$  is divisible by 10. Let the trunk quantity of  $A$  age equal  $N(A)$  and the trunk quantity of  $A+10$  age equal  $N(A+10)$ . For each  $A+i$ ,  $i=0,1,2,\dots,9$  age we will define new trunk quantity of  $n(A+i)$  trees of  $A+i$  age in order for it to be linear according to  $i$ , to form a decreasing sequence and for the equation  $\sum_{i=0}^9 n(A+i) = N(A)$  to be true. The algorithm of this sum's

solution has been implemented in Interpol procedure which is contained in the initial text file gav4.cpp. As a result, we will obtain data on the trunk quantity distribution according to their age. It is worth noting that counting is performed from the bearing start age equaling  $A_{min}=30$  years old till  $A_{max}=200$  years old. The next step is adding seed quantity, sprout quantity and quantity of underbrush starting from 1 year old age to  $A_{min}$ . This is achieved by performing approximation using a power function  $N=92948,7 \times A^{-1,5064}$ . The approximation certainty is  $R^2=0,9884$ .

### 3. Forest renewal process description

The idea of modelling forest renewal process is in an opportunity to obtain information about a pine tree's renewal potential during  $t$  time point if there is information available from the previous time point  $t-1$ . The information is as follows:

- age and quantity of bearing trees;
- quantity of seeds appearing in the initial year;
- quantity of sprouts appearing in the next year;
- quantity of underbrush appearing in the next 3-5 years;
- quantity of trees reaching bearing age.

Above information depends on the whole previous history of the forest renewal process [6, 7, 9]. With that said, if any part of the information is absent, valuable prediction of further forest evolution is practically impossible. Thus, from the mathematical point of view, we are dealing with a multivariate random process.

To predict the process' development it is necessary to account for all factors which influence the process.

Complete information about the state of the forest tract in respect to the quantity of trunks is as follows:

- quantity of trunks  $N(a)$  with age  $A$  if  $A \geq A_{min}$ ;
- quantity of seeds (sprouts, underbrush) with age  $A$  if  $A < A_{min}$ .

If this information is known at the time point  $t-1$ , it is sufficient for obtaining similar information at  $t$  time point. Then, it is possible to trace the changes in the data from the starting year till any subsequent one. Thus, the concept model of the natural forest renewal is quite simple. The other matter is formalizing the model, i.e. obtaining recurrent correlations which allow to mathematically following the process' evolution.

According to classification of all kinds of mathematical models, current model is discrete, dynamic, stochastic and descriptive. Stochasticity of the process is caused by the uncertainty in the quantity of bearing trees depending on age, uncertainty in the quantity of seeds from a single tree, uncertainty in the quantity of sprouts and in the occurrence of normal and bumper crops.

Let the massive  $Au[t]$  characterize normal and bumper crop years. If  $Au[t]=0$  – the year  $t$  is normal, if  $Au[t]=1$  – the year  $t$  is a bumper crop year. For a pine tree bumper crop years occur, approximately, once per 5 years. This means that the bumper crop year may occur in 4 years as well as in 6 years.

To account for randomness a function *ravnom* ( $a,b$ ) has been introduced, which generates uniformly distributed random numbers from  $a$  to  $b$ . Implementation of this function is possible using C++ language.

## RESULTS AND DISCUSSION

### Determining quantity of bearing trees

Based on the initial data we receive a random implementation of the bearing trees percentage depending on their age. The time interval from  $A_{min}$  to  $A_{max}$  can be divided into 3 or 5 stages depending on bearing intensity. Let us examine the case where bearing intensity changes within 5 stages with the increase of the stand age. At the first, the third and the fifth stages the quantity of bearing trees is roughly constant, at the second stage it increases and at the fourth stage it decreases. Thus for a pine tree the first stage is defined by the age of trees from  $A_{n1}=31$  years old to  $A_{n2}=40$  years old. Within this interval the quantity of bearing trees is from  $p_{n1}=5\%$  to  $p_{n2}=10\%$ . The third stage is defined by the maximum bearing age of trees from  $A_{s1}=130$  years old to  $A_{s2}=160$  years old. The percentage of bearing trees within this interval is from  $p_{s1}=70\%$  to  $p_{s2}=90\%$ . The fifth stage corresponds to the age from  $A_{k1}=300$  years old to  $A_{k2}=400$  years old.

The percentage of bearing trees within this interval is from  $p_{k1}=5\%$  to  $p_{k2}=10\%$ .

By using linear extrapolation we will determine the percentage of bearing trees within the second and the fourth intervals.

The second interval corresponds to the age of trees from  $A_{n2}=40$  years old to  $A_{s1}=130$  years old. The range of percentage variation at the beginning of the second stage must be from  $p_{n1}$  to  $p_{n2}$  and the range of percentage variation at the end of the second interval must be from  $p_{s1}$  to  $p_{s2}$ .

The fourth interval corresponds to the age of trees from  $A_{s2}=160$  years old to  $A_{k1}=300$  years old. Thus, the range of percentage variation at the beginning of the fourth stage must be from  $p_{s1}$  to  $p_{s2}$  and the range of percentage variation at the end of the fourth interval must be from  $p_{k1}$  to  $p_{k2}$ .

If the percentage of bearing trees may vary from  $p_1$  to  $p_2$ , then for a normal year we will use the function  $ravnom(p_1, p_2)$ , and for a bumper crop year we will consider that the percentage of bearing trees is maximum and equals  $p_2$ . Mathematically this can be presented as follows. Let  $A_{n1} \leq A \leq A_{n2}$  (first interval), then the percentage of bearing trees can be expressed as a following ratio:

$$P(A) = \begin{cases} ravnom(p_{n1}, p_{n2}), & \text{if the year is a normal one} \\ p_{n2}, & \text{if the year is a bumper crop one} \end{cases}$$

Let  $A_{n2} < A < A_{s1}$  (second interval), then the percentage of bearing trees can be expressed as a following ratio:

$$P(A) = \begin{cases} ravnom(p_1, p_2), & \text{if the year is a normal one} \\ p_2, & \text{if the year is a bumper crop one} \end{cases},$$

where

$$p_1 = p_{n1} + \left( p_{s1} - p_{n1} \right) \cdot \frac{A - A_{n2}}{A_{s1} - A_{n2}}, \quad p_2 = p_{n2} + \left( p_{s2} - p_{n2} \right) \cdot \frac{A - A_{n2}}{A_{s1} - A_{n2}}.$$

Let  $A_{s1} \leq A \leq A_{s2}$  (third interval), then the percentage of bearing trees can be expressed as a following ratio:

$$P(A) = \begin{cases} ravnom(p_{s1}, p_{s2}), & \text{if the year is a normal one} \\ p_{s2}, & \text{if the year is a bumper crop one} \end{cases}$$

Let  $A_{s2} < A < A_{k1}$  (fourth interval), then the percentage of bearing trees can be expressed as a following ratio:

$$P(A) = \begin{cases} ravnom(p_1, p_2), & \text{if the year is a normal one} \\ p_2, & \text{if the year is a bumper crop one} \end{cases},$$

where

$$p_1 = p_{s1} + \left( p_{k1} - p_{s1} \right) \cdot \frac{A - A_{s2}}{A_{k1} - A_{s2}}, \quad p_2 = p_{s2} + \left( p_{k2} - p_{s2} \right) \cdot \frac{A - A_{s2}}{A_{k1} - A_{s2}}.$$

Let  $A_{k1} \leq A \leq A_{k2}$  (fifth interval), then the percentage of bearing trees can be expressed as a following ratio:

$$P(A) = \begin{cases} \text{ravnom}(p_{k1}, p_{k2}), & \text{if the year is a normal one} \\ p_{k2}, & \text{if the year is a bumper crop one} \end{cases}$$

The value of bearing trees percentage during a normal and a bumper crop year depending on age is easily calculated. At that, the age varies from 31 to 200 years old with an interval of one year.

Graphical illustration of  $P(A)$  function is given on Figure 2.

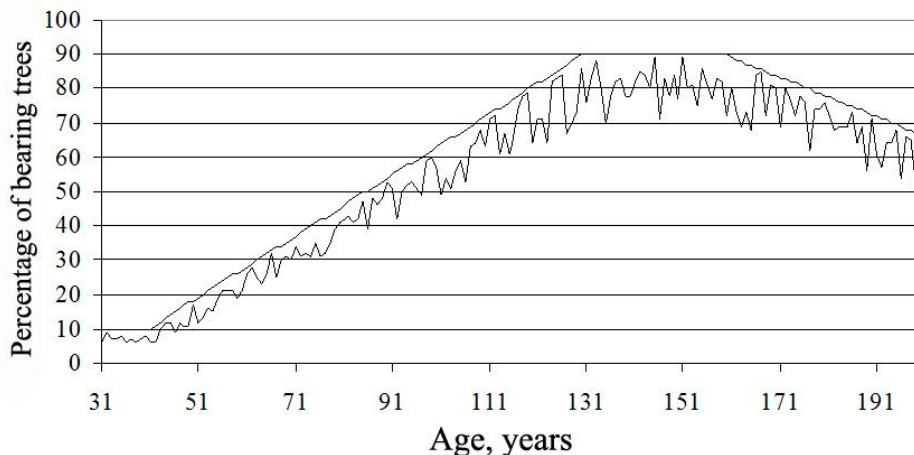


Figure 2. Variation of bearing trees quantity with the change of age

Serrated curve on Figure 2 is one of bearing trees percentage implementations for a normal year. Upon further executions of the program this implementation will be different. A number of such implementations gives an idea about variation of bearing trees percentage depending on their age. The second line which changes smoothly defines the bearing trees percentage for a bumper crop year.

#### Determining seeds quantity from a single tree

Let us examine another important characteristic – a forest's seed productivity. Its variation depending on age also corresponds to a certain implementation of the random process equaling a number of seeds from a single tree of  $A$  age. According to initial data the time interval from  $A_{min}$  to  $A_{max}$  can also be presented in 5 stages. At the first, the third and the fifth stage the quantity of seeds from a tree is roughly constant, at the second stage this quantity increases and at the fourth stage it decreases. For a pine tree the first stage is defined by the age of trees from  $A_{n1}=31$  years old to  $A_{n2}=40$  years old. During this stage the quantity of seeds from a single tree is  $m_{n1}=m_{n2}=400$ . The third stage is defined by the maximum bearing age of trees from  $A_{s1}=110$  years old to  $A_{s2}=130$  years old depending on a forest type. During this stage the quantity of seeds from a single tree reaches  $m_{s1}=m_{s2}=1100$ . The fifth stage corresponds to the age from  $A_{k1}=300$  years old to  $A_{k2}=400$  years old. The quantity of seeds at this point is so small that it can be considered zero:  $m_{k1}=m_{k2}=0$ .

Same as in the previous item, to determine the number of seeds at the second and the fourth stages we will use linear extrapolation.

The second stage corresponds to the age of trees from  $A_{n2}=40$  years old to  $A_{s1}=110$  years old. The range of a single tree seed quantity variation at the beginning of the second stage must equal  $m_{n1}=m_{n2}=400$ , and the range of a single tree seed quantity variation at the end of the second stage must equal  $m_{s1}=m_{s2}=1100$ .

The second stage corresponds to the age of trees from  $A_{s2}=130$  years old to  $A_{k1}=300$  years old. Due to that the range of seed quantity variation at the beginning of the second stage must equal  $m_{s1}=m_{s2}=1100$  and the range of seed quantity variation at the end of the second stage must equal zero:  $m_{k1}=m_{k2}=0$ .

It is necessary to account for a fact that within these intervals the quantity of seeds is a random value with defined mean values. If the seed quantity varies from  $m_1$  to  $m_2$ , then for a normal year we will use the function  $ravnom(m_1, m_2)$  and for a bumper crop year we will consider that the seed quantity from a single tree is maximum and equals  $m_2$ . Then we will obtain the following ratios for determining seed quantity from a single tree.

Let  $A_{n1} \leq A \leq A_{n2}$  (first interval), then the seed quantity from a single tree is expressed as a following ratio:

$$M(A) = \begin{cases} ravnom(m_{n1}, m_{n2}), & \text{if the year is a normal one} \\ m_{n2}, & \text{if the year is a bumper crop one} \end{cases}$$

Let  $A_{n2} < A < A_{s1}$  (second interval), then the number of seeds is expressed as a ratio:

$$(A) = \begin{cases} ravnom(m_1, m_2), & \text{if the year is a normal one} \\ m_2, & \text{if the year is a bumper crop one} \end{cases}$$

where

$$m_1 = m_{n1} + (m_{s1} - m_{n1}) \cdot \frac{A - A_{n2}}{A_{s1} - A_{n2}},$$

$$m_2 = m_{n2} + (m_{s2} - m_{n2}) \cdot \frac{A - A_{n2}}{A_{s1} - A_{n2}}.$$

Let  $A_{s1} \leq A \leq A_{s2}$  (third interval), then the number of seeds is expressed as a ratio:

$$M(A) = \begin{cases} ravnom(m_{s1}, m_{s2}), & \text{if the year is a normal one} \\ m_{s2}, & \text{if the year is a bumper crop one} \end{cases}$$

Let  $A_{s2} < A < A_{k1}$  (fourth interval), then the number of seeds is expressed as a ratio:

$$(A) = \begin{cases} ravnom(m_1, m_2), & \text{if the year is a normal one} \\ m_2, & \text{if the year is a bumper crop one} \end{cases}$$

where

$$m_1 = m_{s1} + (m_{k1} - m_{s1}) \cdot \frac{A - A_{s2}}{A_{k1} - A_{s2}},$$

$$m_2 = m_{s2} + \left( n_{k2} - m_{s2} \right) \frac{A - A_{s2}}{A_{k1} - A_{s2}}.$$

Let  $A_{k1} \leq A \leq A_{k2}$  (fifth interval), then the number of seeds is expressed as a ratio:

$$M(A) = \begin{cases} \text{ravnom}(m_{k1}, m_{k2}), & \text{if the year is a normal one} \\ m_{k2}, & \text{if the year is a bumper crop one} \end{cases}$$

Graphical illustration of  $M(A)$  function is given on Figure 3.



Figure 3. Variation of seed quantity from a single tree with the change of its age

A smooth upper line on Figure 3 reflects the change of seed quantity from a single tree for a bumper crop year. The lower changing line represents an implementation of the seed quantity from a single tree for a normal year. This implementation changes randomly but within preset limits. A combination of such implementations gives an idea about the change of seed quantity depending on the age of trees.

For a more precise forming of these lines it is necessary to account for the forest type and for primary forest estimation characteristics of studied stands. However, this condition doesn't considerably influence prediction of tree quantity.

#### Determining sprout quantity

The percentage of surviving sprouts and underbrush decreases with age  $A$  and towards the bearing age their remainder makes up from  $V_n\%$  to  $V_k\%$  relative to initial seed quantity. By modifying these values it is possible to receive different predictions of trunk quantity. Let  $V_n=40\%$  and  $V_k=55\%$ . Then linear extrapolation will give us the percentage of surviving sprouts depending on their age:



$$V(A) = \begin{cases} 100 - (100 - \text{ravnom}(V_n, V_k)) \times \frac{A - 1}{A_{\min} - 1}, & \text{if the year is a normal one} \\ 100 - (100 - V_k) \times \frac{A - 1}{A_{\min} - 1}, & \text{if the year is a bumper crop one} \end{cases}$$

The age changes from 1 year to  $A_{\min} = 30$  years (for a pine tree).

## CONCLUSIONS

Study of the natural forest renewal is a complex task, no matter what the type of a tree it is. Dynamic modelling was used to predict successfulness of natural renewal depending on maternal stand age, seed yield and bumper crop years' recurrence accounting for the type of the forest. Modelling of the process is tied to the necessity of performing multiple iterations of the developed program for obtaining various initial data. Such necessity is caused by the uncertainty in the bearing trees' quantity at each age stage, in the seed quantity from a single tree, in the share of sprouts from the total seed quantity, in a combination of normal and bumper harvests of seeds at different stages of stand development. Due to stochasticity of the process a large number of program's implementations are needed.

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## **ESSENTIAL OIL COMPONENTS AND ANTIMICROBIAL ACTIVITY OF PEPPERMINT (*MENTHA PIPERITA*) FROM MONTENEGRO**

### **SUMMARY**

Chemical composition of the essential oil of *Mentha piperita* L. (peppermint) from Montenegro was analyzed by gas chromatography-mass spectrometry (GC-MS) and its antimicrobial activity was evaluated. GC-MS analysis showed that major components of peppermint oil were menthol (33.15%), menthon (19.61%), 1,8 cineole (6.37%) and methyl acetate (5.63%).

Clinically isolated bacteria strains, Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli*), were used for the antimicrobial activity tests, and results were compared with peppermint essential oil originated from Spain. The obtained results revealed that the essential oil of *M. piperita* from Montenegro has rather strong antibacterial activity, especially against *Bacillus subtilis*. These results confirm the potential use of *M. piperita* from Montenegro and its essential oil in the medical field as well as in the food industry.

**Keywords:** *Mentha piperita* L., essential oil, chemical composition, antimicrobial activity

### **INTRODUCTION**

The genus *Mentha* (*Lamiaceae*) is composed of 19 geographically widespread species and 13 named hybrids (Chambers and Hummer, 1994). Peppermint (*Mentha piperita* L.) originated from Mediterranean region, but nowadays it is cultivated throughout all regions of the world. It is a hybrid mint, a cross between water mint *Mentha aquatic* and spearmint *M. spicata* L. (Frampton, 2009). *M. piperita* is a perennial herb, 50–90 cm high, and a prototypical member of the mint family (Singh et al. 2015). Peppermint generally grows best in moist, shaded locations and prefers acid, neutral and basic, light, medium soils. Flowering is from mid to late summer, while flowers are purple or pinkish having false spikes with numerous inconspicuous bracts and rarely bear seeds (Clark and Menory, 1980). The medicinal parts are the dried leaves, the fresh flowering plant, the whole plant and the essential oil extracted from the

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aerial parts of the flowering plant. In traditional medicine peppermint and its oil have been used as an aromatic, antispasmodic, antiseptic and also in the treatment of cancers, colds, cramps, indigestion, nausea, sore throat and toothaches (Briggs, 1993). The fresh or dried leaves are the culinary source of mint and are used in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gums, mint teas, beverages, jellies, syrups, candies, ice creams etc. (Hoffmann and Lunder, 1984). Peppermint essential oil and its constituents are commercially used in food, pharmaceutical and cosmetics industries. Thus, menthol is widely used in toothpaste, toothpowder, mouth fresheners, chewing gums, chewing tobacco, candies, analgesic balms, cough drops etc (Soković et al. 2009).

The chemistry of peppermint oil is very complex and highly variable. The relative concentrations vary depending on climate, cultivar, and geographic location (Lis-Balchin et al. 1997). Peppermint volatile oil composed primarily of menthol, menthone, menthofuran and menthyl acetate. Other pharmacologically active ingredients include bitter substances, caffeic acid, flavonoids, polymerized polyphenols, carotenes, tocopherols, betaine, choline and tannins (Soković et al. 2009). Measured low to moderate levels of phenolics with antioxidant activity were reported from peppermint (Zheng and Wang, 2001). Peppermint oil possesses antibacterial activity in vitro, against both Gram-positive and Gram-negative bacteria as well as antiviral and fungicidal activities (Hussain et al. 2010, Iscan et al. 2002, Farshbaf et al. 2004; Soković et al. 2009).

It was previously acknowledged that *M. piperita* essential oil and its major oil components are generally regarded as safe and full toxicology has been obtained (Soković et al. 2009).

Today, due to consumer awareness and negative perception of artificial preservatives, use of herbal extracts as “natural” products are fast developing segment of the industry. The huge production of essential oils (>70,000 tonnes per annum) with estimated market value of more than 700 million US \$, indicate that production and its consumption is increasing (Djilani and Dicko, 2012). Thus there are numerous initiatives all over the world related to the cultivation of aromatic and medicinal plants, including Montenegro.

The aim of this study is to determine the chemical composition together with the antimicrobial properties of the essential oil from leaves of cultivated *M. piperita* from Montenegro, as natural sources of antiseptics with potential applications in the pharmaceutical and food industry.

## MATERIAL AND METHODS

### Preparation of herb material

Fresh leaves of cultivated *M. piperita* were collected manually from Zeta (central part of Montenegro) at the beginning of June. Herb material was milled in a domestic coffee mill and, after sieving in ERWEKA set of sieves, sample with a mean particle diameter size of approximately 0.8 mm was obtained. A prepared batch was kept in an airtight resalable polypropylene bag and stored at

+6 °C for maximum 3 days before use, in order to avoid losses of volatile compounds.

### **Essential oil preparation**

Previously prepared *M. piperita* leaves (80 g) was submitted to hydrodistillation in a Clevenger-type apparatus for 2 hours according to Yugoslav Pharmacopoeia IV (1984). The obtained oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers and stored at 4 °C until analyzed by GC-MS.

Hydrodistilled peppermint essential oil for pharmaceutical purposes, originated from Spain, was purchased in pharmacy (produced by D.B.C.H, Spain) and used for results comparison.

### **Gas chromatography - mass spectrometry (GC-MS)**

The GC-MS analyses were carried out using a Shimadzu 2010+ gas chromatograph-mass spectrometer equipped with a ZB-5ms (30 m x 0,25 mm x 0,25 µm) capillary column. The column temperature was programmed from 35 °C (5 min) to 300 °C at 5 °C/min. The injection port temperature was 260 °C while the interface temperature was 305 °C. The samples of essential oil were injected by splitting and the split ratio was adjusted to 1:100. Helium was used as the carrier gas at a flow rate of 1.2 ml/min and 61.8 kPa inlet pressure. The MS conditions were: the ionisation voltage 70 eV, scanning interval 1.5 s, detector voltage 1.0 kV and *m/z* range 40 - 500. The components were identified by comparing their mass spectral data with those in the WILEY229 and the NIST107 mass spectra libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature and whenever possible, by co-injection with authentic standards (Fluka, Great Britain).

### **Antimicrobial screening**

The antimicrobial activity of the oil was evaluated by the disc diffusion method, using Mueller–Hinton agar for bacteria, by determination of inhibition zones. Two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria were used. Test microorganisms were obtained from Department for medical microbiology of Institute of Public Health in Podgorica. Cefalexin, and erythromycin were used as positive control in order to control the sensitivity of the *S. aureus* and *B. subtilis* and cefalexin and nalidixic for *E. coli*

A 100 µl suspension of any tested bacteria, containing about 10<sup>8</sup> cells/ml, was spreaded on Mueller Hinton Agar (MHA) using sterile swabs. Sterile blank disk 6.0 mm in diameter were impregnated with 5, 10 and 20 µl essential oil/disk and finally placed on the agar surface. Plates were incubated at 37 °C for 24 hours and then the inhibition zones were measured in diameters. Disks soaked in the solvent (5% DMSO) were used as a negative control.

All tests of inhibitory activity were carried out in duplicate and the developing inhibition zones were compared with those of reference disks.

## RESULTS AND DISCUSSION

The chemical composition of the hydrodistilled *M. piperita* essential oil from Montenegro as well as commercial sample of *M. piperita* essential oil from Spain is shown in Table 1. GC-MS analyses of peppermint essential oil from Montenegro revealed the presence of 36 compounds representing 100% of the total oil. The major components were menthol (33.15%), menthone (19.61%), 1,8 cineole (6.37%) and methyl-acetate (5.63%). Other major compounds in obtained essential oil were isomenthone (4.68%), neomenthol (3.90%), piperitone (3.23%), germacrene D (3.77%) and  $\alpha$ -caryophyllene (1.05%).

In our research, commercially available peppermint oil from Spain had higher content of menthol (40.42%), of menthone (14.41%), isomenthone (8.01%), isopulegol acetate (5.32%) and had lower content 1,8 cineole (7,15%) and methyl acetate (1,38%), in comparison to peppermint oil from Montenegro (Table 1). It is noteworthy that oxygenate sesquiterpenes were not detected in oil sample from Spain, while spatulenol (0.52%), veridiflorol (1.54%) and  $\alpha$ -cadinol (0.32%) were present in peppermint oil from Montenegro.

High menthol content is the main criterion in peppermint oil quality. According to European Scientific Organization for Phytotherapy (ESCOP), the menthol content should be 44% (ESCOP 1997).

The quality of aromatic herbs is greatly dependent on the growing area, the cultivation method and the production technology. We assume that the content of menthol would be much higher if herb leaves were collected during full flowering season (late June-July). Previous research confirms that plants subjected to shorter days (sunlight exposure) contained very small quantities of menthone and menthol. Also, it was found that, in order to get better results from leaf oil composition, leaf samples should be taken from the lower part, which contains more menthol and represents the oil content of most parts of mint (Aflatuni, 2005).

It is acknowledged that the specific chemical composition of herbal extracts produced in a particular geographical location is the result of a combination of factors such as genotype, ontogeny, light, temperature, water and nutrients (Pirbaoliti et al. 2013). It was found that three follow-up years are needed in order to understand better the optimum harvesting time (Aflatuni et al. 2000). Previous investigations on *M. piperita* essential oil composition are consistent with our results in which menthol and menthone were found to be the major compounds (Iskan et al. 2002; Soković et al. 2009). However, research of *M. piperita* essential oil from Brazil revealed that methyl acetate was the component obtained at the highest percentage (Scavroni et al. 2005).

Table 1. Chemical composition (%) of *Mentha piperita* essential oil

	Retention time	Compound	from Montenegro %	from Spain %
1.	11.228	$\alpha$ -pinene	0.70	1.06
2.	12.747	Sabinene	0.57	0.50
3.	12.882	$\beta$ -pinene	0.97	1.50
4.	13.432	$\beta$ -myrcene	0.76	0.30
5.	13.747	3-octanol	0.22	0.45
6.	14.619	p-cymene	0.11	0.15
7.	14.777	Limonene	1.77	2.85
8.	14.887	1,8 cineole	6.37	7.15
9.	15.073	cis- $\beta$ -ocimene	0.34	0.03
10	15.420	trans- $\beta$ -ocimene	0.07	-
11	15.778	$\gamma$ - terpinen	0.30	0.06
12	16.200	cis-4-thujanol	0.32	0.20
13	16.671	$\alpha$ -terpinolene	0.11	0.02
14	17.211	Linalool	0.23	0.19
15	18.735	Isopulegol	-	1.88
16	19.025	Menthone	19.61	14.41
17	19.187	Menthofurane	1.64	2.47
18	19.258	Isomenthone	4.68	8.01
19	19.415	Neomenthol	3.90	4.97
20	19.756	Menthol	33.15	40.42
21	19.999	Neoisomenthol	0.49	
22	21.440	Pulegone	2.50	1.00
23	21.897	Piperitone	3.23	1.72
24	22.925	Methyl acetate	5.63	1.38
25	23.429	Isopulegol acetate	0.09	5.32
26	24.065	Bicycloelemene	0.06	0.08
27	25.253	Copaene	0.04	0.11
28	25.472	$\alpha$ -burbonene	0.42	0.05
29	25.617	$\beta$ -elemene	0.23	0.60
30	25.697	Jasmine	0.46	-
31	26.418	$\beta$ -caryophyllene	3.57	2.36
32	27.985	Germacrene D	3.77	0.23
33	28.341	Bicyclogermacrene	1.01	0.09
34	28.885	$\delta$ -cadinene	0.30	0.09
35	30.311	Spatulenol	0.52	-
36	30.739	Veridiflorol	1.54	-
37	32.133	$\alpha$ -cadinol	0.32	-

Yield of *M. piperita* essential oils with respect to grouped components, is shown in Figure 1.

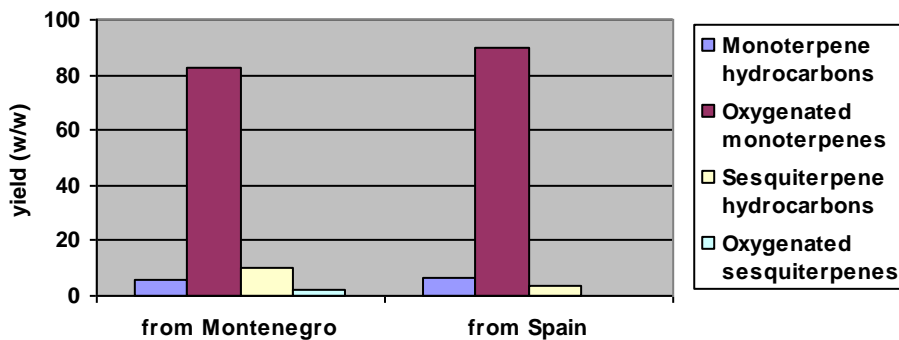


Figure 1. Yield (% , w/w) of *M. piperita* essential oils with respect to grouped components

The *M. piperita* essential oil from Montenegro (Figure 1) consisted mostly of oxygenated monoterpenes (82.6%) and sesquiterpenes (9.86%), while oil from Spain had 89.66% oxygenated monoterpenes (89.66%) and monoterpene hydrocarbons (6.47%).

As previously mentioned, the yield and composition of aromatic plants essential oil are, among other, influenced by harvest time (Araus et al. 2009). Thus, the researchers assume that the content of oxygenated monoterpenes might have been higher if herb was collected at flowering stage, due to the influence of phenological status.

Most of the antimicrobial activity of the essential oils has been attributed to the oxygenated monoterpenes, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antimicrobial properties, as their low water solubility limits their diffusion through the medium (Bakkali et al. 2008). In the literature, it was reported that various chemical compounds have direct activity against many species of bacteria, such as terpenes and a variety of aliphatic hydrocarbons (alcohols, aldehydes and ketones) (Rios and Recio, 2005). The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups, which is related to the present functional group and hydrogen-bonding parameters in all cases, are of main importance in the antimicrobial action of essential oils components (Couladis et al. 2004; Mancini et al. 2015).

It was found that most active antimicrobial compounds of essential oils are terpenes and phenolics and, thus, their mode of action might be similar to that of other phenolic compounds (Shunying et al. 2005). Individual essential oil contains complex mixtures of such compounds however, a little is known about the effect of interaction between individual constituents on antimicrobial activity.



Interactions between constituents may lead to additive, synergistic or antagonistic effects (Delaquis et al. 2002).

The antimicrobial plate diffusion assay for *M. piperita* essential oil, as summarized in the Table 2, showed that different microorganisms tested had different susceptibility to the same essential oil. The essential oil activities against tested microorganisms were increased with increased amount of investigated essential oil.

Table 2. Antimicrobial activity of the *Mentha piperita* essential oil and some standard antibiotics

<i>M. piperita</i> essential oil (µl) from Montenegro								Standard antibiotics (µg)		
								from Spain		
5	10	20		5	10	20	30	30	15	
Inhibition zone (mm)										
<i>Staphylococcus aureus</i>	21	33	42		23	35	45		25	32
<i>Escherichia coli</i>	22	36	41		26	38	46	29	19	
<i>Bacillus subtilis</i>	21	43	50		27	46	52		27	34

Peppermint essential oil from Montenegro shows very high antibacterial activity against *Bacillus subtilis* which were significantly susceptible to the essential oil at concentration of 10 and 20µl, with significant diameters of growth inhibition zones (43 and 50 mm), respectively. Essential oil from Montenegro showed the strongest antimicrobial activity against medically important pathogens *Staphylococcus aureus* and *Escherichia coli*, ranged from 21 to 42 mm and from 22 to 41 mm, respectively. For comparison, used standard antibiotics diameters of growth inhibition zones ranged from 14 to 32 mm (for *S. aureus*) and 22 to 34 mm (for *E. coli*).

Essential oil gained from *M. piperita* from Spain showed stronger antimicrobial activity (Table 2), probably due to the higher content of oxygenated monoterpenes, especially menthol. However, the difference in antibacterial activity between investigated peppermint oils was not high as expected, due to higher content of oxygenated compounds in Spanish oil. It was found that antimicrobial activity of peppermint oil is due to the presence of a mixture of

monoterpenes and oxygenated monoterpenes, particularly menthol (Iskan et al. 2002; Mimica-Dukić et al. 2003; Sivropoulou et al. 1995; Yadegarinia et al. 2006). The antimicrobial activity of the peppermint essential oil also could be associated with presence of 1,8 cineole and linalool, well-known chemical with their pronounced antimicrobial properties (Viljoen et al. 2003; Damjanović-Vratnica et al. 2011).

The components present in lower amount in oil, such as p-cymene, limonene, neoisomenthol and oxygenated sesquiterpenes might contribute to the antimicrobial activity, involved in some type of synergism with the other active compounds, which could be an explanation for rather similar antimicrobial activity of investigated peppermint essential oils. Identification of such compounds with wide biological activity is critical for mankind as it helps in the search for chemical structures (Damjanović-Vratnica et al. 2011) which should assist in designing new drugs as therapeutic against human pathogens.

### CONCLUSIONS

Chemical composition of the hydrodistilled essential oil of *Mentha piperita* L. (peppermint) from Montenegro was analyzed by GC-MS and major components identified were menthol (33.15%), menton (19.61%), 1,8 cineole (6.37%) and methyl-acetate (5.63%).

This study has shown that *M. piperita* essential oil possesses significant activity against different microorganisms, especially against *Bacillus subtilis*, which suggest that investigated peppermint essential oil could be used as preservative materials on foods, since it is natural, and generally non-toxic to humans.

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## ASSESSMENT OF YIELD AND QUALITY OF SPRING BARLEY DEPENDING OF FOLIAR FERTILIZATION

### SUMMARY

Barley is the fourth most important cereal in the world, right behind maize, wheat and rice, but before sorghum, oat and rye. In Republic of Macedonia, barley is sown on a quarter of the entire cereal production area. On the National variety list, there is only one registered variety of spring barley -Makedo. Barley grain yield can vary from year to year. It is highly dependent on of the climate conditions, as well as the agronomic measures applied during the vegetation period. One of the most important measures is the fertilization of the crops.

Starting from the fact that the use of chelate fertilizer solutions, using the spray-on foliar method is in the early development phase, an experiment was conducted using spring barley Makedo variety, and three types of chelate fertilizer solutions (Agrosal N12P5K7+ME, Agrosal NH<sub>4</sub>NO<sub>3</sub> 50% and Agrosal N31P0K0+ME), applied in 6 different concentrations (0.5, 1, 3, 5, 10 and 15 percent).

The average grain yield was 1 347 kg/ha-1, the best results of this trait were registered at the crops treated with Agrosal N12P5K7+ME in a 1 percent solution, while the lowest yield was measured on the crops treated with Agrosal N31P0K0+ME in the concentration of 0.5 percent. The quality highly varied depending of the different applied treatments. Highest protein content was registered on the crops treated with Agrosal N31P0K0+ME in a 10 percent solution, whilst the lowest on the crops treated with Agrosal N12P5K7+ME, in a 0.5 percent solution.

**Keywords:** spring barley, foliar fertilizer, yield, quality

### INTRODUCTION

Barley is the fourth most significant cereal in the world, right after corn, wheat and rice (FAO, 2013). Barley accounts for around 41 000 ha, of the total production of cereals in the Republic of Macedonia (State Statistical Office of the Republic of Macedonia, 2014). The majority parts of the varieties are imported

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autumn varieties. On the National variety list in Republic of Macedonia is registered the two rows spring barley - variety Makedo as the only domestic variety (MAFWE, 2008).

The efficient cereal production, including barley, largely depends on the applied agro-technics, especially the diet. Numerous tests have been performed, which confirmed that proper and balanced cereals diet is essential to improve the yield and quality and can directly and indirectly affect the efficiency of the other agro-technical measures (Negriľã and Negriľã, 1995; Popescu et al., 1997). Basic nutrients, such as nitrogen, phosphorus, potassium, sulfur and magnesium, are crucial elements in many processes in the development of the plant and the formation of the yield (Randahwa and Arora, 2000), but besides these elements, microelements play a large role in the quality of final product as well.

Common practice in cereal production is introduction of nutrients through the soil. However, recently it has been discovered that different nutritive solutions containing the elements, enable easy availability of nutrients to plants, which results in positive outcomes (Alaru et al., 2003; Arif et al., 2006). Because of the fact that soil application of nutrient solutions can lead to loss of nutrients (Dines et al., 2002; Follet and Delgado, 2002), over the last decades it has been confirmed for the fact that foliar application is the preferred option and it can reduce losses (Brar and Brar, 2004; Kinaci and Kinaci, 2001; Cakmak, 2008; Babaeian et al., 2011). Fast and efficient input of required elements into the plants, in the form of nutrient solutions is commonly performed through the leaf. This method has higher efficiency and lower cost and at the same time, it does not pollute the environment (Abbou El-Nour, 2002; Bozogi et al., 2011).

Foliar fertilization, i.e. supplementation of nutrients through the leaf, represents an efficient fertilization technique which highlights the availability of nutrients. In more developed countries, this application is simplified through a large-scale sprinkling irrigation (linear machines, ranger machines, hydromatics etc.), a process known as fertigation, or simultaneous irrigation and feeding. In this context, Tanaskovik and Chukaliev (2014) point out that in the United States fertigation of agricultural crops (corn, soybeans, etc.) is conducted via sprinkling irrigation systems, where instead of conventional sprinklers, micro-sprinklers are set near the surface. Quite often, besides fertigation, pesticides are applied via this system, a process known as chemigation. Unfortunately, due to the separation of production area on small plots, such a technique where really big irrigation systems can efficiently and economically operate is not applicable in our country.

Awasti and Brahm (1994) conclude that the barley yield is increased by increasing the dose of nitrogen. In addition to this element, a number of studies so far have proven the usefulness of treating cereals with nutrient solutions containing other important elements (Diaz-Zorita et al., 2001; Kinaci and Kinaci, 2001; Demirer et al., 2004), whereby the yield and the quality are enhanced with positive effects.

Foliar application of nitrogen at different development stages of wheat and triticale development improve seed quality and increase yield (Brar and Brar, 2004; Saeed et al., 2012). Well balanced nitrogen and phosphorus fertilization increases the nitrogen content of the wheat grain, but also affects the gluten formation (Hera et al., 1996; Brucher and Moroy, 1988).

Nitrogen deficiency, which occurs due to constant dynamics of this element in the soil, can cause poor growth and poor plants vitality, yellowing of leaves, formation of tiny leaves, falling leaves, forming a less branched root system, small and poor quality fruits etc. The nitrogen supply of the crop is one of the essential measures for successful and quality agricultural production.

From the farmers' perspective, it is difficult to achieve high yields and high protein content in grain at the same time, because these properties are usually inversely proportional. Bhatia and Rabson (1976) and Sinclair and DeWitt (1995) have shown that the simultaneous increase in yield and protein content was incompatible in terms of energy. For best growth and development of plants, specific amounts of certain nutrients at certain times are required (Sajid et al., 2008).

The research results of Bosev et al. (2013) showed that different treatments with nutritive solutions have significant effects on wheat and triticale. Applications of different concentrations of nutritive solutions have shown increased protein synthesis and content of wet gluten in wheat and triticale (Bosev et al., 2012). Other researchers also point out that foliar application of nutrient solutions has positive impact on the yield of wheat grain (Yassen et al., 2010; Matilo et al., 2006; Slaton et al., 2011). Römheld and El-Fouly (1999) came to the conclusion that the effectiveness of foliar application is better than that of soil application of nutrient solutions, because in this way, the intake of necessary nutrients is focused directly at the site where it is needed most - in the leaf, and has relatively rapid absorption.

In Macedonia, there is data deficiency regarding the effects of the use of nutritive solutions in barley. Considering the fact that in Macedonia the use of nutritive solutions in cereals is in nascent, while in other crops has seen rapid rise, the aim of this research was to determine the effects of different nutritive solutions at different concentrations on grain yield and protein content in brewing barley. Through the results of this research it will be able to see the influence of different concentrations of nutritive solutions, applied at different stages of plant development, on the analyzed traits. Determination of the necessary dosages and the type of solution required for obtaining higher yields and adequate quality of barley will result in lowering the cost of fertilization, which will further lead to a reduction of the production costs of brewing barley. Such measures can ultimately lead to the spread of the areas sown with brewing barley in Macedonia.

## **MATERIAL AND METHODS**

The experiments of this research were conducted in the Skopje region (41°56'58"N and 21°25'06"E, 550 m above sea level), with the two rows brewing

barley, variety Makedo. The experiment was designed as CRBD, with three fertilizers and six concentrations, in three replications. The blocks represent the nutritive solution, which are marked with the letters A, B and C, and refer to the following nutritive solutions:

Agrosal N12P5K7+ME (A)- liquid mineral fertilizer which, in addition to basic nutritive elements, contains microbiogenetic elements in chelated form (Table 1).

Table 1. Chemical content of Agrosal N<sub>12</sub>P<sub>5</sub>K<sub>7</sub>+ME (%)

Total Nitrogen (N <sub>2</sub> )	12.00 ± 0.7%
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	5.00 ± 0.7%
Potassium (K <sub>2</sub> O)	7.00 ± 0.7%
Microelements in chelated form: Mg, Cu, Zn, Mn, Ca, Co, Fe, B	

Agrosal NH<sub>4</sub>NO<sub>3</sub>50% (B)- liquid nitrogen fertilizer where nitrogen is in ammonia and nitrate form (Table 2).

Table 2. Chemical content of Agrosal NH<sub>4</sub>NO<sub>3</sub>50% (%)

Ammonia Nitrate (NH <sub>4</sub> NO <sub>3</sub> )	48.00 % - 52.00%
Total Nitrogen(N <sub>2</sub> )	16.80 % - 18.20%

Agrosal N<sub>31</sub>P<sub>0</sub>K<sub>0</sub>+ME (C)- liquid nitrogen fertilizer, in which nitrogen is found in all three forms (ammonia, nitrate and amide), and contains iron and magnesium (Table 3).

Table 3. Chemical content of Agrosal N<sub>31</sub>P<sub>0</sub>K<sub>0</sub>+ME (%)

Total Nitrogen(N <sub>2</sub> )	31.00 ± 1.5%
Ammonia nitrate (NH <sub>4</sub> NO <sub>3</sub> )	37.00 ± 2.0%
Microelements in chelated form: Mg, Fe	

The variants of the concentrations used in the study are marked with numbers 1 to 6 and refer to the following concentrations of the solution: 0.5% (1) 1% (2) 3% (3) 5% (4) 10% (5) and 15% (6).

Prior to setting up the experiment, in order to determine the application irrigation rate with the nutrient solution, an agro-chemical analysis of the soil was done, and retention of moisture and bulk density were determined. The plots were 5 m<sup>2</sup> each, and each plot had 8 rows positioned at a distance of 12.5 cm. During the vegetation, the plots were kept clean of weeds. The treatment with nutrient solution was done manually, in the following phases of plant growth: tillering, stem extension, head visible and early maturity of the grain. During the harvest, all plants were harvested from the plots in order to determine the yield of grains (kg ha<sup>-1</sup>) and the protein content (%).

The analysis of the yield of grain results was calculated to 14% moisture, and the protein content was determined by the method of kjeldal. The results were statistically processed by the method of analysis of variance (ANOVA), and



mean values for the properties in respect of all the factors involved in the survey were analyzed by LSD-test.

## RESULTS AND DISCUSSION

### Analysis of variance

In order to determine the effect of the factors (nutritive solution and concentration) as well as their interactive impact on analyzed characteristics, an analysis of variance – ANOVA was conducted. This analysis provides insight into the impact on individual examined traits, as well as the interaction influence of the factors on their performance.

Table 4. Influence of the source of variability on the analyzed traits

Source of variation	Replication	Fertilizer	Concentration	Interaction	Error
Df	2	2	5	10	34
Yield	37742ns	267350**	210108.6**	316168**	21220.7
Proteins	0.099ns	10.861**	2.415**	5.999**	0.114

Based on the obtained values (Table 4), it can be concluded that the fertilizer concentration had a very significant impact on the tested properties, meaning that it is crucial for their expression. Nutrient solution showed highly significant influence on both yield and quality. The interaction of the concentration with the nutrient solution had highly significant effect on the tested properties, which confirms the fact that apart from the right choice of the nutrient solution, it is crucial to determine the concentration of the same. These two factors together are the key to getting good results in brewing barley cultivation.

### Grain yield

Barley grain is the main product and the main reason for growing barley as well, except when used in silage, or as biomass for biogas production, when the whole plant is being used. The yield is limited by the genetic potential of the plant itself, but it largely depends on the technical measures applied to the crop. So far, there are numerous surveys confirming the link between foliar application of nutritive solutions and yield. Thus, Shah and Saeed (1989) came to the conclusion that the barley grain yield has increased by foliar application of nitrogen solutions and Fathi et al. (1990) and Szafranski (1995) concluded that this characteristic is directly related to the dosage of nitrogen applied.

The results of our study are shown in Table 5. In terms of the interaction of the two factors in the experiment, the highest grain yield was seen at A2 (2355 kg ha<sup>-1</sup>), while the lowest in C1 (988 kg ha<sup>-1</sup>). Statistically, these values were significantly different in comparison to all other combinations of nutritive solution and concentration.

If the effect of the concentration in the blocks is analyzed in details, in block A, the highest value was achieved with the plants treated with two concentrations (2355 kg ha<sup>-1</sup>), and the lowest using concentration 4 (1047 kg ha<sup>-1</sup>)

<sup>1</sup>). Blocks treated with solutions B and C obtained similar results with each other, that is, the highest yields were observed in treatment 4, and the lowest in treatment 2.

Table 5. Grain yield (kg ha<sup>-1</sup>)

	1	2	3	4	5	6	Average
A	1444	2355	1221	1047	1342	1244	1442
B	1104	1269	1146	1318	1260	1163	1210
C	988	1284	1592	1643	1383	1454	1391
Average	1179	1636	1320	1336	1328	1287	1 348

Fertilizer LSD<sub>0.05</sub>=82 Concentration LSD<sub>0.05</sub>=56 Interaction LSD<sub>0.05</sub>=51  
 Fertilizer LSD<sub>0.01</sub>=195 Concentration LSD<sub>0.01</sub>=94 Interaction LSD<sub>0.01</sub>=77

In terms of the impact of the concentration of the solutions, the average values of the yield of grain indicate that the highest value of grain yield (1636 kg ha<sup>-1</sup>) was observed in treatment 2, while the lowest has been reported in treatment 1 (1179 kg ha<sup>-1</sup>). The differences are statistically significant compared to other applied concentrations. The highest average yield was obtained in the block treated with the fertilizer A (1442 kg ha<sup>-1</sup>), followed by the fertilizer C (1391 kg ha<sup>-1</sup>). The lowest yield was achieved in the block treated with B (1210 kg ha<sup>-1</sup>), statistically significantly lower yield compared with other fertilizers. Mean values for blocks have confirmed the importance of balanced diet, i.e. the participation of all elements, as well as the justification of the application of foliar nutrient solutions. Namely, the use of relatively low concentrations resulted in higher yields. Additionally, the use of multiple components nutrient solutions (NPK), required lower concentrations of the solution compared to the solutions dominated by nitrogen.

### Protein content

The protein content is one of the important features of the cereals, which is an indicator of their quality. Two rows brewing barley, compared to wheat and other cereals used in bakers' industry, differs in a way that with it, this property is coveted to have as lower values as possible, if used in brewing industry. The reason for this is because the protein can cause biochemical instability of the brewing, which usually manifests as foggy brewing (Beer Haze), what means clouding of beer caused by proteins breakdown.

The results obtained by Jasvinder (2012) in the experiments with different N<sub>2</sub> doses on the two rows brewing barley, show that the protein content was proportional to the amount of nitrogen applied. Similar results have been obtained by Tarjoc and Tabara (2011) in the tests they had performed on brewing barley with foliar application of different nutritive solutions.

From the results presented in Table 6, it can be concluded that the highest protein content in terms of interaction effect of the two factors was obtained in treatment C5 (12.91%), while the lowest in treatment A5 (8.07%), which is in

line with the previous investigations conducted by several authors. Statistically these results are significantly different from the other set for this parameter.

Table 6. Protein content (%)

	1	2	3	4	5	6	Average
A	8.82	9.55	12.26	8.95	8.07	10.57	9.71
B	10.61	11.31	9.91	11.73	11.86	11.06	11.08
C	10.93	8.96	11.79	11.63	12.91	9.89	11.02
Average	10.12	9.94	11.32	10.77	10.95	10.51	10.60

Fertilizer  $LSD_{0.05}=0.19$  Concentration  $LSD_{0.05}=0.13$  Interaction  $LSD_{0.05}=0.13$

Fertilizer  $LSD_{0.01}=0.45$  Concentration  $LSD_{0.01}=0.22$  Interaction  $LSD_{0.01}=0.18$

If we analyze each block separately, in order to recognize the influence of the concentration in a particular block, we can say that, in block A, the highest percentage of protein showed plants in treatment 3 (12.26%) and the lowest in treatment 5 (8.07%). In block B, the highest results were obtained with the dose 5 (11.86%), as opposed compared to the results obtained in the previous block. Similar values have been achieved with dosage 4 (11.73%), so that statistically non significant differences were found between these values. The plants treated with the dose 3 (9.91%), had the lowest content of protein. In block C, the highest values were measured in plants treated with nutritive solution concentration 5 (12.91%) and the lowest in plants with a dose 2 (8.96%). These results support the claim that protein synthesis is closely dependant on the amount of nitrogen applied on plants.

From the perspective of the influence of nutrient solutions on average values of protein content, the highest share of protein was measured in the block B (11.08%). Similar results were found in block C (11.02%), and the lowest in block A (9.71%), which is the only one that showed a statistically significant difference compared to blocks B and C. In terms of the effect of concentration, the highest average values had dose 3 (11.32%), which significantly differed from other concentrations, and the lowest values were obtained in dose 2 (9.94%). The remaining doses showed similar values ranging from 10.12% to 10.95%.

## CONCLUSIONS

From the results of the tests conducted on the *Makedo* two-rows brewing barley, one may perceive which nutritive solutions and in what way they affect the analyzed properties, if applied foliar at different concentrations. The highest yield of grain is determined in plants treated with 1% solution of Agrosal  $N_{12}P_5K_7+ME$ , and the highest yield in terms of the impact of nutrient solution showed the plants treated with Agrosal  $N_{12}P_5K_7+ME$ . The highest concentration of protein is observed in the plants treated with 10% solution of Agrosal  $N_{31}P_0K_0+ME$ , and the lowest in the plants treated with 10% solution of Agrosal  $N_{12}P_5K_7+ME$ .

If barley is grown in order to be used in the brewing industry, then the most appropriate treatment is with Agrosal  $N_{12}P_5K_7+ME$  in 10% solution concentration, because this combination of nutrient solution and dosage results in low protein content. If the purpose of cultivation is the use of this crop as fodder, in that case Agrosal  $N_{31}P_0K_0+ME$  and concentration of 10% solution is recommended, because this treatment provides plants whose grains have the highest proportion of protein.

On the other hand, treatment with Agrosal  $N_{12}P_5K_7+ME$  at a dose of 1%, showed the highest yields and acceptable low percentage of protein, which would be a good combination for brewing industry production of as well as for fodder.

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## THE INFLUENCE OF INOCULATION ON CHEMICAL COMPOSITION, QUALITY AND PROTEOLYSIS IN SILAGES MADE FROM WHOLE MAIZE PLANT AND ALFALFA

### SUMMARY

The paper presents the results for ensiling of the whole maize plant alone or in mixing with alfalfa. In this experiment maize FAO maturity group 600 and alfalfa at the end of butonization phase (wilted material with 390 gkg-1 dry matter), was used for ensiling. The experiment was organized as two-factorial (3 × 2), with three replications, where factor A was the different amount of alfalfa (A1=0%, A2=15%, A3=30%,) and factor B was inoculation (B1= no inoculant, B2= with inoculant). All silages after the treatment were compressed in plastic experimental siloses with volume of 60 dm<sup>3</sup>. After 56 days the experimental siloses were opened and representative samples were taken for chemical analyses. Chemical composition and silage quality were analysed in the Laboratory for nutrition of domestic animals on the Faculty of Agriculture of the University of Belgrade. Statistical analysis was performed by software Statsoft (2006), where the analysis of variance examined the significance of the factors. Along with the increase of the share of alfalfa in silages, there was increase of crude proteins, lipids, cellulose, ash, pH value and production of ammonia, with the decreasing share of NFE (p<0.05). In treatments with inoculant increase in amount of lactic acid was noticed, as well as lower amount of ammonia nitrogen and acetic acid, which can be significant for aerobic stability of silage. All silages, with a slight variation in the point number, were ranked in the 1st quality class according to the Flieg method.

**Keywords:** maize plant, alfalfa, inoculants, silage, quality

### INTRODUCTION

Maize and alfalfa are important for cattle nutrition throughout the world, and so are in Montenegro where area of their cultivation depends on climate, altitude and soil quality (Dubljević et al., 2013a, 2013b). High energy value of entire plant, cob and grain is being excellently supplemented with high protein content of alfalfa, why those two feeds are regularly combined in cattle diet (TMR – total mixed ratio), mainly in the form of silage of entire maize plant, alfalfa hay or haylage and maize grain flour (Dewhurst, 2013). This possibility

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can be used during the silaging of entire maize plant by adding certain amount of green or wilted alfalfa fourth or fifth cut. Technology of combining grains and legumes during silaging process was researched in number of experiments (Đorđević et al., 2010a). Maize and alfalfa silaging enables better balancing of energy and protein but negative influence of alfalfa on non-degradable protein fractions is expressed (Slottner and Bertilsson, 2006). However total combined silage quality mainly stays unchanged which was determined by Koljajić et al. (1998) when 20 and 50% of wilted alfalfa was included in maize silage. Alfalfa share during silaging process mostly depends on available amounts which are low in fourth and fifth cut because of high temperatures and low precipitation during summer (Dinić et al., 2014). Therefore the choice of silaging time is chosen according to entire maize plant silaging optimal phase, and that is grain waxing phase (Đorđević et al., 2010b). In this case alfalfa nutritional value often can be lowered because it varies during growth (Peyraud et al., 2009; Božičković et al., 2015).

Inoculant usage became usual practice during silaging. Inoculants speed and direct fermentation in legume silage, while in maize silage they increase aerobic stability (Lynch et al., 2011; Đorđević et al., 2009a,b).

Goal of this paper was to research maize silaging possibility with addition of small amounts of wilted alfalfa (15 and 30%), in order to achieve better nutritional value of silage and maximal usage of all available feeds at the farm. In order to achieve better silage quality usage of inoculants was planned

### MATERIAL AND METHODS

Whole maize plant (FAO maturity group 600) and alfalfa experiment was set as two-factorial ( $3 \times 2$ ;  $n=3$ ), where A factor was amount of wilted alfalfa in silaging biomass ( $A_1=0\%$   $A_2=15\%$   $A_3=30\%$ ), and inoculant was used as B factor ( $B_1$ =without inoculant;  $B_2$ =with inoculant). Inoculant used was BioStabil Plus and it contains homo-fermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) which intensifies and directs fermentation, as well as hetero-fermentative lactic acid bacteria (*Bacillus brevis*) whose products increase aerobic silage stability. For silaging 60 dm<sup>3</sup> plastic containers closed with threaded lid were used.

Samples for laboratory analysis in order to determine the nutritional value and quality of silage were taken 56 days after ensiling. Chemical analysis of silage samples was performed in the laboratory of animal nutrition at the Agricultural faculty of the University of Belgrade. The parameters of the chemical composition were determined according to AOAC (2002) methods, the amount of lactic, acetic and butyric acids were determined by distillation method according to Wiegner (1926), the amount of ammonia nitrogen by a modified Kjeldahl's method (Dulphy and Demarquilly, 1981). The quality of silage was evaluated using the Flieg (Đorđević and Dinić, 2003) methods. Statistical analyse of obtained results was done with analysis of variance procedure with software package Statistica v.6. (Statsoft, 2006).



## RESULTS AND DISCUSSION

Compared to starting material, whole maize plant silage (with and without inoculant) had couple of percent higher amount of moist, which can be explained by loss of volatile matters during drying of sample at 105°C with the goal to determine amount of dry matter content (table 1). With the increase of alfalfa amount in silage, level of dry matter increased. In all silages determined amount of dry matter was higher than 350 gkg<sup>-1</sup>, while at the start extraction of juices was stopped and maximal control over butyric acid fermentation was achieved (Đorđević and Dinić, 2003).

Amount of crude protein was increased in silages along with the increase of alfalfa share ( $p < 0.05$ ). Silage containing 30% of wilted alfalfa had by 60% higher level of protein than silage made of whole maize plant. Silage that contained inoculant averagely contained significantly higher level of crude protein, which can be explained by lower level of proteolysis, e.g. low losses of volatile matters (NH<sub>3</sub>N), in lower pH value conditions.

Inoculation effect did not significantly influence the amount of fat, cellulose, nitrogen free extract (NFE) and mineral matters. However, with increase of alfalfa share, fat, cellulose and ashes level was significantly increased while NFE level dropped. Amount of crude fat in all silage was higher compared to starting sample, which was due to relative increase of same during the loss of volatile matters, also as result of lactic acid extraction (as non-volatile) by diethyl ether (Soxlet method) used for determining amount of crude fat (Đorđević et al., 2003).

Table 1. Chemical composition of starting material and silages, gkg<sup>-1</sup> DM

Starting material	Inoculant	DM gkg	Proteins	Lipids	Cellulose	NFE	Ash
Whole maize plant (WMP)		358.26	74.52	60.71	168.35	649.58	46.84
Alfalfa (A)		388.30	216.47	61.04	252.18	345.10	125.29
Silages							
A <sub>1</sub> (100% WMP)	B <sub>1</sub> (-)	360.31	72.28	66.22	171.27	645.67	44.56
	B <sub>2</sub> (+)	363.07	76.56	57.27	164.42	656.51	45.24
A <sub>2</sub> (85% WMP+15%A)	B <sub>1</sub> (-)	365.35	95.31	71.32	186.22	589.82	57.33
	B <sub>2</sub> (+)	363.96	98.43	78.61	183.73	580.96	58.27
A <sub>3</sub> (70% WMP+30%A)	B <sub>1</sub> (-)	370.20	116.56	92.50	195.84	534.02	61.08
	B <sub>2</sub> (+)	375.11	120.23	80.18	198.11	540.93	60.55
Average for A <sub>1</sub>		361.69c	74.42c	61.74c	167.84c	651.09a	44.90c
Average for A <sub>2</sub>		364.66b	96.87b	74.96b	184.98b	585.39b	57.80b
Average for A <sub>3</sub>		372.66a	118.40a	86.34a	196.98a	537.48c	60.82a
Average for B <sub>1</sub>		365.29	94.72B	76.68	184.44	589.84	54.32
Average for B <sub>2</sub>		367.38	98.47A	72.02	182.09	592.80	54.69
Significance for A		*	**	*	**	**	**
Significance for B		ns	*	ns	ns	ns	ns

ns – no significance; \* ( $p < 0.05$ ); \*\* ( $p < 0.01$ )

pH value varied in the interval 3,91 – 4,28, which secured stable conditions for silage quality without additional (butyric) fermentation (table 2). According to McDonald (1991) for butyric fermentation high level of moist is needed (over 70%), as well as pH value over 4,5. With increase of alfalfa share amount of ammonia nitrogen increased. That can be explained by increase of alfalfa protein part whose characteristic is higher level of hydrolysis, which is one of the important specific traits of this species (Owens et al., 2002). Ammonia nitrogen in silage is main indicator of protein degradation; it is created as result of reaction between proteolytic enzymes originating from plant cells and microorganisms, above all butyric clostridia. Ammonia presence in silage which does not contain butyric acid is result of plant enzyme reactions.

Increase of alfalfa share in silage did not significantly influence the production of lactic and acetic acid shown in absolute value. However, when alfalfa was added relative share of acetic acid increased which can be considered positive as it helps increasing the aerobic stability of silage. On the other hand, inoculant use influenced increase of lactic acid production and decrease of acetic acid production which helped achieve lower pH values.

To assess silage quality Flieg method was used, which takes relative share (percent) of lactic, acetic and butyric acid. This method is considered as most objective method for determining maize silage quality. According to used method all silages were appointed with highest (I) class, even with small variations in number of points (table 3).

Table 2. Parameters of biochemical changes in silages, gkg<sup>-1</sup> DM

Silages	Inoculant	pH	NH <sub>3</sub> -N, gkg <sup>-1</sup> N	Lactic acid	Acetic acid	Butyric acid
A <sub>1</sub> (100% WMP)	B <sub>1</sub> (-)	3.96	86.15	55.18	28.34	0.00
	B <sub>2</sub> (+)	3.91	87.42	54.87	18.46	0.00
A <sub>2</sub> (85% WMP+15% A)	B <sub>1</sub> (-)	4.14	103.11	52.75	42.57	0.00
	B <sub>2</sub> (+)	4.08	98.37	59.42	22.64	0.00
A <sub>3</sub> (70% WMP+30% A)	B <sub>1</sub> (-)	4.28	108.70	56.31	36.91	0.00
	B <sub>2</sub> (+)	4.25	105.18	55.06	24.35	0.00
Average for A <sub>1</sub>		3.94c	86.78c	55.02	23.40	0.00
Average for A <sub>2</sub>		4.11b	100.74b	56.08	32.60	0.00
Average for A <sub>3</sub>		4.26c	106.94a	55.68	23.88	0.00
Average for B <sub>1</sub>		4.13A	99.32A	54.75B	35.94A	0.00
Average for B <sub>2</sub>		4.08B	96.99B	56.45A	21.82B	0.00
Significance for A		**	**	ns	ns	-
Significance for B		*	*	*	*	-

ns – no significance; \* (p<0.05); \*\* (p<0.01)

Table 3. Relative ratio of acids (%) and silage quality by Flieg

Silages	Inoculant	Ratio of acids, %			Points	Quality class by Flieg
		Lactic	Acetic	Butyric		
A <sub>1</sub> (100% WMP)	B <sub>1</sub> (-)	66.07	33.93	0.00	49	I
	B <sub>2</sub> (+)	74.83	25.17	0.00	50	I
A <sub>2</sub> (85% WMP+15% A)	B <sub>1</sub> (-)	55.34	44.66	0.00	44	I
	B <sub>2</sub> (+)	72.41	27.59	0.00	50	I
A <sub>3</sub> (70% WMP+30% A)	B <sub>1</sub> (-)	60.41	39.59	0.00	46	I
	B <sub>2</sub> (+)	69.33	30.66	0.00	48	I

## CONCLUSIONS

Whole maize plant in waxing grain phase represents good material for silaging as it contains a lot of fermenting carbohydrates which during fermentation give averagely 70% of lactic acid out of all acid amounts. When 30% of wilted alfalfa is added increase of crude protein share by 60% happens, while quality of the silage remains the same. When share of alfalfa is increased ammonia nitrogen amount increases which has to be taken in to consideration while balancing the diet for ruminant as part of degradable proteins. Use of selected inoculants lead to increase of lactic acid and decrease of acetic acid amount which did not affect the silage quality.

Based on obtained facts it is recommended to add wilted alfalfa during maize silage (up to 30% of fresh mass), it will increase protein amount in silage, achieve better balance between energy and protein without significantly changing the silage quality. Use of inoculants for this type of silage is not significant for quality but can be significant for aerobic stability; therefore research should be continued in that direction.

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## **RESPONSE OF LEAF NUMBER AND PLANT HEIGHT OF POLKA DOT PLANT (*HYPOESTES PHYLLOSTACHYA*) UNDER VARIOUS INDOOR LIGHTS**

### **SUMMARY**

Variegated foliage plants such Polka Dot Plant (*Hypoestes phyllostachya*) as are often used in interiorscaping in low light environments. The changes in leaf number and plant height under various light types (blue, red and blue+red LED (light-emitting diode) and fluorescent) were investigated to elucidate their optimum indoor light environment. The changes in plant height (was different from leaf number. In general, plant height increased with increasing time. Leaf number showed no significant changes in first sampling decades under different light treatments. The analysis of variance results indicated significant differences for plant height in different light treatments for all sampling decades except the first, second and third decades, but the results of analysis of variance for leaf numbers indicated non-significant differences among different light treatments. The comparison of means for plant height in the first, second and third decades indicated that there are no significant differences among light treatments while in next five decades, red light treatment showed the long plant height. Also, comparison of means for leaf numbers under various light treatments via Duncan's new multiple range test indicated that there is not any significant differences among different light treatments in first five decades and seventh decade while in the sixth decade, blue light treatment had the highest leaf numbers following to red and blue+red light treatments. In the eighth decade, blue and red light treatments had the highest leaf numbers following to blue+ red light treatment. This study revealed that the most suitable light treatment for obtaining short and high leaf numbers in Polka Dot Plant were blue or blue+red treatments.

**Keywords:** Blue light, Fluorescent, Red light, Polka Dot Plant.

### **INTRODUCTION**

Polka Dot Plant (*Hypoestes phyllostachya*) is a lively and beautiful little plant with brightly spotted leaves that stand out especially well against other plants. It is a perennial herb, native to Madagascar, with ovate leaves marked with lavender-pink spots (Moronkola et al. 2009). Polka Dot Plant is common

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houseplants with colourful foliar displays and it is highly hybridized to produce a variety of colours and types of leaf spotting. It is not especially difficult to grow, and their main drawback is their relatively short lifespan (Kim et al. 2012). Polka Dot Plant is also called freckle face plant; this houseplant can grow in any type of indirect light but has best colour in lower light situations. When it is grown as a potted plant, a growth regulator is normally used to control plant size or to give good height control (Armitage and Carlson, 1980). Control of plant height is one of important problems related with the production of Polka Dot Plant in many circumstances. In its common native habitat, the plant can get up to 3 feet in height, but pot grown specimens will usually be smaller.

Urbanization is associated with a substantial increase in impervious surface in world cities (Booth and Jackson 1997). Since many people spend most of their lives indoors, indoor air represents a major proportion of their exposure to air pollution and poor indoor air quality may pose serious health risks (Wood and Burchett, 1995). The importance of indoor air quality to human health has become of increasing interest where inhabitants often spend over 90% of their time indoors and indoor air has been reported to be as much as 12 times more polluted than that outdoors (Orwell et al. 2004; Zabiegała, 2006). Indoor air pollutants include volatile organic compounds, particulate matter, ozone, radon, lead, and biological contaminants (Destailats et al. 2008) and exposure can cause acute illnesses and chronic diseases (Suh et al. 2000). Plants can effectively improve the indoor air quality by reducing volatile organic compounds (Thomsen et al. 2011), thus reducing the risk of sick building syndrome (Kim et al. 2011). Foliage plants are often used as house plants due to their attractive foliage as well as their ability to grow under limited indoor light (Chen and Henny, 2008).

The most limiting factor for plant growth is reduced light intensity regarding the environmental conditions for indoor plants because the typical indoor light intensity is less than  $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  while the outdoor light intensity is higher than  $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of photosynthetic photon flux (PPF) at sunny days (Manaker, 1997). Due to requirement of plant growth for light, plants need a particular light environment for suitable growth (Maloof et al. 2001). Plants can adjust to varying light circumstances through physiological and morphological changes, which is causing either in increased light capture or improvement of light utilization. Light acclimatization is the process needed to cause morphological changes enabling plants to withstand under low light conditions and foliage plants prior to placement indoors improves their survival and quality. Different responses of acclimation to low light consist on long plant height, higher shoot to root ratio, large leaf size increased, changes in leaf numbers, leaf dry weight, increased total chlorophyll content, and a decrease in the chlorophyll a:b ratio (Evans and Poorter, 2001; Nemali and van Iersel, 2004).

The objective of present research was to investigate the optimum light intensity for Polka Dot Plant foliage plant in an indoor light environment. These results will improve understanding of the light requirements for better performance of foliage Polka Dot Plant under limited indoor light environments.



## MATERIAL AND METHODS

Rooted cuttings of Polka Dot Plant were obtained from a local garden center in Tabriz, Iran. All the seedlings were transplanted into 3-inch-diameter round plastic pots filled with vermiculite (30% by volume) and soilless substrate (70% by volume) and after acclimatization for 2 weeks in the greenhouse with shade at University of Tabriz, Iran. All the plants were moved to an indoor laboratory at a temperature of 25 °C and were hand-watered every other day. To obtain suitable indoor light treatments, two phosphor fluorescent lamps [Dulux L 36W (OSRAM GMBH, Germany) and FL40 EX-D (AEG, Germany)] were used as the light source at the 15 cm above canopy of the plants with light period was 12 h day and 12 h night. Four treatments including (i) blue light, (ii) red light, (iii) blue+red light and (iv) fluorescent light were applied and plant height and leaf numbers were measured every decade (each ten days). The datasets were first tested for normality by the Anderson and Darling normality test using Minitab version 14 (Minitab Institute, 2005) statistical software. Data from each trial were subjected to analysis of variance (ANOVA) using appropriate models. The experimental design was completely randomized design (CRD) with eight replications. Data were analyzed general linear models and regression in SAS version 9.1 (SAS Institute, 2004).

## RESULTS AND DISCUSSION

The results of Anderson and Darling normality test indicated that dataset were normal and there is no need for transformation (data not shown). The analysis of variance results indicated highly significant differences for plant height in different light treatments for all sampling decades except the first, second and third decades (Table 1)

**Table 1.** Analysis of variance for plant height of polka dot plant under various light treatments in eight decades of growt

SOV	DF	D1	D2	D3	D4	D5	D6	D7	D8
Treatment	3	15.08 <sup>ns</sup>	25.50 <sup>ns</sup>	52.13 <sup>ns</sup>	3226.42 <sup>**</sup>	7353.11 <sup>**</sup>	14140.21 <sup>**</sup>	32531.42 <sup>**</sup>	46018.78 <sup>**</sup>
Error	28	13.81	25.54	46.70	283.58	676.39	1414.97	2686.47	3830.95
CV (%)		27.0	27.2	27.3	36.9	40.7	45.6	45.4	40.7

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup>; Significant 1% and 5% of probability level and non-significant.

Controlling of plant height trait is one of important problems in Polka Dot Plant and so applying different light treatments can be changed it. According to Table 2, the comparison of means for plant height of polka dot plant under various light treatments in the first, second and third decades of growth indicated that there are no significant differences among light treatments based on

Duncan's new multiple range test. In fourth decade, red light treatment with 75.4 mm had the most plant height and was not better than the other light treatments (Table 2) according to least significant differences (LSD) test. In other word, the low plant height was seen in the blue, red+blue and fluorescent light treatments and there are not any significant differences among these treatments. Similar trend was observed for plant height trait in the fifth decade and red light treatment with 108.8 mm had the long plant height in comparison to the other light treatments according to LSD test (Table 2). The plant height of the red light treatment in the sixth, seventh and eighth decades were 145.0, 208.8 and 264.6 mm, respectively, and were higher than the other light treatments based on LSD test (Table 2). Therefore, it seems that various light treatments did not affect height of Polka Dot Plant significantly till the first month of growth (three decades), but it can be reduced by applying blue, red+blue and fluorescent light treatments instead of only red light treatment from the fourth decade to last decade (about two months of growth).

Table 2. The comparison of means for plant height of polka dot plant under various light treatments in eight decades of growth.

Treatments	D1		D2		D3		D4		D5		D6		D7		D8	
Blue	13.3	A	17.9	A	24.1	A	32.3	B	44.0	B	54.8	B	70.4	B	100.4	B
Red	15.6	A	21.1	A	28.5	A	75.4	A	108.8	A	145.0	A	208.8	A	264.6	A
Red+Blue	13.8	A	18.6	A	25.1	A	35.0	B	47.3	B	62.4	B	84.4	B	117.0	B
Fluorescent	12.4	A	16.7	A	22.6	A	39.9	B	55.4	B	67.6	B	93.0	B	125.9	B

\*Mean with the similar letters in each column have not significant differences at 0.05 probability level by least significant differences (LSD) test for F-test significant traits and Duncan's new multiple range test (MRT) significant traits

The results of analysis of variance for leaf numbers of polka dot plant indicated non-significant differences among different light treatments (Table 3). Regarding high magnitudes of coefficient of variations (CV), logarithmic and square root transformations were performed, but significant differences did not observed for leaf numbers in the eight decades (data not shown).

Table 3. Analysis of variance for leaf numbers of polka dot plant under various light treatments in eight decades of growth

SOV	DF	D1	D2	D3	D4	D5	D6	D7	D8
Treatme nt	3	21.28 <sup>ns</sup>	31.03 <sup>ns</sup>	42.08 <sup>ns</sup>	121.83 <sup>ns</sup>	303.79 <sup>ns</sup>	705.58 <sup>ns</sup>	763.78 <sup>ns</sup>	1629.21 <sup>ns</sup>
Error	28	12.08	17.33	24.62	112.21	169.09	363.10	434.09	617.12
CV (%)	21.1	21.1	20.9	42.6	49.9	60.3	57.0	56.7	21.1

However, comparison of means for leaf numbers of polka dot plant under various light treatments via Duncan's new multiple range test (MRT) indicated that there is not any significant differences among different light treatments in first five decades (D1, D2, D3, D4 and D5) and seventh decade (Table 4). In the sixth decade, blue light treatment had the highest leaf numbers (41.5) following to red and blue+ red light treatments (32.6 and 33.5, respectively) while fluorescent light treatment had the lowest (18.9) leaf numbers (Table 4). In the eighth decade, blue and red light treatments had the highest leaf numbers (54.6 and 53.4, respectively) following to blue+ red light treatment (43.5) while fluorescent light treatment had the lowest (23.8) leaf numbers (Table 4). Therefore, it seems that various light treatments did not affect leaf numbers of Polka Dot Plant significantly till the sixth decade of growth, but it can be increased by applying blue, red and red+blue light treatments instead of only fluorescent light treatment in the sixth and eighth decades.

Table 4. The comparison of means for leaf numbers of polka dot plant under various light treatments in eight decades of growth.

Treatments	D1		D2		D3		D4		D5		D6		D7		D8	
Blue	15.9	A	19.1	A	22.9	A	27.5	A	29.9	A	41.5	A	45.9	A	54.6	A
Red	18.9	A	22.7	A	27.2	A	28.0	A	31.6	A	32.6	AB	41.5	A	53.4	A
Red+Blue	15.3	A	18.3	A	22.0	A	24.5	A	24.9	A	33.5	AB	35.4	A	43.5	AB
Fluorescent	15.9	A	19.1	A	22.9	A	19.5	A	17.9	A	18.9	B	23.4	A	23.8	B

\*Mean with the similar letters in each column have not significant differences at 0.05 probability level by duncan's new multiple range test (mrt) for non f-test significant

According to Fig. 1, plant height of Polka Dot Plant under various light treatments did not changed until the third decade, but from the third to the eighth decade, blue light treatment increased plant height significantly while the other three light treatments have not any significant differences with each other and did not increased plant height. The trend of leaf number over eight decades was relatively complicated (Fig.2); fluorescent light treatment had the low leaf number over eight decades, but the other light treatments had incensement trend and increase over time. Artificial lighting is gaining relevance in horticulture, since it allows cultivation wherever natural light is not sufficient (indoor cultivation). Although, various plants need various light treatments, it has been confirmed that the optimal ratio between blue and red light is of great relevance in determining yield performance (Tarakanov et al. 2012). Moreover, increased crop growth is also related to improved light interception rather than increased photosynthetic rates (Hogewoning et al. 2012). A great opportunity for the financial sustainability of artificial lighting is provided by the chance of quality improvement and light is one of the most important variables affecting physiological processes in plants (Kopsell and Kopsell, 2008). Different light regimes may help to optimize growth and a control developmental transition

makes the implementation of light type especially to design the controlled circumstance targeted to production (Samuoliene et al. 2010). Among different light treatments considered in the present study, plant height was increased to a greater extent in plants grown under red light while the other light treatments (blue, red+blue and fluorescent) (Table 2), confirming that the proper balancing of red and blue components of the light spectrum would be beneficial to plants' production (Hogewoning et al. 2012). The present work confirmed the efficiency superiority of LED compared to the traditional fluorescent lamps, enabling an increase of about two folds leaf number productivity (Table 4).

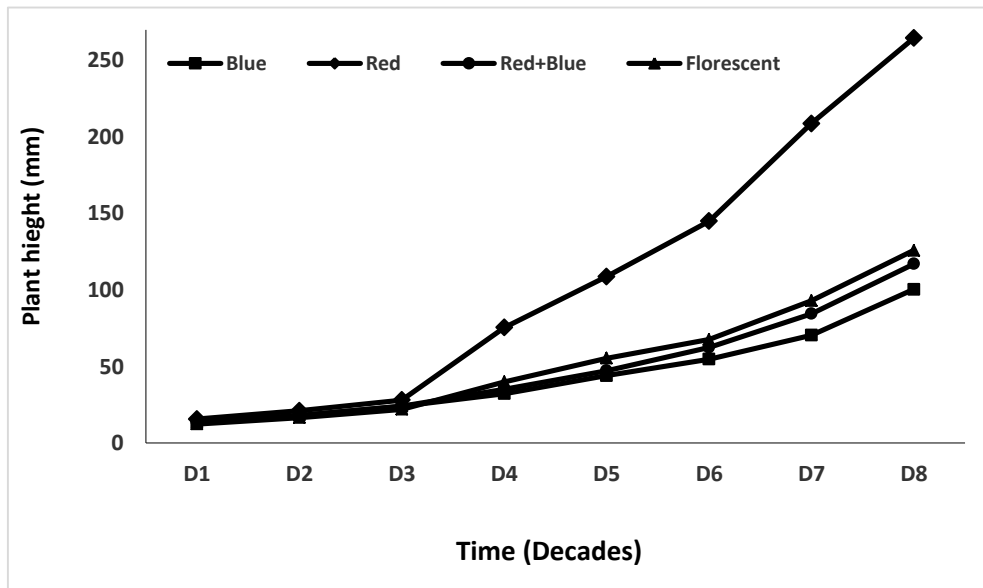


Figure. 1. Effect of various light treatments on plant height in polka dot plant

According to Samuoliene et al. (2010), a species-specific mixture of red and blue spectral components is necessary for proper plant development and the effect of the blue light in promoting leaf number has been addressed in a range of recent reports, although often with controversial results (Tarakanov et al. 2012). Furthermore, the improvement on the biomass of Welsh onion (*Allium fistulosum* L.) shoot with blue, rather than red and green, overnight supplemental lighting was reported by Sase et al. (2012). Different activities of plants consist on physiological and biochemical processes are strictly related with the quality of the incident light (Horton, 2000), and identification of the optimal spectral composition shall take into account how plant functions varied across light treatments. The increased crop growth (more leaf numbers in sixth and eighth decades) under LED lighting (blue, red and red+blue) should be related to improved light interception rather than increased photosynthetic rates (Hogewoning et al. 2012).

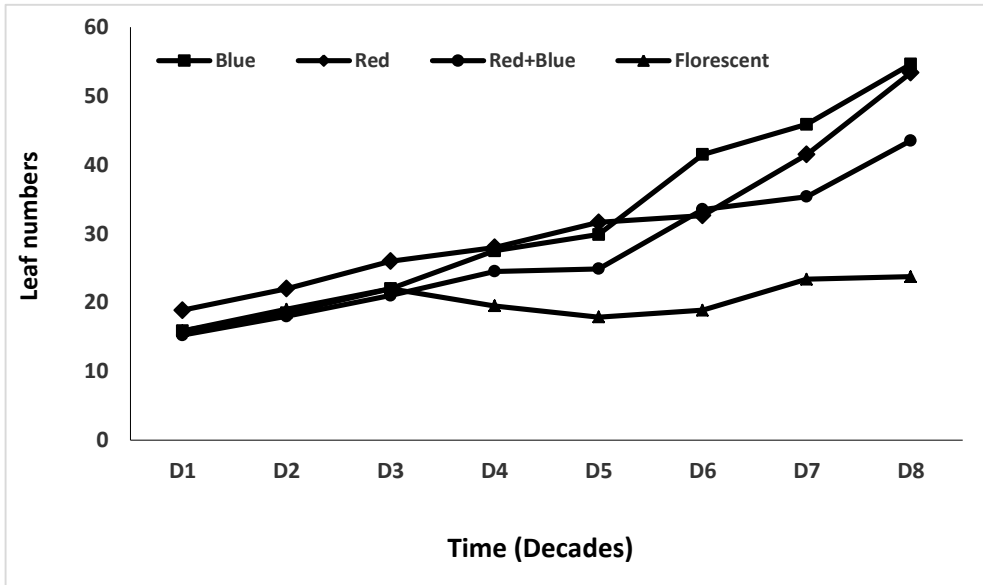


Figure 2. Effect of various light treatments on plant leaf numbers in polka dot plant.

## CONCLUSIONS

This study addressed the applicability of blue, red and blue+red LED lights and fluorescent light for indoor production of Polka Dot Plant. Through of analyses (addressing plant height and leaf number), it was possible to determine the most suitable light blue or blue+red treatments. Consistently, LED lights (blue and blue+red) improved plant leaf number and reduced unwanted traits (e.g. long plant height).

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## THE COMBINING ABILITY OF MAIZE (*ZEA MAYS L.*) INBRED LINES FOR GRAIN YIELD AND YIELD COMPONENTS

### SUMMARY

A full diallel cross comprising ten (10) inbred lines was studied for different characters to determine the nature of gene action in parents and hybrid genotypes. The analysis of variance revealed significant differences for general combining ability (GCA) and specific combining ability (SCA) indicated the presence of additive as well as non-additive gene effects for controlling the traits. However, relative magnitude of these variances indicated that additive gene effects were more prominent for all the characters studied except grain yield/plant. The ratio of the components revealed that the magnitudes of SCA components were much higher than that of GCA in all crosses except number of kernel row per ear. A wide range of variability of GCA effects was observed among the parents. For grain yield (GY) parents L2, L5, L6, L7 and L9, showed significant positive GCA effect. Thirteen five crosses exhibited significant positive SCA effects for grain yield (GY). These crosses involved high × high, high × low and low × low general combining parents (GCA). Although the cross L6 × L10 involved low × high general combiners, exhibited the highest significant positive of SCA effect (14.14 tha<sup>-1</sup>). The cross L1 × L10 involved the two inbred lines with lower general combiners and also showed the lower SCA effects (7.61tha<sup>-1</sup>).

**Keywords:** Combining abilities, GCA, gene effects, maize, SCA

**Abbreviations:** GCA-general combining ability; SCA-specific combining ability

### INTRODUCTION

The information on general combining ability (GCA) and specific combining ability (SCA) is important for hybrid development. Combining ability study of inbreds and populations are important for hybrid breeding in order to understand the heterotic patterns of the germplasm. Many procedures have been used by plant breeders in attempt to increase the yield of maize (Gaedelmann and Peterson, 1980; Aliu et al, 2008). The improvement of maize yields depends on the knowledge of the type of the gene action involved in its inheritance and also the genetic control of the related traits such as capacity of production (Rezaei et al., 2004). Grain yield of maize is a complex trait. It includes a number of

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components, which are inherited in a quantitative manner (Živanovic et al., 2006). The General Combining Abilities (GCA) and Specific Combining Abilities (SCA) effects are important indicators of potential value for inbred lines in hybrid combinations. The value of GCA tends to express Differences in GCA effects have been attributed to additive, interaction of additive x additive, and higher-order interactions of additive genetic effects in the base population, while differences in SCA effects have been attributed to dominant and epistatic gene effects (Spitko et al, 2010). Non additive gene effects for grain yield were found to be significant in maize (Kalla et al., 2001) which suggested that several combinations among parental lines by their mean performance and genetic nature had the potential for the development of more yielding and earlier genotypes. Evaluation of crosses among inbred lines is an important step towards the development of hybrid varieties in maize (Hallauer, 1990). This process ideally should be through evaluation of all possible crosses (diallel crosses), where the merits of each inbred line can be determined. Diallel crosses have been widely used in plant breeding to investigate combining abilities of the parental lines in order to identify superior parents for use in hybrid development programmes. Diallel mating design has been devised, also, in genetic research to investigate the inheritance of important traits among a set of genotypes and gene effects (Malik et al., 2005). Diallel crosses have been used in genetic research to determinate the inheritance of a trait among a set of genotypes and to identify superior parents for hybrid or cultivar development (Weikai Yan & Manjit Kang, 2003). Combining ability has been investigated by several authors in maize (Kang et al., 1995; Kim and Ayala, 1996; Betrán et al., 2002; Bhatnagar et al., 2004; Glover et al., 2005). The main objective of our study was to estimate General combining ability (GCA) and Specific combining ability (SCA) among these maize inbred lines and, consequently, to identify superior single-cross hybrids (SCH) developed from them.

## MATERIAL AND METHODS

### Plot Layout and Stand Establishment

Plant materials used as parents for crosses in this study were 10 selected superior maize inbred lines (Parents) (L1, L2, ...L10) with medium maturity, originating from the Agriculture University of Tirana, Albania. Crosses among these inbred lines were based on a diallel. During the first 3 years, we evaluated adaptability of inbred lines to specific agro-ecological conditions of Kosovo, in the locality Ferizaj (580 m a.s.l). In the fourth year, we conducted diallel crosses (with 10 inbreds) following the method of Griffing (1956). The field experiments with F1 hybrids and their parents (10 diverse maize lines and their 45 F1 crosses) were conducted during the fifth year. The total of means of the single crosses was calculated based on formula: 
$$Tx = \frac{p(p-1)}{2}$$

Whereas: Tx is a total of means of the single crosses and  $p$  = number of parents



The experiments were based on a randomized complete block design (RCBD) with three replications. The distances between plants were 60×30 cm or 55.000 plants<sup>-1</sup>. The experimental plots for each replication were 5.4 m<sup>2</sup>. The seeds were sowing deep 3-5 cm. The standard agronomic practices were applied. Measurements on plot basis were recorded on the following agronomic traits: grain yield (GY) t ha<sup>-1</sup>, ear length (EL) (cm), number of rows per ear (NRE), and number of kernels per row (NKR). Grain yield evaluation was performed by measurement of ears mass for each elementary plot using average sample from each replication, in order to calculate grain yield with 14% moisture. Analyses of other above mentioned traits were conducted using 10 ears per genotype from each replication.

### Statistical analyses

Differences among observed individuals, within each combination, were analyzed using the mathematic model of Griffing (1956):

$$X_{ij} = \mu + gi + gj + sij + e,$$

$X_{ij}$  – value of the progeny derived from the crossing of  $i$ -th female parent with  $j$ -th male parent

$\mu$  – grand mean,

$gi$  – the GCA effects of the  $i$ -th female parent,

$gj$  – the GCA effects of the  $j$ -th male parent,

$sij$  – the SCA effects specific to the hybrid of the  $i$ -th female line and the  $j$ -th male line,

$e$  – experimental error.

ANOVA for GCA and SCA was calculated as presented in Table 1.

Table1. Model of ANOVA for GCA and SCA according to Griffing's method 2 (Aliu et al.,2008)

Source	d.f.	S.S.
GCA	$n-1$	$\frac{1}{n+2} \left[ \sum (y_{i.} + y_{.i})^2 - \frac{4}{n} y_{..}^2 \right]$
SCA	$\frac{n(n-1)}{2}$	$\sum \sum y_{ij}^2 - \frac{1}{n+2} \sum (y_{i.} + y_{.i})^2 + \frac{2}{(n+1)(n+2)} y_{..}^2$
Error	$\left[ \frac{n(n+1)}{2} - 1 \right] \times (r-1)$	$\frac{\text{Total S.S.} - \text{Treatm. S.S.} - \text{Replic. S.S.}^*}{r}$

Statistical analyses package were conducted using program – MSTAT-C , version 2.10(Russell, 1996).

## RESULTS AND DISCUSSION

Analyses of variance revealed that mean square values were significant (LSD $p=0.01$ ) for the genotypes for all traits under study. Analysis of variances for combining ability (Table 1) revealed that both GCA and SCA variances were highly significant for all the characters studied indicating importance of additive as well as non-additive type of gene action in controlling the traits. Effect of a replication was insignificant for all analyzed traits and suggested uniformity of a soil and agronomic practice used. The ratio of the components revealed that the magnitudes of SCA components were much higher than that of GCA in all crosses except number of kernel row per ear. (Table 2). This indicated predominance of additive gene action for all the characters except kernel rows per ear (NKR). Debnath et al. (1988), Sanghi et al. (1983), Roy et al. (1998) and Das and Islam (1994) also reported predominance of non-additive gene action for grain yield in the same crop.

Table 2. Analysis of variance for combining ability of different characters in maize line

Source of Variation	d.f	Mean sum of squares			
		GY	EL	NRE	NKR
Replication	2	0.23	0.25	0.02	0.14
Crosses	44	13.78**	11.08**	42.17**	1.95**
GCA	9	10.36**	15.14**	8.75**	21.27**
SCA	44	74.00**	33.95**	47.42**	7.95**
Error	108	0.22	0.15	0.11	0.12
GCA/SCA		0.14	0.45	0.18	2.67

\*\* Significant at  $p=0.05$ ,  $p=0.01$  level respectively.

The GCA effects and performance of the parents revealed that none of the parents were found to be a good general combiner for all the characters studied (Table 3). A wide range of variability of GCA effects was observed among the parents. For grain yield (GY) parents L2, L5, L6, L7 and L9, showed significant positive GCA effect and simultaneously possessed high mean value indicating that the performance of the parents could prove as a useful index for combining ability and suggesting they contributed good alleles and their importance in grain yield improvement. Roy et al. (1998) and Hussain et al. (2003) also have observed similar results. Grain Yield (GY), Ear length (EL), Number of row per ear (NRE) showed that ratio of GCA/SCA what led to conclusion that additive gene effects are importance in inheritance of this trait. El-Badawy (2013) and EL-Hosary and Elgammaal (2013) showed that the additive gene effects represented the major role in the inheritance of grain yield and other agronomic

traits. Theoretically, additive gene effect can be fixed in pure lines, while non-additive can be expressed in hybrids.

Table 3. Estimates of general combining ability effects (GCA) and mean performance

	GY		EL		NRE		NKR	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
L1	3.0	-0.05	12.7	0.023	13.0	-0.38	17.6	0.483**
L2	3.4	0.095	13.1	0.038*	12.0	-0.53	18.3	-0.056
L3	3.1	-0.21	13.2	0.336**	12.7	-0.74	19.7	0.078
L4	2.1	-0.72	17.2	0.113**	12.0	-0.74	16.7	-1.95
L5	4.5	0.14**	14.7	-0.91	14.7	0.424**	24.3	0.144**
L6	4.7	0.64**	12.2	-0.11	15.3	0.576**	15.7	-0.236
L7	4.8	0.35**	18.2	1.122**	15.3	-0.46	24.0	2.194**
L8	3.5	-0.03	13.1	0.062**	15.6	0.154**	17.0	0.65**
L9	3.3	0.08	15.2	0.258**	13.3	1.062**	15.3	-1.02
L10	4.0	-0.34	14.1	-0.92	15.3	0.664**	21.7	-0.28
LSD 0.05	0.11		0.02		0.01		0.12	
LSD 0.01	0.13		0.04		0.04		0.26	
SE (gi)	0.69		0.91		0.85		2.19	

\*, \*\* significant at 0.05 and 0.01 probability levels, respectively

Parents (lines) L5, L6 and L10 showed significant negative GCA for Ear length (EL) and are good contributor and can be used in maize breeding for reducing ear length. Parent (line) L7 might be useful in developing new hybrid genotypes because it was characterized with high GCA values (1.122) and suggesting it contributed good alleles for ear length. The largest significant (LSD<sub>p=0.01</sub>) positive GCA effects for number of row per ear (NRE) were observed for the inbred lines L9 on value 1.062. Also, the other inbred lines L5, L6, L8 and L10 had the positive values but compare to line P9 the values were lower (Table 3). For number of kernels per row (NKR) it was detected for L3, L5, L7 and L8 showed largest positive significant GCA effects for NKR suggesting usefulness in breeding programmes for the increase of this trait. It is the confirmation of the earlier acknowledged fact that large SCA effect often includes one parent with large GCA effect and another with small GCA (Glover et al., 2005). Specific combining ability effects (SCA, effects are shown in Table 4. For grain yield generally the crosses showing significant positive of SCA

effects also possessed high mean performance and significant negative SCA effects possessed low mean performance. This reflects that high value of the crosses indicated their potentiality. Thirteen five crosses exhibited significant positive SCA effects for grain yield (GY). These crosses involved high  $\times$  high, high  $\times$  low and low  $\times$  low general combining parents (GCA). The average values of GY at all crosses were  $10.8 \text{ tha}^{-1}$ . Although the cross L6  $\times$  L10 involved low  $\times$  high general combiners, exhibited the highest significant positive of SCA effect ( $14.14 \text{ tha}^{-1}$ ). The cross L1  $\times$  L10 involved the two inbred lines with lower general combiners and also showed the lower SCA effects ( $7.61 \text{ tha}^{-1}$ ). The differences between these hybrid combinations were  $+6.53 \text{ tha}^{-1}$  or expressed in relative values was 60.46%. While differences between the parents (MP) and hybrid combination ( $F_1$ ) for GY were ( $d = F_1 - MP = +7.16 \text{ th}^{-1}$ ). Ivy and Hawlader (2000) also reported that good general combining parents do not always show high SCA effects in their hybrid combinations. On the contrary, Paul and Duara (1991) reported that the parents with high GCA always produce hybrids with high estimates of SCA. Roy et al. (1998) also found significant positive SCA effects in high  $\times$  low general combiners. Hybrid combination L6  $\times$  L10 and L1  $\times$  L4 showed largest positive significant ( $LSD_p = 0.01$ ) SCA effect for ear length (EL), while with lower SCA effects was characterized the combination L5  $\times$  L7 ( $15.9 \text{ cm}$ ) The differences between hybrid combination L6  $\times$  L10 and L1  $\times$  L4 were  $6.40 \text{ cm}$  or expressed in relative values was 31.37%. The differences among the parents (MP) and hybrid combination ( $F_1$ ) for EL were ( $d = F_1 - MP = +6.06 \text{ cm}$ ) (Table 4). For NRE the average values were  $16.7 \text{ rows/ear}$ . With higher SCA was determined the hybrid combination L5  $\times$  L9 (higher  $\times$  higher of GCA) on value  $19.2 \text{ rows/ear}$ , while on minimal values was L7  $\times$  L10 (lower  $\times$  higher) on value  $14.3 \text{ rows/ear}$ . The differences between combination for NRE were  $+4.9 \text{ rows/ear}$  or in relative values 29.34%. The differences among the parents (MP) and hybrid combination ( $F_1$ ) for NRE were ( $d = F_1 - MP = +2.8 \text{ rows/ear}$ ). For NKR, the crosses L6  $\times$  L10 exhibited higher significant SCA effects ( $45 \text{ kernel/row}$ ) of the hybrid. In this crosses are involved low  $\times$  low general combining parents. With low SCA was determined the combination L1  $\times$  L10 ( $29.5 \text{ kernel/row}$ ) and if compared this combination on L6  $\times$  L10, the differences between them are  $+15.5 \text{ kernel/row}$  or expressed in relative values are 41.11%. While the differences among the parents (MP) and hybrid combination ( $F_1$ ) for NKR were ( $d = F_1 - MP = +18.67 \text{ kernel/row}$ ). Aliu et al., (2008), has studied some maize inbred lines and from results found that it was not possible to prove the rule that inbreeds with good GCA usually had the good SCA. Results are presented in Table.4.

Table 4. Estimates of SCA and mean performance of the maize inbred lines

Crossing	GY( $\text{tha}^{-1}$ )		EL (cm)		NRE (rows/ear)		NKR(kernel/ear)	
	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
L1x L2	12.01	2.11**	21.2	1.74**	14.4	-0.53	41.3	6.17**
L1x L3	11.73	2.15**	22.1	2.37**	14.3	-0.41	41.8	6.54**
L1x L4	10.45	1.37**	22.3	2.56**	16.5	1.86**	39.0	5.73**
L1 xL5	13.85	3.91**	22.2	3.6**	17.0	1.14**	41.4	6**
L1xL6	12.42	1.98**	21.4	2.12**	16.5	0.52*	40.4	3.12 <sup>NS</sup>
L1 xL7	9.00	-1.14	21.1	0.55*	14.9	0.006	40.5	4.97*
L1 xL8	12.93	3.16**	21.8	2.34**	16.1	0.51*	40.8	3.47 <sup>NS</sup>
L1xL9	11.69	1.81**	19.9	0.25 <sup>NS</sup>	18.3	1.79**	37.7	-5.4
L1 xL10	7.61	-1.84	15.9	-2.56	16.0	-0.11	29.5	2.61 <sup>NS</sup>
L2xL3	10.68	1.01**	21.6	2.12**	15.3	0.8**	37.4	4.97*
L2xL4	11.52	2.34**	20.9	1.39**	15.4	0.84**	37.7	6.08**
L2xL5	11.82	1.78**	20.9	2.34**	16.4	0.67**	39.9	0.09 <sup>NS</sup>
L2xL6	11.72	1.2**	20.8	1.44**	16.9	0.99**	34.5	6.33**
L2xL7	10.84	0.59**	21.6	1.07**	15.5	0.63**	43.2	7.44**
L2xL8	11.06	1.2**	21.5	1.96**	16.2	0.71**	42.8	4.25*
L2xL9	12.66	2.67**	21.7	1.87**	17.9	1.57**	37.9	-4.39
L2xL10	9.74	0.19 <sup>NS</sup>	17.0	-1.21	15.8	-0.16	30.0	1.71 <sup>NS</sup>
L3xL4	10.4	1.53**	20.2	0.48*	16.4	2.01**	34.6	-1.25
L3xL5	11.46	1.73**	20.9	2.08**	15.9	0.41*	33.7	0.36 <sup>NS</sup>
L3xL6	9.03	-1.2	20.0	0.37 <sup>NS</sup>	14.9	-0.81	34.9	1.79 <sup>NS</sup>
L3xL7	10.9	1.05**	21.4	0.57*	15.2	0.57**	38.8	5.07*
L3xL8	8.79	2.83**	21.3	1.53 <sup>NS</sup>	15.3	0.08	40.5	5.01*
L3xL9	11.49	1.81**	21.5	1.52**	17.5	1.31**	38.8	8.57**
L3xL10	10.9	1.65**	21.5	2.72**	15.1	-0.62	43.1	3.14 <sup>NS</sup>
L4xL5	10.41	1.17**	18.1	-0.49	14.9	-0.58	36.1	4.65*
L4xL6	11.96	2.23**	20.6	1.16**	16.6	0.93**	37.2	0.32 <sup>NS</sup>
L4xL7	11.51	2.08**	19.7	-0.93	14.6	-0.02	35.3	1.27 <sup>NS</sup>
L4xL8	8.26	-0.79	19.2	-0.37	14.5	-0.74	34.7	2.08 <sup>NS</sup>
L4xL9	9.45	0.28*	20.2	0.39 <sup>NS</sup>	16.6	0.41*	33.8	4.4*
L4xL10	10.97	2.22**	19.1	0.57*	15.7	-0.02	36.9	4.36*
L5xL6	12.41	1.82**	19.4	1.02**	16.7	-0.79	39.7	5.06*
L5xL7	10.51	0.21 <sup>NS</sup>	18.7	-0.94	15.0	0.98**	37.1	0.03 <sup>NS</sup>
L5xL8	11.65	1.74**	18.6	0.05 <sup>NS</sup>	17.4	1.84**	38.3	2.74 <sup>NS</sup>
L5xL9	9.65	-0.36	17.4	-1.32	19.2	0.47*	34.4	0.51 <sup>NS</sup>
L5xL10	8.82	-0.77	16.5	-1.06	17.4	0.32 <sup>NS</sup>	34.6	0.03 <sup>NS</sup>
L6xL7	12.52	1.72**	22.6	2.22**	16.3	-0.16 <sup>NS</sup>	38.4	1.74 <sup>NS</sup>
L6xL8	9.67	-0.74	19.7	0.31 <sup>NS</sup>	16.4	0.56**	36.8	1.62 <sup>NS</sup>
L6xL9	12.2	1.66**	21.3	1.72**	16.7	1.09**	40.9	7.43**
L6xL10	14.14	4.03**	22.3	4.12**	18.2	-0.65	45.0	10.78**
L7xL8	12.52	2.4**	21.8	2.38**	14.9	0.77**	44.5	6.92**
L7xL9	12.47	2.24**	21.6	0.77**	17.2	-1.69	38.6	2.73 <sup>NS</sup>
L7xL10	12.05	2.23**	20.8	1.23**	14.3	0.58**	39.9	3.22 <sup>NS</sup>
L8xL9	11.48	1.63**	22.2	2.55**	17.7	0.31	39.1	4.71*
L8xL10	10.47	1.04**	20.5	1.99**	17.0	0.4*	38.4	3.3 <sup>NS</sup>
L9xL10	11.04	1.49**	20.4	1.66**	18.0	0.39*	37.9	4.47*
LSD 0.05	0.26		0.43		0.38		4.25	
LSD 0.01	0.34		0.61		0.52		5.38	
SE (gi)	0.13		0.23		0.21		1.35	

\*, \*\* significant at 0.05 and 0.01 probability levels, respectively

## CONCLUSIONS

From the results it was concluded that the following parental lines L5, L6 and L7 were having good general combining ability for grain yield. These parents had resulted in the production on higher values. So, these inbred lines (parents) could be used extensively in hybrid breeding program with a view to increasing the yield level. Among the crossing combination L6xL10, L1xL5, and L1xL8 were found to be good specific combiners and these line could be used for heterosis breeding programs in maize and represent a highly valuable of genetic material.

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## **DETERMINATION OF EFFECTIVE INDICES IN THE DROUGHT MONITORING THROUGH ANALYSIS OF SATELLITE IMAGES**

### **SUMMARY**

The aim of this research was to determine the effective drought indices such as NDVI, VCI, EVI, TVX, VTCI, VHI, TCI and NVSWI based on some criteria. The satellite-based drought indices have different data from vegetation and temperature conditions. Linear and trend analysis showed that satellite-based drought indices based on vegetation condition were not more efficient indices such as NDVI and VCI. It can be said that the indices with combination of vegetation and temperature condition have more efficiency. A good agreement was observed among drought indices and precipitation in the arid and semi-arid climatic regions. Application of NDVI, VCI and EVI was limited for real time drought monitoring. Investigation the topographic effect on drought indices indicated that VTCI and NDVI had maximum impressed from height variations. The synthesized drought indices based on effective indices led to better performance. Drought indices investigation using different criteria indicated the high performance of NVSWI, TCI, VHI and TVX. The indices have high correlation with each other. Therefore, using one of them is suitable for drought monitoring.

**Keywords:** Linear Analysis, Precipitation, Climatic Regions, Correlation.

### **INTRODUCTION**

Drought is an insidious hazard of nature that has heavy damage and losses in many parts of world. The severity of drought is related to a specific climatic region and local energy and water balance status. In general, drought can be defined as a period of abnormally dry weather, which further results in vegetation cover condition changes (Shahabfar et al., 2012). Over the last three decades, the frequency and intensity of drought have increased. Drought with slow onset is different from other natural hazards, which referred to as creeping phenomenon. Unlike other natural disasters, it starts unnoticed and develops cumulatively (Shakya and Yamaguchi, 2006). Drought is a complex phenomenon that the American Meteorological Society group classifies into four categories: meteorological, agricultural, hydrological and socioeconomic drought. Meteorological drought is a precipitation deficit, agricultural drought is a total soil moisture deficit, hydrological drought is a shortage of stream flow and

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

socioeconomic drought is associated with the shortage of some economic goods affected by the drought process (Du et al., 2013)

Because of drought negative impact on agricultural, ecological, environmental aspects, drought investigation and monitoring with efficient method is necessary. Classic drought monitoring approaches were based on the point-based data predominantly using meteorological observations and regional hydrologic records which include the Palmer Drought Severity Index (PDSI) and Standardized Precipitation Index (SPI). The indices were in situ drought index, the spatial drought condition need to be estimated using spatial interpolation techniques, such as inverse distance weighted; ordinary kriging model. Their accuracy and level of spatial detail are functions of the density and the distribution of the station network. However, because of its frequency and spatial coverage, remote sensing has become the most promising tool for large-scale drought monitoring (Qin et al., 2008). Satellite based drought indices has different data requirement which are based on vegetation or temperature condition. Associated with the drought and land surface temperature (LST) increases slightly earlier than plant cover decrease. During dry conditions (there is less soil moisture availability), rising leaf temperature are good indicator of plant moisture stress and precede the onset of drought. This thermal response can occur even when plants are green, as stomata closure to minimize water loss by transpiration results in a decreased latent heat flux. At the same time, due to the requirement that the energy flux must balance, there will be an increase in the sensible heat flux, which may result in increased leaf temperatures. This increase in leaf temperature can be used for stress detection in crops. This land surface energy flux balance finally results in high land surface temperature (Wan et al., 2004). Bayarjard et al. (2006) used a vector analysis for a comprehensive study of NOAA-AVHRR derived drought indices. The groups of drought indices are based on vegetation state derived from the reflective channel surface, brightness temperature derived from the thermal channel, the combination between the reflective and thermal channel. It was found that the combination of satellite derived drought indices can identify wider drought occurred areas rather than PDSI. The relationship between the satellite-based vegetation condition index (VCI) and a number of frequently used meteorological drought indices was evaluated using data from all 254 Texas counties during 18 growing-seasons (March to August, 1982–1999). In particular, the response of the VCI was compared to that of the PDSI, Moisture Anomaly Index (Z-index), SPI, percent normal and deciles. Overall the VCI is most strongly correlated with the 6-month SPI, 9-month SPI and PDSI. It appears that the climate region is the most important determinant of the nature of the relationship between the VCI and PDSI. These results demonstrate that care must be taken when using the VCI for monitoring drought because it is not highly correlated with station-based meteorological drought indices and it is strongly influenced by spatially varying environmental factors (Quiring and Ganesh, 2010).

Rhee et al. (2010) proposed a new remote sensing-based drought index, the Scaled Drought Condition Index (SDCI) for agricultural drought monitoring in both arid and humid regions using multi-sensor data. The index combines the land surface temperature data and the Normalized Difference Vegetation Index (NDVI) data from the moderate resolution imaging spectroradiometer (MODIS) sensor and precipitation data from TRMM. SDCI performed better than existing indices such as NDVI and Vegetation Health Index (VHI) in the arid region of Arizona and New Mexico as well as in the humid region of North Carolina and South Carolina. The year-to-year changes and spatial distributions of SDCI over both arid and humid regions generally agreed to the changes documented by the United States Drought Monitor (USDM) maps (Rhee et al., 2010). Shahabfar et al. (2012) evaluated the remote sensing drought indices of MODIS such as the Perpendicular Drought Index (PDI) and Modified Perpendicular Drought Index (MPDI) against meteorological drought indices including Z-score (Z), China-Z Index (CZI) and Modified China-Z Index (MCZI) over 180 meteorological stations from February 2000 to December 2005. The results showed that there is a statistically significant correlation between the PDI and MPDI and regional surface dryness and drought conditions. Du et al. (2013) were defined the synthesized drought index (SDI) in Shandong province, China from 2010 to 2011 which was defined as a principal component of VCI, temperature condition index (TCI) and precipitation condition index (PCI). SDI integrates multi-source remote sensing data from MODIS and tropical rainfall measuring mission (TRMM) and it synthesizes precipitation deficits, soil thermal stress and vegetation growth status in drought process. Therefore, this method is favorable to monitor the comprehensive drought. Results showed that SDI is not only strongly correlated with 3-month scales standardized precipitation index (SPI3), but also strongly correlated with variation of crop yield and drought-affected crop areas (Du et al., 2013). Dutta et al. (2015) were used NOAA-AVHRR NDVI data for monitoring agricultural drought through NDVI based vegetation condition index. VCI was calculated for whole Rajasthan using the long term NDVI images which reveals the occurrence of drought related crop stress during the year 2002. The VCI values of normal (2003) and drought (2002) year were compared with meteorological based SPI, Rainfall Anomaly Index and Yield Anomaly Index; then a good agreement was found among them. The correlation coefficient between VCI and yield of major rain-fed crops ( $r > 0.75$ ) also supports the efficiency of the remote sensing derived index for assessing agricultural drought (Dutta et al., 2015).

Therefore, drought indices can be applied effectively in the drought monitoring. On the other hand, a comprehensive study for evaluating the performance of drought indices and selection of one or some indices as the effective indices is necessary for drought monitoring. Effective indices can be modeled with some method such as artificial neural network and fuzzy regression which leads to drought forecasting. This research seeks to identify the effective drought indices based on MODIS data that can be used for meteorological

drought monitoring in different climatic regions. A linear correlation analysis is applied to evaluate the performance of indices.

## MATERIAL AND METHODS

### Remote Sensing Drought Indices

Drought is difficult to monitor so various indices have been proposed to detect the drought intensity. This misadventure can be monitored effectively using drought indices. Compared to in situ indices, drought indices derived from remote sensing data are more suitable for spatial drought conditions monitoring (Quiring and Ganesh, 2010). The groups of drought indices are derived from the reflectance channel, the thermal channels and the combination of the reflectance and thermal channels. The indices classification is illustrated in Figure 1.

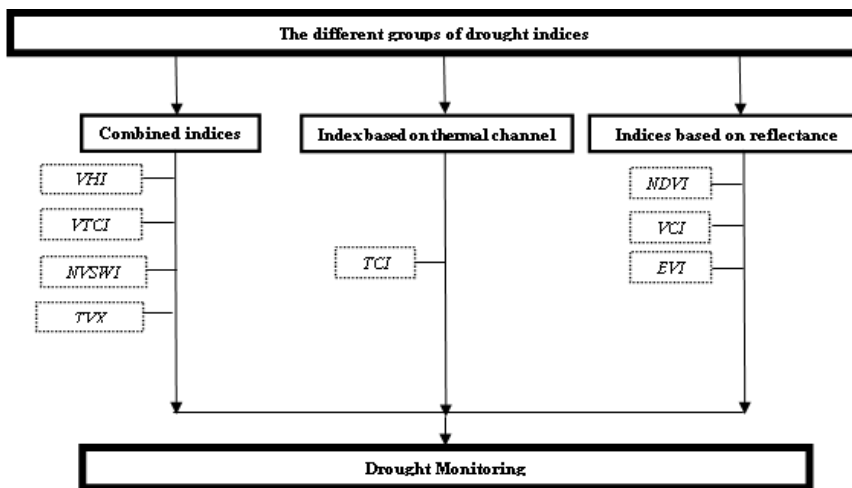


Figure 1. Drought indices classification for drought monitoring.

Each drought index has different data requirement and utilizes method to measure drought. In the following sections, drought indices are explained.

#### 1-NDVI

NDVI has been most widely used for drought monitoring which can be derived using near-infrared and red radiation. Because when sunlight strikes a plant most of the red wavelengths in the visible portion of the spectrum are absorbed by chlorophyll in the leaves, while the cell structure of leaves reflects the majority of near-infrared radiation. Healthy plants absorb much of the red light and reflect most near-infrared radiation. In general if there is most reflected radiation in the near-infrared wavelengths than in the visible wavelengths, the vegetation is likely to be healthy (dense). If there is very little difference between the amount of reflected radiation in the visible and infrared wavelength, the vegetation is probably unhealthy (sparse) (Quiring and Ganesh, 2010). NDVI not

only maps the presence of vegetation on a pixel basis, but also provides measures of the amount or condition of vegetation within a pixel. NDVI can be defined as (Tripathi et al., 2013):

$$NDVI = \frac{\rho_{nir} - \rho_{red}}{\rho_{nir} + \rho_{red}}$$

Where  $\rho_{nir}$  and  $\rho_{red}$  represent pixel reflectance in the near infrared and red channel respectively. NDVI values range from -1 to +1, with values near zero indicating no green vegetation and values near +1 indicating the highest possible density of vegetation.

## 2-VCI

Despite the potential applications of the NDVI, numerous shortcomings have also been revealed. For heterogeneous land cover, the NDVI, which reflect vegetation greenness and vigor, are normally higher in the area with more favorable climate, soil and more productive ecosystem (forest) compared to the areas with less favorable environmental conditions (dry steppe). These differences should be taken into consideration when NDVI is used for monitoring annual weather impact on vegetation (Unganai and Kogan, 1998). Thus, Kogan (1990) proposed a vegetation condition index based on the relative NDVI change with respect to minimum historical NDVI value. This normalized index indicates percent change of the difference between the current NDVI index and historical NDVI time series minimum with respect to the NDVI dynamic range. It was defined by the following formula:

$$VCI = \frac{NDVI - NDVI_{min}}{NDVI_{max} - NDVI_{min}}$$

Where  $NDVI_{max}$  and  $NDVI_{min}$  are maximum and minimum NDVI respectively calculated by the corresponding pixels in same month from the entire NDVI records. VCI changes from 0 to 1, corresponding to the changes in vegetation condition from extremely unfavorable to optimal. In case of an extremely dry month, the vegetation condition is poor and the VCI is close or equal to 0 (Du et al., 2013)

## 3-Enhanced Vegetation Index (EVI)

A major finding on atmospheric effect minimization is the use of the difference in blue and red reflectance as an estimator of the atmosphere influence level. This concept is based on the wavelength dependency of aerosol scattering cross sections; in general the scattering cross section in the blue band is larger

than that in the red band. When the aerosol concentration is higher, the difference in the two bands becomes larger. The EVI formula is written as:

$$EVI = G \cdot \frac{\rho_{nir} - \rho_{red}}{\rho_{nir} - c_1 \rho_{red} - c_2 \rho_{blue} + L}$$

Where  $\rho_{red}$ ,  $\rho_{nir}$ ,  $\rho_{blue}$  are the reflectance of the red, near-infrared, and blue channels, respectively.  $C_1$  and  $C_2$  are coefficients and  $L$  is the canopy background brightness correction factor, and  $G$  is the Gain factor (Frag et al., 2014; Matsushita et al., 2007).

#### 4-Temperature Condition Index (TCI)

Land surface temperature is a good indicator of the energy balance at the earth's surface because it is one of the key parameters in the physics of land surface process on regional and global scales. The TCI was proposed to estimate the thermal impact of drought. The TCI was developed in order to approximate the thermal band contribution in assessment of vegetation condition. It was computed as:

$$TCI = \frac{LST_{max} - LST}{LST_{max} - LST_{min}}$$

Where  $LST$ ,  $LST_{max}$  and  $LST_{min}$  are land surface temperature, maximum and minimum  $LST$  of each pixel, respectively in same month during the study period. The values of TCI vary from 0 to 1. The low values of TCI imply serious condition of drought (Du et al., 2013).

#### 5-Vegetation Health Index (VHI)

The synthesizing VCI and TCI together, the TCI and VCI provide a reliable additive drought detection and crop condition assessment scheme. On the base of VCI and TCI, VHI can be calculated by the following expression:

$$VHI = \alpha VCI + (1 - \alpha)TCI$$

Where  $\alpha$  and  $(1-\alpha)$  indicate the relative contribution of VCI and TCI to the value of VHI, respectively.

In the previous studies, equal values of  $\alpha$  are used to account for the contributions of both VCI and TCI. However, the impacts of the same temperature and the same time period of drought vary depending on how an area is vegetated. For pixels of dense vegetation coverage, drought assessment relies more on the information provided by vegetation condition and therefore the contribution of VCI in VHI increases as the vegetation coverage increases. (Feng, 2011).

### 6-Normalized Vegetation Supply Water Index (NVSWI)

The Vegetation Supply Water Index (VSWI) combines a vegetation index (NDVI) with the thermal image-based parameter land surface temperature and is commonly used to its simplicity and ability to represent two potential properties of vegetation stress in one index, but suffer from the mismatch in time scales, since vegetation greenness is fairly stable in the short to medium term but temperature fluctuate diurnally, and according to weather conditions as well as slope, aspect and terrain properties. The VSWI is also specific to the land cover type and measurement time of the image scene, and cannot be used as an absolute measure of drought severity. Thus, attempts to normalize the VSWI have contextualized the index within a defined period of available records.

$$VSWI = \frac{NDVI}{LST}$$

$$NVSWI = \frac{VSWI - VSWI_{min}}{VSWI_{max} - VSWI_{min}}$$

Where VSWI,  $VSWI_{min}$ ,  $VSWI_{max}$  are vegetation supply water index, minimum and maximum of VSWI.

NVSWI of zero indicates severest drought during the study period and NVSWI of 1 indicates wettest conditions (Nichol and Abbas, 2015).

### 7-Vegetation Temperature Condition Index (VTCI)

An approach developed which integrates land surface reflectance and thermal properties for drought monitoring. VTCI is defined as the ratio of land surface temperature difference among pixels with a specific NDVI in a sufficiently large study area. VTCI is defined as:

$$VTCI = \frac{LST_{NDVI,max} - LST_{NDVI_i}}{LST_{NDVI,max} - LST_{NDVI,min}}$$

$$LST_{NDVI,max} = a + bNDVI_i$$

$$LST_{NDVI,min} = \hat{a} + \hat{b}NDVI_i$$

Where  $LST_{NDVI,max}$  and  $LST_{NDVI,min}$  are maximum and minimum land surface temperature of pixels which have same  $NDVI_i$  value in a study region, receptively and  $LST_{NDVI_i}$  denotes LST of one pixel whose NDVI value is  $NDVI_i$ . Coefficient a,b,  $\hat{a}$  and  $\hat{b}$  can be estimated from an area large enough where soil moisture at surface layer should span from wilting point to field capacity at pixel level. In general, the coefficients are estimated from the scatter plot of LST and

NDVI in the area.  $LST_{max}$  can be regarded as the warm edge where there is less soil moisture availability and plants are under dry conditions,

$LST_{min}$  can be regarded as the cold edge where there is no water restriction for plant growth. The value of VTCI have ranges from 0 to 1; the lower the value of VTCI, the higher the occurrence of drought (Wan et al., 2004).

### 8-Temperature Vegetation Index (TVX)

Among indices which are used remotely sensed thermal and reflected radiation, TVX is a simple index with calculation their ratio. TVX can be defined as:

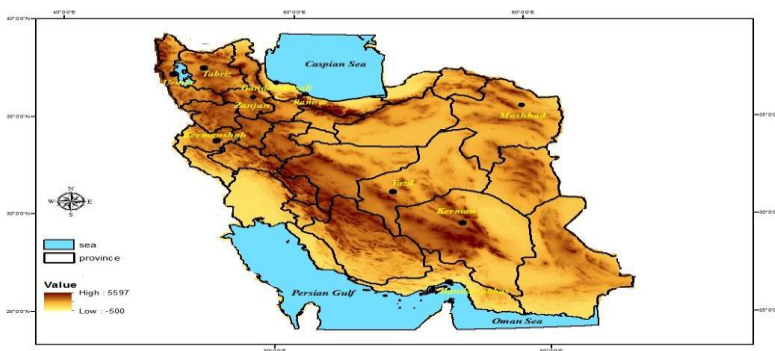
$$TVX = \frac{LST}{NDVI}$$

Where LST is land surface temperature, NDVI is normalized difference vegetation index.

TVX is graphically displayed in the LST–NDVI feature space by iso-lines of increasing slopes. TVX is negatively related to water condition. For stressed surfaces, land surface temperature increases or NDVI decreases as a result of decreased evaporation and vegetation fraction, therefore TVX dramatically increases. The major advantage of TVX is that it integrates both the reflective bands and thermal bands of remote sensing data, which offers more spectral information for drought detection. The main drawback is that there are several other factors influencing TVX values such as land cover change, sensor drift, atmospheric effect, cloud and etc. When the NDVI value is very small, the TVX value tends to infinite values. So, at places where NDVI is very small, one can use the arctangent of LST/NDVI which is expressed in degrees (Qin et al., 2008).

### CASE STUDY

For evaluating the performance of drought indices, data of some metrological stations which are located in Iran were used. Figure 2 shows the location of meteorological stations.



**Figure 2.** Spatial distribution of meteorological stations.



The stations are the different climatic regions such as arid, semi-arid and wet (based on the De Martonne classification: *Bandar-abass, Kerman, Yazd*-arid; *Bandar-anzali, Ramsar* –wet; *Kermanshah, Tabriz, Mashhad, urmia, Zanjan*- semi-arid).

Iran is one of the countries which suffer from severe drought. Iran's precipitation is approximately one third of global average and distribution of the monthly rainfall has been changed in recent years. The drought indices are derived from Terra MODIS data because of the sensor's moderate spectral and spatial resolution. Some process must be conducted on the images for determination the vegetation and drought indices. The nearest neighborhood method has been applied for the correction of pixels size from nadir pixels for geometric correlation (geo-referencing) using ENVI software. Growing months April, through September from 2006 to 2010 were used to assess the drought indices.

### RESULTS AND DISCUSSION

In this study, some criteria have been used for determination of effective drought indices which one of them is the use of linear correlation coefficient concepts. Therefore, for meteorological drought monitoring with emphasis on precipitation, linear correlation between precipitation and drought indices for each meteorological stations were calculated which are listed in Table 1.

Table 1. Correlation coefficients among precipitation – drought indices.

Stations	NDVI	EVI	VTCl	NVSWI	VCI	TCl	VHI	TVX
Bandar -abass	0.212	-0.159	-0.025	0.326	0.213	0.26	0.348	-0.261
Bandar- anzali	-0.131	-0.45*	0.2	0.074	-0.131	0.129	-0.008	-0.082
Kermanshah	0.673*	0.66*	-0.131	0.835*	0.673*	0.848*	0.786*	- 0.663*
Tabriz	-0.216	-0.39*	-0.242	0.664*	-0.216	0.763*	0.639*	- 0.621*
Kerman	0.051	-0.45*	-0.206	0.619*	0.052	0.719*	0.51*	-0.59*
Mashhad	0.247	0.358	-0.151	0.691*	0.247	0.77*	0.682*	-0.72*
Urmia	-0.063	-0.163	0.285	0.87*	-0.064	0.822*	0.715*	-0.82*
Ramsar	-0.25	-0.58*	-0.135	0.23	-0.251	0.269	0.055	-0.213
Yazd	0.121	0.02	0.45*	0.6*	0.122	0.65*	0.493*	-0.57*
Zanjan	0.34	0.025	0.23	0.87*	0.344	0.849*	0.778*	-0.8*

\*significantly different at the 5 % probability level.

The maximum number of significant correlation coefficients is related to TCl, NVSWI, TVX, VHI and then EVI respectively. The number of stations with significant correlation coefficient is one in the context of NDVI, VTCl and VCI indices. It can be said that NDVI has two components: ecology and weather which can be the reason of NDVI performance with low accuracy (Rhimzadeh et al., 2008). Kogan (1990) proposed geographic filtering to eliminate that portion of NVDI spatial variability that is related to the contribution of geographic resources to the amount of vegetation. This contribution fluctuates considerably

depending mainly on climate, soils, vegetation type and topography of an area. The VTCI performance can be related to the selected area because VTCI can be physically interpreted in an area large enough to provide wide ranges of NDVI and soil moisture at surface layer (Wan et al., 2004). Drought monitoring using VTCI, NDWI and NDVI indices in the Sefidroud River basin which is located in Iran, indicated that NDVI has the minimum correlation coefficient with precipitation (Parviz et al., 2011). In a study for drought monitoring in Iran using the perpendicular drought indices, VCI and EVI had poor relationship with precipitation (Shahabfar et al., 2012). Quring and Ganesh (2010) demonstrated that care must be taken when using the VCI for monitoring drought because it is not highly correlated with station-based meteorological drought indices and it is strongly influenced by spatially varying environmental factors. The VCI is suitable for monitoring agriculture drought but it has been shown to be inappropriate for monitoring meteorological drought in some regions (Quring and Ganesh, 2010).

The maximum and minimum number of significant correlation coefficient is related to Kermanshah, Tabriz, Kerman and Ramsar, Bandar-anzali stations respectively. It is worth noting that the number of significant correlation coefficient varies among different climatic regions and increasing the number of significant correlation coefficients from wet to semi-arid and arid regions. Shahabfar et al. (2012) indicated that VCI had the highest correlation with precipitation only in some stations located in mountains and semi mountains regions. In the other case, the correlation coefficients of precipitation and drought indices were calculated basis on climatic classification and the results are shown in Table 2.

Table 2. Correlation coefficients of precipitation – drought indices based on climatic classification.

Climate	NDVI	EVI	VTCI	NVSWI	VCI	TCI	VHI	TVX
Arid	0.393*	-0.2	-0.598*	0.57*	0.421*	0.767*	0.752*	-0.723*
Semi- Arid	0.639*	0.492*	-0.17	0.939*	0.39*	0.904*	0.9*	-0.856*
Wet	-0.185	-0.537*	-0.01	0.142	-0.183	0.18	0.02	-0.146

\*significantly different at the 5 % probability level.

The maximum and minimum number of significant correlation coefficients is related to arid, semi-arid and wet regions, respectively (Table 2). Among drought indices, EVI only has significant correlation coefficient in wet region with no significant correlation coefficient in arid region. Shahabfar et al. (2012) indicated that EVI showed poor relationship with precipitation data, as a

vegetation index rather than a drought index. In the different climatic regions, the VTCI have the minimum number of significant correlations. The results of correlation analysis based on climate and time aspect are listed in Table 3.

Table 3. Correlation coefficient based on climate and time aspect.

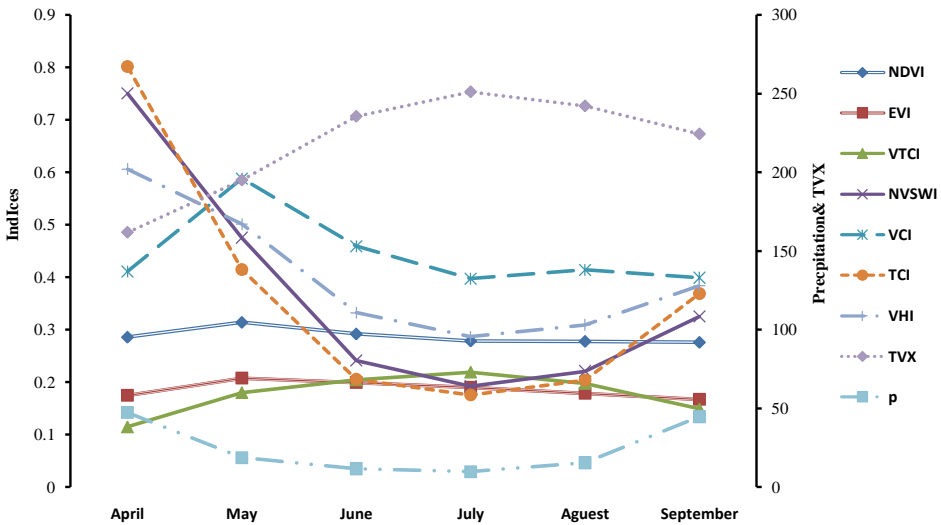
<i>NDVI</i>	April	May	June	July	August	September
Arid	0.52	-0.7	0.92*	-0.2	-0.317	
Semiarid	0.96*	0.76	0.05	0.64	0.315	-0.33
Wet	-0.8	0.26	-0.43	0.81	-0.72	0.2
<i>VTCI</i>	April	May	June	July	August	September
Arid	-0.241	-0.317	0.287	0.379	-0.383	
Semiarid	0.033	0.047	0.128	-0.361	-0.036	0.14
Wet	-0.074	-0.214	-0.352	-0.293	-0.162	-0.524
<i>NVSWI</i>	April	May	June	July	August	September
Arid	0.58	-0.57	0.914*	-0.12	-0.008	
Semiarid	0.98*	0.94*	0.47	0.73	0.26	0.65
Wet	0.77	0.569	0.73	0.62	-0.004	0.26
<i>VCI</i>	April	May	June	July	August	September
Arid	0.45	-0.71	0.96*	0.33	-0.28	
Semiarid	0.84	0.76	0.028	0.69	0.211	-0.4
Wet	-0.77	0.58	-0.44	0.79	-0.72	0.18
<i>EVI</i>	April	May	June	July	August	September
Arid	-0.143	-0.71	0.178	-0.56	-0.063	
Semiarid	0.94*	0.63	0.083	0.53	0.27	-0.33
Wet	0.89*	-0.31	-0.59	0.64	0.03	-0.26
<i>VHI</i>	April	May	June	July	August	September
Arid	0.54	-0.56	0.97*	-0.23	0.026	
Semiarid	0.98*	0.91*	0.55	0.72	0.24	0.5
Wet	0.24	0.73	0.32	0.7	-0.31	-0.046
<i>TVX</i>	April	May	June	July	August	September
Arid	-0.56	0.56	-0.86	0.099	0.103	
Semi- arid	-0.98*	-0.83	-0.29	-0.44	-0.4	-0.29
Wet	-0.84	-0.51	-0.63	-0.59	0.095	0.277
<i>TCI</i>	April	May	June	July	August	September
Arid	0.29	0.79	0.44	0.54	0.917*	
Semiarid	0.906*	0.82	0.96*	0.51	0.068	0.96*
Wet	0.78	0.37	0.84	0.17	0.42	-0.44

\*significantly different at the 5 % probability level.

The maximum number of significant correlation coefficients in all climatic regions is related to TCI and then VHI and NVSWI. VTCI has not significant correlation coefficients in any climatic regions and months (Table 3). In wet

regions, significant correlation coefficients of EVI are in April and maximum precipitation is in September. Maximum number of significant correlation coefficients of arid and semi-arid regions is related to June and April.

Also, maximum precipitation in arid and semi-arid climate is in April. Therefore, the similarity between the month with maximum number of significant correlation and the month with maximum precipitation is high in semi-arid climatic region. The maximum and minimum values of coefficient are in April and August, respectively. Therefore, it can be said that the correlation coefficient values are increased from dry to wet seasons. Investigation the equation of indices indicated the direct relationship among precipitation and drought indices except in TVX index which increasing of indices implies the precipitation increasing. Therefore, the monthly variation of all drought indices and precipitation are illustrated in Figure 3a.



Maximum values of precipitation and VHI, NVSWI and minimum value of TVX happened in April (Figure 3a). It can be said that NVSWI, TCI, VHI, TVX has temporal coordination with precipitation. Better performance of VCI rather than NDVI is evident from the Figure 3a.

The reason of VCI performance can be related to the effect of index normalization. Drought indices with direct relationship are decreased from April to July and after that month, indices increases with precipitation increasing according to Figure 3b. The trend of drought indices with direct relationship indicated the decreasing of indices values from April to July and then with precipitation increasing from August, the indices values are increased

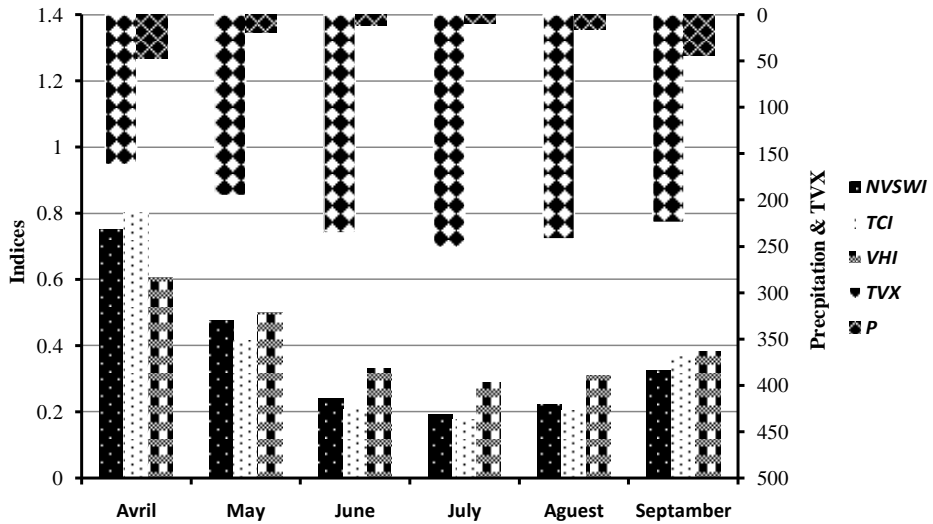


Figure 3. Monthly variation of all drought indices and precipitation (a), some indices (b)

Maximum and minimum values of precipitation are in April and July respectively. From drought indices with direct relationship, TCI has the minimum and maximum values. In this regard, TCI has more similarity with precipitation. Therefore, it can be said that TCI has more sensitivity to precipitation. TVX with inverse relationship has maximum and minimum value in July and April which is similar with minimum and maximum value of precipitation. In this aspect, TVX and TCI can be regarded as the selected indices. The precipitation and TCI average percentage of reduction and TVX average percentage of increasing from April to July are 37.75%, 38.21%, 15.97% respectively. In addition, the precipitation and TCI average percentage of increasing and TVX average percentage of reduction from July to September are 47.47%, 121.9%, 5.46% respectively. It is evident that TCI is more similar with precipitation.

Based on the other criteria, comparison among drought indices and precipitation was conducted annually. The mean precipitation of ten meteorological stations indicated that the maximum value of precipitation is in 2007 and then in 2009 but the minimum value of precipitation is in 2008. The annual variation of drought indices is illustrated in Figure 4.

NDVI and EVI has constant annual trend and they cannot show the variation of maximum and minimum precipitation completely (Figure 4). Inverse relationship of TVX with precipitation preserves the trend of precipitation in all years.

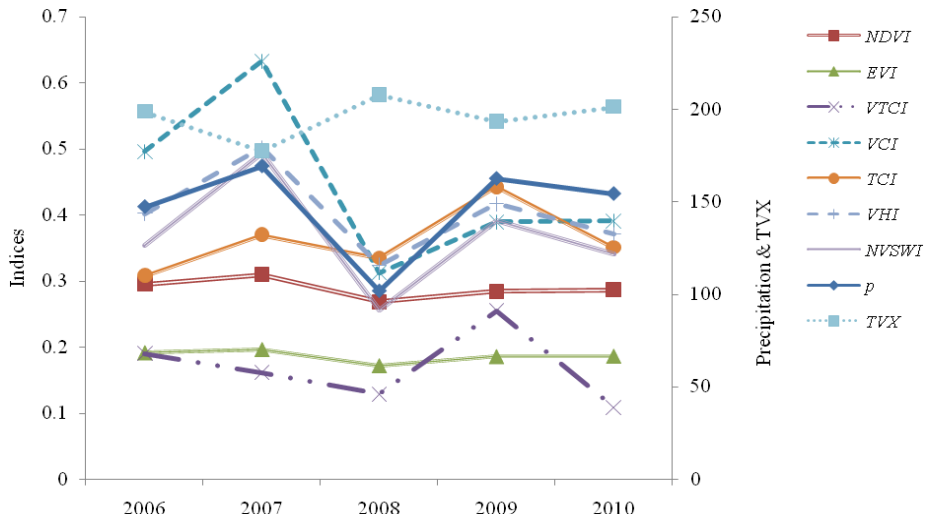


Figure 4. Annually variation of drought indices and precipitation.

VCI, VHI, NVSWI indices have similar trend with precipitation. In the other case, the slope of precipitation and drought indices time series were calculated using the trend line of time series and their results are shown in Figure 5.

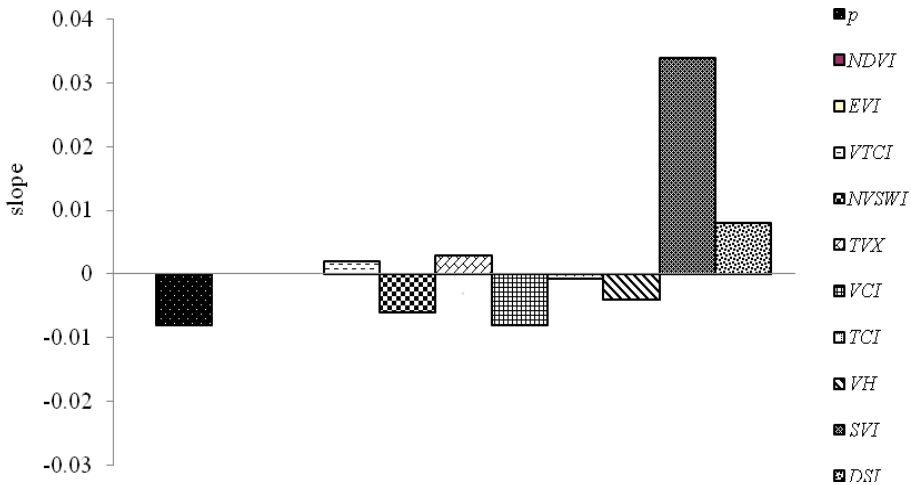


Figure 5. Slope of precipitation and drought indices.

The slope of NDVI and EVI time series is zero and they cannot be the efficient indices in explanation of precipitation variations. The sign of precipitation slope is negative which the slope of NVSWI, TCI, and VHI with negative sign and TVX with positive sign is coordinate with precipitation. The other criterion is the use of correlation coefficients which the correlation coefficients of drought indices with precipitation in all meteorological stations are listed in Table 4.

Table 4. Correlation coefficient of precipitation- drought indices in all stations.

NDVI	EVI	VTCI	NVSWI	TVX	VCI	TCI	VHI
0.01	-0.398*	0.16	0.66*	-0.59*	0	0.719*	0.6*
*significantly different at the 5 % probability level.							

EVI, NVSWI, TVX, TCI, VHI indices have significant correlation coefficients. Maximum values of correlation coefficients are related to TCI, NVSWI, VHI, TVH and the difference of correlation coefficient among indices is low (Table 4). Based on the other criteria for effective index determination, the trend of drought index and precipitation time series were investigated. Trend determination of time series conducted basis on Spearman and Pearson correlation test and the results of drought indices and precipitation are listed in Table 5.

Table 5. Trend analysis of precipitation and drought indices.

Correlation coefficient	Precipitation	NDVI	EVI	VTCI	NVSWI	VCI	TCI	VHI	TVX
Pearson	-0.004	-0.38*	-0.23	0.122	-0.2	-0.5*	-0.022	-0.27	0.22
Spearman	-0.049	-0.38*	-0.17	0.085	-0.2	-0.4*	0	-0.25	0.218
*significantly different at the 5 % probability level.									

According to Table 5, precipitation time series has not significant trend but there is significant trend in VCI and NDVI time series. It can be noted that there is not any similarity between trend of mentioned indices and precipitation. Also, the significant of Pearson coefficient among drought indices was investigated (Table 6).

The maximum number of significant correlation coefficients is related to NVSWI, VHI and TVX indices with other indices. The number of significant correlation coefficients is six which is acceptable from eight indices. EVI and VTCI indices have the minimum significant correlation coefficients. In the other case, sensitivity of drought indices to topographic effects was investigated. In this regard, meteorological stations were divided into three groups: first, second and third groups with the height average: -11.26, 1188.3, 1450.850 m, respectively.

Then the average of drought indices in each height was calculated and the results are illustrated in Figure 6.

Table 6. Correlation coefficients among drought indices.

Indices	NDVI	EVI	VTCl	NVSWI	TVX	VCI	TCI	VHI
NDVI	1	0.338*	-0.157	0.471*	-0.54*	0.93*	0.142	-0.6*
EVI		1	-0.014	0.059	-0.135	0.71*	-0.22	0.17
VTCl			1	-0.38*	0.46*	0.053	-0.42*	-0.38*
NVSWI				1	-0.97*	0.39*	0.92*	0.98*
TVX					1	-0.46*	-0.87*	-0.98*
VCI						1	0.025	0.53*
TCI							1	0.85*
VH								1

\*significantly different at the 5 % probability level.

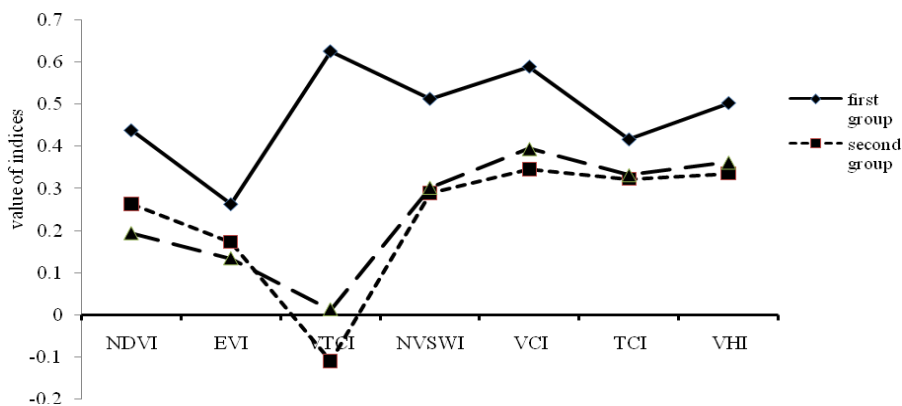


Figure 6. Average of drought indices in each height groups.

The highest variation is related to VTCl. Matsushita et al. (2007) were employed the coefficient of variation for evaluation the difference between the topographic effects on the EVI and NDVI (Figure 5). The larger coefficient of variation values was associated with a larger topographic effect and vice versa. The variation of coefficient of variation from minimum to maximum are: 1- TCI (0.14) 2- VH (0.22) 3-VCI (0.29) 4- TVX (0.31) 5- NVSWI and EVI (0.34) 6- NDVI (0.42) 7- VTCl (2.23). In this case, the maximum coefficient of variation is related to VTCl and TCI, VHI, VCI have the minimum coefficient of variation. The other criterion is the investigation of real time drought index. In this regard, the correlation coefficient of drought index with precipitation were examined in various precipitation schemes including 1-determination the correlation coefficient with precipitation with a lag time 2- determination the correlation coefficient with precipitation current month plus last two months. With one lag time, the number of significant correlation coefficients increase such as: NDVI: from one to four, VCI: from one to three, EVI: from five to six. In the second scheme the number of significant correlation coefficients increase such as:



NVDI: from one to three, VCI: from one to three, SVI: from one to two, NVSWI: from seven to eight. NDVI and then VCI indices have the maximum variations. It can be said that these indices are not only related to recent precipitation events but also related to past precipitation value and indicated that these indices cannot be used as a real time drought monitoring approach. The latest criterion is the use of the synthesized drought indices based on effective indices. The acceptable performance of TCI, NVSWI and VHI are illustrated based on some criteria in the previous sections. The average of correlation coefficient related to the synthesized index for all stations are listed in Table 7.

Table 7. Correlation coefficients of synthesized index.

Index	NVS WI	TCI	NVSWI	TCI	NVSWI	TCI	NVS WI	TCI	NVS WI	TCI	NVSWI	TCI
Weight	0	0	0.5	0.5	0.7	0.3	0.3	0.7	0.8	0.2	0.6	0.4
Coefficient	0.57	0.607	0.623		0.624		0.612		0.628		0.627	
Index	NVS WI	VHI	NVSWI	VHI	NVSWI	VHI	NVS WI	VHI	NVS WI	VHI	NVSWI	VHI
Weight	0	0	0.5	0.5	0.6	0.4	0.4	0.6	0.3	0.7	0.2	0.8
Coefficient	0.57	0.49	0.55		0.54		0.524		0.567		0.573	
Index	VHI	NVS WI	TCI	VHI	NVSWI	TCI	VHI	NVS WI	TCI	VHI	NVSWI	TCI
Weight	0	0	0	0.2	0.6	0.2	0.25	0.5	0.25	0.5	0.25	0.25
Coefficient	0.49	0.57	0.607	0.598			0.601			0.59		
Index	VHI	NVS WI	TCI	VHI	NVSWI	TCI	VHI	NVS WI	TCI	VHI	NVSWI	TCI
Weight	0.25	0.25	0.5	0.2	0.2	0.6	0.15	0.15	0.7	0.05	0.05	0.9
Coefficient	0.617			0.619			0.619			0.613		
Index	VHI	NVS WI	TCI	VHI	NVSWI	TCI						
Weight	0.1	0.8	0.1	0.1	0.5	0.4						
Coefficient	0.589			0.639								

The synthesized indices were conducted using two and three indices. Using two indices, the synthesized index was based on simple weighting to each index.

The result of synthesized index using two indices indicated that the number of coefficient correlations is not changed in this case. But the average correlation coefficient comparison of different stations indicated that there is not any improvement in the synthesized of VHI and NVSWI except in the weights: 0.2-0.8 which led to 52% increasing regard to NVSWI and 14.48% increasing

regard to correlation coefficient. There is the correlation coefficient improvement using NVSWI and TCI in all weights such as increasing correlation coefficient in weights 0.2-0.8 which led to 9.23% and 3.34% increasing regard to NVSWI and TCI respectively. The comparison of average correlation coefficient indicated the improvement of correlation coefficient using three indices rather than using only VHI. The maximum improvement is related to 0.1-0.5-0.4 with 23.31% increasing regard to VHI, 10.79% increasing regard to NVSWI, 5% increasing regard to TCI. NVSWI and TCI indices have the maximum improvement on correlation coefficient in the synthesized index. Du et al. (2013) proved that the synthesized index is a comprehensive drought monitoring indicator.

### CONCLUSIONS

Drought is one of the natural disasters which have negative impact on several aspects. Drought can be investigated effectively using drought indices. In this regard, finding the indices which can reflect the comprehensive information of drought is necessary. Therefore, selection of one or some indices as the effective indices is important for drought monitoring. Among drought indices, TCI, NVSWI, TVX, VHI and NDVI, VTCI, SVI have maximum and minimum number of significant correlation coefficients, respectively. It can be said that, satellite based drought indices based on vegetation condition were not more efficient indices such as NDVI and VCI, whereas drought indices based on combination of vegetation condition and land surface temperature has better performance. The goal of drought investigation is regarding the precipitation variation using drought indices.

Drought indices in arid and semi-arid regions have high significant correlation coefficient rather than wet regions. Temporal coordinates between months with maximum number of significant correlation of drought indices and months with maximum precipitation is obvious in semi-arid regions. The number of significant correlation coefficient of NDVI, VCI and EVI increases with lag time. Therefore, application of indices in real time drought monitoring is limited. The investigation of topographic effect on drought indices indicated that TCI, VHI and VCI indices have minimum effect on height variations and VTCI, NDVI has maximum impact from height variations. Therefore, the effect of height must be removed from the indices. The synthesized drought indices based on effective indices led to better performance. Trend analysis of precipitation and drought indices showed any trend in precipitation time series. Drought indices must preserve the trend but NDVI and VCI has significant trend Therefore, the care must be taken for drought monitoring using the mentioned indices. Drought indices investigation using different criteria indicated the high performance of NVSWI, TCI, VHI and TVX. The indices have high correlation with each other. Therefore, using one of them is suitable AQW4 for drought monitoring. It can be said that the indices with combination of vegetation indices and land surface temperature have more efficacy

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## APPLICATION OF PLANT BIOTECHNOLOGY TECHNIQUES IN ANTIOXIDANT PRODUCTION

### SUMMARY

Nowadays, antioxidant compounds are receiving increased attention in scholarly literature as well as in research. Antioxidants are a diverse group of compounds that can neutralize free radicals and thus help prevent diseases that are a consequence of oxidative stress. The most common antioxidant compounds are vitamins (A-carotenoids, C and E), thiols molecules (thioredoxins, glutathione), phenolic compounds (phenolic acids and flavonoids), enzymes and metal ions, as well as others. Plants have been shown to be an excellent source of antioxidant compounds, such as carotenoids, polyphenols, vitamins and betalains. Plant biotechnology uses the genetic engineering of agricultural crops as a means of producing foods rich in antioxidant nutrients, whilst plant cells and tissue culture techniques are used for the in vitro increment of antioxidant compounds in plant cells. There are numerous inspiring and promising reports about the possibilities of plant biotechnology that should provoke and encourage more research focused on antioxidant production from plants. The exogenous antioxidant molecules of important to human health (since endogenous antioxidants can be produced by the human cell itself) and the use of genetic engineering and plant cell culture techniques in antioxidant production in commonly used crops are presented in this paper.

**Keywords:** antioxidants, plant biotechnology, genetic engineering, plant tissue culture

### INTRODUCTION

There has been a growing interest in the role of antioxidants in chronic diseases caused by oxidative stress. Oxidative stress is the consequence of an imbalance between free radical generation and the antioxidant protection system that leads to numerous diseases, such as Alzheimer's and Parkinson's Disease, inflammatory diseases, cardiovascular disorders and so on (Uttara et al., 2009; Hybertson et al., 2011; Djordjevic et al., 2008). ROS, reactive oxygen species,

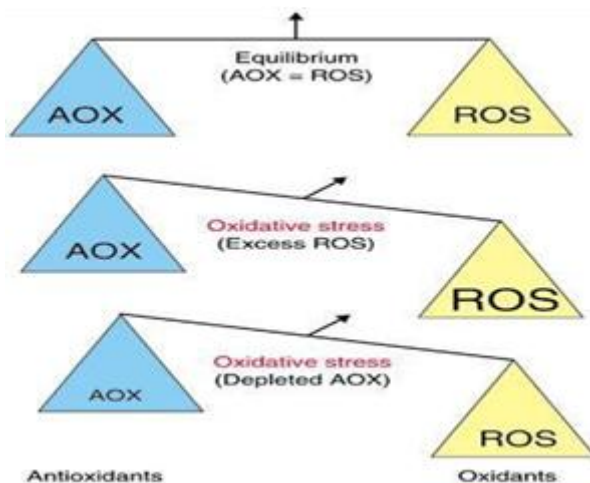
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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

and RNS, reactive nitrogen species, are produced in cells either through normal cell activities (e.g. through mitochondria and peroxisomes working by the action of the enzyme xanthine oxidase and so on) or in pathological states (inflammation, infections and ischemia/reperfusion injury). Also, free radicals can be generated by external factors, such as smoke from cigarettes, environmental pollutants, radiation (X,  $\gamma$  and UV rays), pesticides, ozone, industrial solvents, metals and so forth (Droge, 2002; Valko et al., 2006; Valko et al., 2007). Cells are constantly exposed to free radicals, so a balance between free radical species and antioxidants is important for cell homeostasis (Figure 1). Antioxidants are synthetic or natural substances that inhibit the oxidation of other molecules by numerous mechanisms. They prevent ROS and RNS reacting with macromolecules (proteins, lipids and DNA). In cells, homeostasis is held back by the synergic activity of non-enzymatic antioxidants and the enzymes of antioxidant molecules. Antioxidants produced by human cells are called endogenous antioxidants (enzymes and proteins), whilst antioxidants taken in by food are called exogenous antioxidants, or non-enzymes antioxidants (Fang et al., 2002; Lobo et al., 2010; Kunwar et al., 2011; Yeddes et al., 2013).

The importance of exogenous antioxidant molecules for human health (since endogenous antioxidants can be produced by the human cell itself) and the use of genetic engineering and plant cell culture techniques in antioxidant production in commonly used crops are presented in this paper.



**Figure 1:** Equilibrium between ROS and antioxidants

### Genetic/Metabolic Engineering in Plants

Food and health will always be a priority for humankind. Genetic engineering, amongst other methods, is employed for nutritional improvement of commonly used plant crops (Ryu et al., 1994; Altman et al., 2012). For this purpose, plants must have a foreign gene incorporated into them, to produce the transgenic plant Through the use of genetic techniques and metabolic

engineering, it is possible to produce plants with desired traits (such as increased production of antioxidant compounds). This transgenic plant or GM (genetically modified) crop can be created following the most common procedure, via the *Agrobacterium tumefaciens*-mediated transformation method, as shown in Figure 2 (Kirakosyan et al., 2009; Madigan et al., 2006; Jhansi Rani et al., 2013).

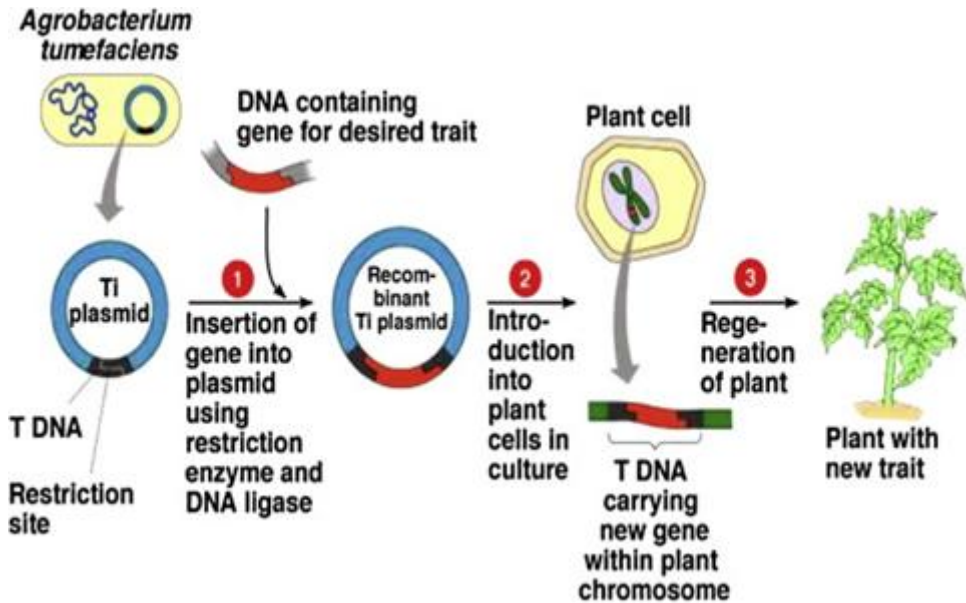


Figure 2: Process of *A. Tumefaciens*-mediated plant transformation

### Genetic/Metabolic Engineering of Flavonoid Compounds

Flavonoids are a very diverse class of phenolic compounds that perform antioxidant activities. They consist of two aromatic rings connected by a heterocyclic ring. Their structure is also referred to as C6-C3-C6 (Figure 3). They can be subdivided into different subgroups such as flavones, flavonols, flavanones, flavanonols, flavanols, or catechins and anthocyanins. Finally, flavonoids with an open C ring are called chalcones (Ververidis et al., 2007; Madhuri et al., 1999).

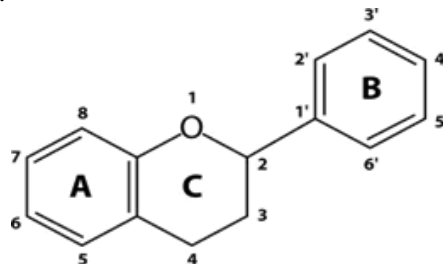


Figure 3: General structure of flavonoids

Fruits and vegetables are the main dietary sources of flavonoids for humans, along with tea and wine. It has been shown that foods enriched with flavonoids have preventive effects on human health that are the result of their antioxidants properties. The biosynthetic pathway of flavonoid synthesis is well-known and a simplified scheme is presented in Figure 4. Thus, it is possible to optimise genetic and regulatory processes within cells to increase the cells' production of flavonoids (Dias et al., 2014; Treutter, 2010).

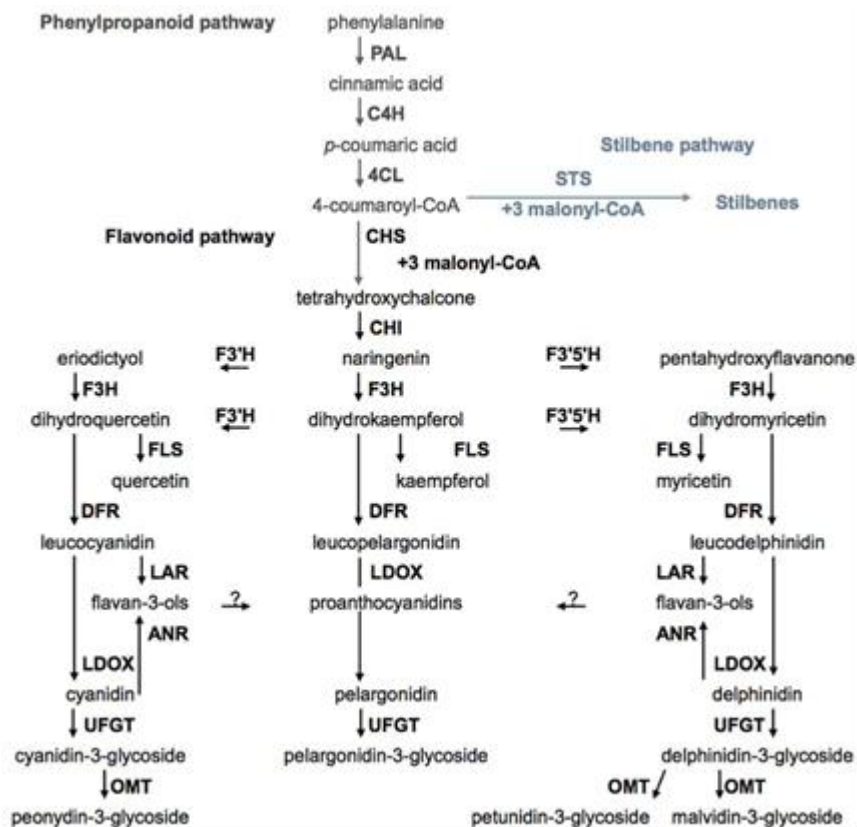


Figure 4: Biosynthetic pathways of flavonoids. Phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumaroyl:CoA-ligase (4CL), stilbene synthase (STS), chalcone synthase (CHS), chalcone isomerase (CHI), flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3'5'H), flavanone-3-hydroxylase (F3H), flavonol synthase (FLS), dihydroflavonol reductase (DFR), leucoanthocyanidin reductase (LAR), anthocyanidin reductase (ANR), leucoanthocyanidin dioxygenase (LDOX), dihydroflavonol 4-reductase (DFR), flavonoid glucosyltransferase (UFGT), O-methyltransferase (OMT).



The genetic/metabolic engineering of flavonoid compounds can be achieved through three different approaches: 1) by endogenous synthesis of flavonoids using structural and regulatory genes, 2) blocking the flavonoid pathway by the RNA interference technique and 3) the production of novel flavonoids by inserting new branches in the pathway (Bovy et al., 2007).

### **Regulatory gene engineering**

There are two types of regulatory proteins which are important for the engineering of the flavonoid biosynthesis of regulatory genes – one from c-MYB (cell-myeloblastosis) families and the other from c-MYC (cell-myelocytomatosis) transcription factor families. Transcription factor AtMYB12 (from c-MYB families) a flavonol-specific transcriptional activator in *Arabidopsis thaliana*, activates a biosynthetic pathway of polyphenols, chlorogenic acid and flavonols when it is engineered in tomatoes. In GM tomatoes, the engineering of this regulatory gene directs so-called ectopic expression, which means synthesis in the tomato parts that normally do not produce any flavonoids. AtMYB12 is very promising for the improvement of polyphenol content in industrially important crops (Bovy et al., 2007; Luo et al., 2008).

Simultaneous ectopic heterologous expression of the maize transcription factor genes, LC (a member of the MYC gene families) and C1 (a member of the MYB gene families) resulted in high levels of the flavonol-kaempferol in the flesh of a transgenic tomato. However, in ripe LC/C1-transformed tomatoes, anthocyanins were not detected (though in the leaves, anthocyanins were present), probably due to insufficient expression of the gene that codes for flavanone-3',5'-hydroxylase (required for the modification of the B ring) and a greater affinity of the dihydroflavonol reductase for the substrate dihydromyricetin (Bovy et al., 2007; Bovy et al., 2002). The anthocyanins content of the transgenic tomato could be increased by overexpression of AN1 transcriptional factors. The AN1 gene encodes a MYB-factor protein (c-MYB families) and regulates a variety of genes involved in anthocyanin accumulation (Mathews et al., 2003; Buteli et al., 2008; Sangera et al., 2013).

### **Engineering of structural genes**

In the tomato's peel, it is possible to detect following flavonoids: naringenin-chalcone, quercetin-rutinoside (rutin) and quercetin-3-trisaccharide, but the tomato flesh shows very low expression levels for flavonoids in general. This is because the activity of the endogenous flavonoid biosynthetic genes chalcone synthase (chs), chalcone isomerase (chi) and flavonol synthase (fls). Transcripts were undetectable in the fruit flesh. The chi (chalcon isomerase) gene from *Petunia* was introduced in the tomato and its overexpression resulted in elevated levels of flavonols up to 70-fold of quercetin glycosides and smaller, but still substantial, increases in kaempferol glycosides in the fruit peel. The flavonoid level in tomato flesh can be incremented using four flavonoid

biosynthetic genes from *Petunia* (CHS, CHI, F3H and FLS). When these genes were assembled in the tomato, the flavonoid production was increased in both the peel (primarily quercetin glycosides) and the flesh (primarily kaempferol glycosides). Interestingly, when expressed separately, none of these four genes was sufficient to lead to flavonol production in the fruit flesh, so the metabolic complex comprising of CHS, CHI, F3H and FLS in tomato flesh plays a key role (Muir et al., 2001; Bovy et al., 2007).

The introduction of two structural genes into the tomato — the naringenin-8-dimethylallyl-transferase (N8DT) gene and chalcone isomerase (CHI) gene — and their overexpression lead to accumulated rutin compared to wild-type tomatoes (Kawasaki et al., 2014). It is possible to gain up to a 78-fold increase in total fruit flavonols through ectopic expression of a single structural gene CHI (Verhoeven et al., 2002).

### **Blocking specific steps in flavonoid biosynthesis using the RNA interference technique**

This approach uses the RNA molecule which is complementary with the RNA transcripts of some genes and commonly important in flavonoid biosynthetic pathways. This antisense RNA is called RNA interference (RNAi). In tomatoes, when the CHS1 gene (encode for the first enzyme in flavonoid synthesis – chalcone synthase) was inhibited, the result was decreased activity of chalcone synthase and the total flavonoid level (naringenin chalcone and quercetin rutinoside) was reduced relative to the wild-type. The flavonol level was decreased after introducing a FLS (flavonol synthase) iRNA construct, but it was detected as anthocyanins accumulation due to low activity of the FLS and DFR (dihydroflavanol 4-reductase) enzymes. RNAi used for the F3H (flavanone 3-hydroxylase) gene showed a decrease of 20% as compared to the wild type. The DET1 (De-etiolated1) regulatory gene was inhibited using RNAi technology and the result was an increased level of flavonoids ( $\beta$  carotene and lycopene) (Casacuberta et al., 2015; Schijlen et al., 2007; Bovy et al., 2007; Davaluri et al., 2005).

### **Introducing novel branches in flavonoid biosynthetic pathways (stilbenes and isoflavon production)**

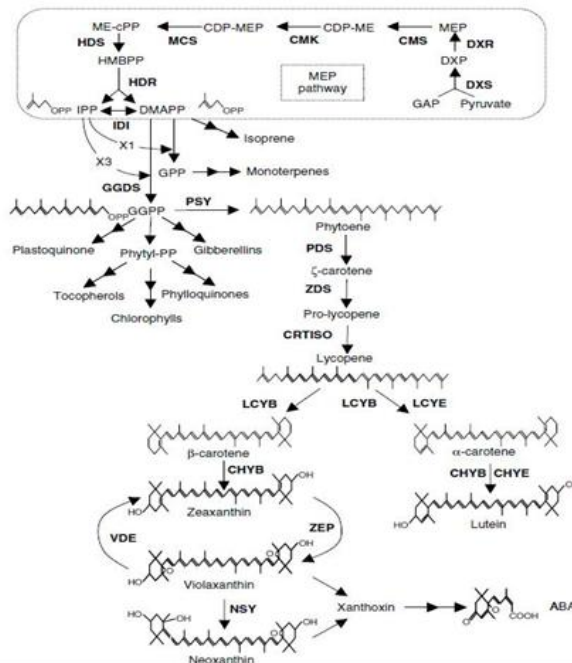
Stilbenes are phenolic compounds with antioxidant properties and resveratrol is such a compound. The key enzyme in resveratrol synthesis is stilbene synthase (StSy), but generally plants do not have StSy because they do not have any StSy genes (Treutter et al., 2010), so the production of resveratrol might be achieved if StSy genes could be incorporated into crops. The StSy gene was engineered from the grape to the tomato which resulted in resveratrol and its derivative production. By this genetic manipulation, the content of other phenolic compounds (such as naringenin and chlorogenic acid) in the tomato was not changed. Also, the StSy gene from *Parthenocarpissushenryana* was introduced into

lettuce which resulted in the synthesis of revarstatol in transgenic lettuce (Bovy et al., 2007; Giovinazzo et al., 2005; Schijlen et al., 2006; Liu et al., 2006).

Soybean isoflavon synthase genes have also been engineered in the popular crop tomato which lead to isoflavon production in transgenic tomatoes without any side effects on the endogenous phenolic content (Shih et al., 2008; Bovy et al., 2007).

### Genetic/metabolic engineering of carotenoids

Carotenoids or tetraterpenoids are yellow, orange and red organic pigments synthesised by plants. Some of them, such as  $\alpha$ -carotene,  $\beta$ -carotene and  $\beta$ -cryptoxanthin, are provitamin A carotenoids which means they can be converted by the body to retinol (vitamin A). Lutein, zeaxanthin and lycopene have no vitamin A activity. Carotenoids are important antioxidant molecules in a lipid environment, along with  $\alpha$  tocopherol, and, because of this, they are important ingredients in the human diet (Eldahshan et al., 2013; [www.scielo.cl/scielo.php?pid=S0718-16202009000200001&script=sci\\_arttext](http://www.scielo.cl/scielo.php?pid=S0718-16202009000200001&script=sci_arttext)). In order to increase the levels of nutritionally relevant carotenoids in crop plants, it is important to understand biosynthetic pathways and the regulation of this process so different strategies can be employed. Carotenoid biosynthesis and its related pathways are shown in Figure 5.



**Figure 5:** Carotenoid biosynthesis and related pathways in plants. GAP, glyceraldehyde 3-phosphate; DXP, deoxyxylulose 5-phosphate; MEP, methylerythritol4-phosphate;

CDP-ME, 4-diphosphocytidyl-methylerythritol; CDP-MEP, 4-diphosphocytidyl-methylerythritol 2-phosphate; ME-cPP, methylerythritol2,4-cyclodiphosphate; HMBPP, hydroxymethylbutenyl 4-diphosphate; IPP, isopentenylidiphosphate; DMAPP, dimethylallyldiphosphate; GPP, geranyldiphosphate; GGPP, geranylgeranyldiphosphate; ABA, abscisic acid. Enzymes are indicated in bold: DXS, DXP synthase; DXR, DXPreductoisomerase; CMS, CDP-ME synthase; CMK, CDP-ME kinase; MCS, ME-cPP synthase; HDS, HMBPP synthase; HDR, HMBPP reductase; IDI, IPPisomerase; GGDS, GGPP synthase; PSY, phytoene synthase; PDS, phytoenedesaturase; ZDS,  $\alpha$ -carotene desaturase; CRTISO, carotenoid isomerase; LCYB, lycopene  $\beta$ -cyclase; LCYE, lycopene  $\epsilon$ -cyclase; CHYB, carotenoid  $\beta$ -ring hydroxylase; CHYE, carotenoid  $\epsilon$ -ring hydroxylase; ZEP, zeaxanthineoxidase; VDE, violaxanthin de-epoxidase; NSY, neoxanthin synthase.

Metabolic engineering, along with genetic engineering of structural genes, are mainly used for the increment of carotenoid products. The structural gene, crtB (phytoene synthase is product of this gene), which is isolated from *Erwinia uredovora*, has been overexpressed in the transgenic tomato. The result was total carotenoid content increment up to 2.4 times as compared to the control. The obtained transgenic tomato is suitable for the production of processed products (Fraser et al., 2002; Giuliano et al., 2008). Since lycopene and other carotenoids are hydrophobic, its absorption in the body is low which means low bioavailability. Tomato processing, which includes the addition of fat, increases bioavailability and thus the function of these compounds (Richelle et al., 2002). To increase carotenoid content in plant crops, structural genes are mainly used, such as a crtB (isolated from) gene, whose product is a key enzyme in the biosynthetic pathway of carotenoids. When the crtB gene is overexpressed in the transgenic tomato, the ripe tomato fruit contains significant amounts of lycopene, while oxidised carotenoids (xanthophylls) are present only in trace amounts. The engineering of the structural genes of lycopene  $\beta$ -cyclase (LCY-b, from *Arabidopsis*) and  $\beta$ -carotene hydroxylase (b-chy from pepper) resulted in the increase of  $\beta$ -carotene,  $\beta$ -cryptoxanthin and zeaxanthin in the transgenic fruit. A change in carotenoid composition could be detected by the naked eye because the transgenic fruit, compared to the control, changed colour from red to yellow. The transformed lines demonstrated unchanged levels of expression of the endogenous biosynthetic genes of carotenoids which tells us that the production of carotenoids is the result of the expression of the introduced genes and not due to deregulation of the endogenous genes (Dharmapuria et al., 2002; Giuliano et al., 2008). The approach by which structural genes are silenced by RNAi strategy in order to increase the content of carotenoids was achieved in potatoes. A potato contains low levels of carotenoids – mainly lutein and violaxanthin (that have no vitamin A activity). Because the potato is a widely used crop across the world, research was performed which managed to increase the level of  $\beta$ -carotene by 38 times and incremented the total level of the carotenoids by 4.5 times by blocking the CHY1 and CHY 2 genes. CHY1 and CHY 2 genes code for the enzyme  $\beta$ -carotene hydroxylase which is, along with the  $\epsilon$  cyclisation of lycopene, a key

regulatory step in the carotenogenesis of potatoes. Silencing  $\beta$ -carotene hydroxylase increases total carotenoid and  $\beta$ -carotene levels in potatoes (Diretto et al., 2007; Giuliano et al., 2008; Apel et al., 2009).

**Table 1:** List of genetically engineered crops with enhanced carotenoid/flavonoid content

GM product	Nutrient	Gene introduced
Tomato	$\beta$ -carotene	<i>BLcy/Arabidopsis</i> + $\beta$ <i>Chy</i> /Pepper <i>DET1</i> /Tomato (RNAi)
	Lutein	<i><math>\beta</math>-Lcy/Arabidopsis</i> <i>CRY2</i> /Tomato
	Anthocyanin	<i>Del/Snapdragon</i> + <i>Ros1</i> /Snapdragon
	$\beta$ -cryptoxanthin, zeaxanthin	<i>B-Lcy/Arabidopsis</i> + $\beta$ <i>Chy</i> /Pepper
	Lycopene	<i>ySAMDC</i> , <i>Spe2</i> /Yeast
	Phytoene	<i>CrtB/E. uredovora</i>
	isoflavone/genistin	<i>GmIFS2</i> /Soybean
	Stilbenes	<i>STS/Vitisvinifera</i>
	Flavonoid/quercetin kaempferol	<i>CHI</i> /Petunia
	Flavonoid	<i>DET1</i> /Tomato (RNAi)
	Flavonol	<i>MYB12/Arabidopsis</i>
	Potato	Carotenoid
Carotenoids		<i>CrtI</i> , <i>CrtBandCrtY/E. uredovora</i>
$\beta$ -carotene: carotenoids		<i>LCY-e/potato</i> (antisense)
Brassica	$\beta$ -carotene, Lutein	<i><math>\epsilon</math>-CYC/Brassica napus</i> (RNAi)
	Carotenoid	<i>crtB</i> /bacteria
	$\beta$ -carotene	<i>crtI</i> + <i>crtb</i> /bacteria <i>DET1</i> (RNAi suppression)
Canola	Carotenoid	<i>idi</i> + <i>crtW</i> + <i>crtZ</i> (synthetic)/marine bacteria + <i>crtE</i> + <i>crtB</i> + <i>crtI</i> + <i>crtY/Pantoeaanatis</i>
Flax	Carotenoid	<i>crtB/P. ananatis</i>
Carrot	Ketocarotenoid	<i>CrtO/Haematococcuspluvialis</i>
Lettuce	Resveratrol	<i>STS/Parthenocissushenryana</i>
Apple	trans-piceid	<i>Vst1/V. vinifera</i>
Kiwi	Piceid	<i>Stibene synthase/Vitis spp</i>
Papaya	Piceid	<i>Vst1/V. vinifera</i>
Strawberry	<i>p</i> -coumaryl alcohol, <i>p</i> -coumaryl-1-Acetate	<i>CHS/strawberry</i> (antisense)

The engineering of regulatory genes for carotenoid amelioration is not well-developed in the case of flavonoids because the transcription factors that control the synthesis of isoprenoid compounds are not known.

However, researchers managed to isolate the regulatory Or gene from cauliflower and incorporate it into potatoes and thus to increase by six times the content of carotenoids in the transgenic potatoes. Besides this, the introduction of this gene leads to the differentiation of plastids hromoplaste and facilitated the identification of key steps in the biosynthesis of carotenoids. The Or gene product is a protein, DnaJ which contains a cysteine-rich domain. It is interesting that the DnaJ transcription factor does not increase the level of carotenoids by stimulating endogenous biosynthesis, but by affecting the formation of isolated structures within the hromoplast in which carotenoids have been accumulated (Lu et al., 2006; Lopez et al., 2008; Sanghera et al., 2013).

In tomatoes, the regulatory gene DET1 (TDET1) has been suppressed using fruit specific promoters, combined with RNA interference (RNAi) technology. Repression of this regulatory gene increases the level of carotenoids. Such transgenic tomato plants show that both lycopene and  $\beta$  carotene were generated at higher levels than in wild-type fruit with no negative effects on fruit yield and quality (Davaluri et al., 2005).

It is interesting to mention the approach by which the final product (in this case, carotenoid compounds) is elevated by increasing the supply of precursors in the biosynthetic pathway. The increase in carotenoids (phytoene and carotene) is achieved by increasing the precursor 1-deoxy-D-xylose-5-phosphate (DXP) which was synthesised from pyruvate and glyceraldehyde-3-phosphate in the MEP pathway by DXP synthase enzymes. The DXP synthase was isolated from bacteria and introduced by engineering into the tomato (see Figure 5) (Enffisi et al., 2005).

The list of genetically engineered crops with enhanced carotenoid/flavonoid content is shown in Table 1 (Sanghera et al., 2013).

### **Plant tissue culture**

Many years ago, it was thought that plant cells could not compete with microorganism cells in terms of the production of important metabolites due to the low rate of multiplication of plant cells. However, the production of secondary metabolites unique to plants demands the use of plant cell cultures, so different types of plant tissue cultures were used, both for commercial herbal products and in basic plant research. In plants, many somatic cells are totipotent, meaning that they contain all genes capable of being expressed by the plant and they can develop into the whole plant under the appropriate conditions. To create a successful culture of plant cells, tissues and organs, it is necessary to use the appropriate plant-starting material which is usually an explant, a small fraction of an intact plant taken under sterile conditions. The culture of plant cells, tissues or organs can be used for production of antioxidant compounds under strictly controlled conditions (Matkowski et al., 2008; Moscatiello et al., 2013; Orhan,

2012; [http://www.researchgate.net/publication/51077608\\_Plant\\_cell\\_cultures\\_bio\\_reactors\\_for\\_industrial\\_production](http://www.researchgate.net/publication/51077608_Plant_cell_cultures_bio_reactors_for_industrial_production), 2009).

### **Strategies for increased antioxidant production by plant tissue culture**

The following factors are important for the optimisation of antioxidant production in plant tissue culture:

- Optimisation of biosynthesis by culture conditions – environmental and nutritional factors influence the biosynthetic pathways of secondary metabolites. The media composition has to be optimised for both an intensive biomass increase and the accumulation of the desired metabolite.
- Determining whether the desired metabolite is produced in greater quantities in differentiated or undifferentiated cells.
- Selection of high-producing cell lines with a selecting agent.
- Precursor feeding and biotransformation – the absence of the precursor in the culture may be due to insufficient expression of the gene involved in the synthesis of the metabolites due to the absence of the stimulus in the environment.
- Elicitation and stress-induced production.
- Transformation with *Agrobacterium rhizogenes*-hairy roots. A number of species have been transformed with *A. rhizogenes* in order to achieve transformed root induction.

Now we will present some antioxidant compounds that are produced from *in vitro* cultures (Matkowski et al., 2008; Ryu et al., 1994; Altman et al., 2012).

### **Production of phenolic acid by plant tissue culture**

Rosmarinic acid, a phenolic compound and a two caffeic acid molecule, is very important for medicine and pharmacology. Rosmarinic acid has been produced from cell suspension culture, callus culture and from the transformed roots of different species (see Table 2). Since it has been shown that two effective methods to improve the accumulation of secondary metabolites in plant cell cultures are precursor feeding or elicitor treatment, researchers managed to produce rosmarinic acid by *Lavandula vera* MM cell suspension culture using vanadyl-sulfate as an abiotic elicitor. Rosmarinic acid production was increased by up to 3.92 g/L (2.8 times higher as compared to the control cultivation) and it was observed that the extracellular content of this molecule was 3.3 times higher when compared to the control variant, whilst the intracellular content represented no significant amount in the culture medium (Matkowski et al., 2008; Georgiev et al., 2006; Georgiev et al., 2006).

Cynarine and chlorogenic acid are phenolic antioxidant compounds with antimicrobial activities. An experiment produced cynarine and chlorogenic acid from calluses (calluses were obtained from disks of young cardoon leaves) and from leaves of *Cynara cardunculus* var. *cardunculus*, and the calluses gave a higher content of cynarine than the *in vivo* leaves, so *in vitro* tissue cultures can be used as a source to obtain cynarine for pharmaceutical purposes. However, the

antioxidant properties of the in vivo leaves showed higher activity than the calluses, probably because leaves contain other phenolic compounds which increased the antioxidant activity of the in vivo leaves (Trajtemberg et al., 2006; Siahpoush et al., 2011; Matkowski et al., 2008).

### **Flavonoid production by plant tissue culture**

Flavonoids can be produced by using callus cultures, cell suspension cultures and/or organ cultures. Amongst flavonoids, flavone C-glycosides are very important therapeutic molecules for disorders related to oxidative stress. Flavone C-glycosides are rare in plants, but they can be produced by callus cultures of *Passiflora quadrangularis*. The level of the flavones orientin, isoorientin, vitexin and isovitexin is increased when a callus is treated with UV-B irradiation. The antioxidant activities of the treated callus is higher by about 50 % when compared to the untreated calluses (Matkowski et al., 2008; Antognoni et al., 2007).

Rutin, from the flavon group of flavonoids, was produced from hairy root cultures of buckwheat (*Fagopyrum esculentum*). Researchers examined the effect of different media and the effect of the plant hormone, auxin, on rutin production and it was concluded that hairy root cultures are a good alternative approach for rutin production (Lee et al., 2007). Anthocyanins were also obtained from selected callus cultures of *Ajuga reptans* and from a cell suspension culture which could only be established in a light-dark cycle. Also, anthocyanins can be produced from *Glehnia littoralis* and *Ipomoea batatas* by using callus and cell suspension cultures (Callebaut et al., 1990, Terehara et al., 2001).

It was found that *C. roseus* anthocyanin biosynthetic enzymes are expressed both in vivo and in vitro. This was concluded after a comparison was made between anthocyanin production in stable cell suspension lines of *Catharanthus roseus* and from flowers obtained both from somatic embryogenesis and field-grown plants. The anthocyanin content was 1.5 times higher in the flowers from both plant types than that obtained from cell suspensions (Matkowski et al., 2008; Filippini et al., 2003).

### **Carotenoid production by plant tissue culture**

Carotenoids production was achieved from calluses of *Tagetes erecta*'s yellow and white flowers. For this purpose, the callus was treated with 9  $\mu\text{M}$  4-dichlorophenoxyacetic acid (2,4-D) and 8  $\mu\text{M}$  benzyladenine (BA). From the yellow flower callus, the main carotenoids were lutein and zeaxanthin, whereas the white flower callus gave lutein, zeaxanthin,  $\beta$ -cryptoxanthine and  $\beta$ -carotene. It can be concluded that the white callus of *Tagetes erecta* could be used as a good source of different carotenoids ( Benítez-García, I. et al., 2014).

### **Betalains**

Betalains are nitrogen containing compound pigments widely used as non-toxic food colourants. There are two classes of betalains – betaxantins and betacyanins. Betalains show high biological value and, in vitro simulation of



gastrointestinal conditions, their radical scavenging activity decreased from 75% inhibition of DPPH (2,2-diphenyl-1-picryl-hydrazil) to about 38%. These results open up possibilities for the use of betalains as additives in different food systems. The biosynthesis of betalains is poorly known, so genetic engineering is not applicable yet. On the other hand, betalain has been produced from the hairy root culture of *Beta vulgaris* L (red beetroot). The suitable technique for cultivation of hairy roots is immersion which has previously been implemented and the result was 18.8 mg/g dry biomass (DB) and betalains (9.6 mg/g DB betacyanins and 9.2 mg/g DB betaxanthins) (Yeddes et al., 2013; Clifford et al., 2015; Pavlov et al., 2006).

Some selected examples of the production of antioxidants from different plant culture systems are shown in Table 2:

Table 2: Selected examples of the production of antioxidants from different plant culture systems

Species	Compounds	Culture system
<i>Ajuga reptans</i>	Anthocyanins	Flower cell culture
<i>Anchusa officinalis</i>	Rosmarinic acid	Cell suspension
<i>Anthoceros agrestis</i>	Rosmarinic acid and its glucosides	Cell suspension
<i>Arachis hypogea</i>	Piceatannol (a stilbene)	Callus
<i>Artemisia judaica</i>	Flavonoids	Shoot cultures in bioreactor
<i>Beta vulgaris</i>	Betalains	Cell suspension, hairy roots
<i>Carthamus tinctorius</i>	Kinobean A	Cell suspension
<i>Cistanche deserticola</i>	Phenylethanoid glycosides	Cell suspension
<i>Cynara cardunculus</i>	Cynarin, chlorogenic acid	Callus
<i>Daucus carota</i>	Anthocyanins	Callus, cell suspensions
<i>Fagopyrum esculentum</i>	Rutin	Hairy roots
<i>Glehnia littoralis</i>	Anthocyanins	Callus, cell suspension
<i>Hemidesmus indicus</i>	Rutin	Callus, shoot culture
<i>Hyssopus officinalis</i>	Rosmarinic acid, lithospermic acid B	Hairy roots
<i>Ipomoea batatas</i>	Anthocyanins	Callus, cell suspension
<i>Lavandula officinalis</i>	Rosmarinic acid	Callus, cell suspension, bioreactor
<i>Ocimum basilicum</i>	Rosmarinic acid	Bioreactor culture of nodal explants and cell suspension
<i>Passiflora quadrangularis</i>	Flavone-C-glycosides	UV irradiated callus
<i>Petroselinum sativum</i>	Flavonols and flavones	Cell suspension
<i>Stevia rebaudiana</i>	Flavonoids	Callus
<i>Vaccinium pahalae</i>	Anthocyanins	Cell and aggregate suspension
<i>Vitis vinifera</i>	Stilbenes, procyanidins	Cell suspension

## CONCLUSIONS

Antioxidant compounds, such as carotenoids and flavonoids, are present in fruits and vegetables. The enrichments of antioxidants in agricultural products are achieved through genetic engineering and breeding transgenic crops. On the other hand, some antioxidant compounds can be produced for therapeutic application using the methodology of plant cell culture (callus, cell suspensions, tissue/organ culture and so on). The concept of functional food gives us the opportunity to research the possibilities of genetic engineering techniques and the methodology of plant cell cultures and to take this work in desired directions.

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## DEOXYNIVALENOL IN GRAINS OF OATS AND WHEAT PRODUCED IN SLOVAKIA

### SUMMARY

The mycotoxin survey focused on natural occurrence of Deoxynivalenol (DON) in mature grains of oats and wheat produced by Slovak fields in 2013. DON is one of the most predominant mycotoxins occurring in grains of cereals produced by *Fusarium* fungi after the attack of plants. A total of 10 oat samples from 9 locations and 178 wheat samples from 89 locations were collected in 2013. The samples were collected directly from growers. A commercial ELISA kit was used to determine the DON concentration in wheat samples with the limit of detection < 0.2 mg.kg<sup>-1</sup> (ppm) and limit of quantification 0.2 mg.kg<sup>-1</sup> (ppm). Mycotoxin was found in 30.0% (max. 0.49 mg.kg<sup>-1</sup>) of oat and 82.0 % (max. 5.10 mg.kg<sup>-1</sup>) of wheat samples. The natural mean DON contamination of oat samples was lower than in wheat samples. Only wheat samples had higher DON content than defined by the regulations of the European Union (EU) for this mycotoxin. Results indicated that the location had a significant effect on the DON content ( $p < 0.000$ ) in wheat grains what was connected with climatic conditions.

**Keywords:** mycotoxin, *Avena sativa* L., *Triticum aestivum* L., locations

### INTRODUCTION

Oats and wheat are among the most significant crops grown in Slovakia. However, the oat-growing area has diminished in the past few years. Grain of these crops is used for food and feed purposes. Nowadays, oat grain is often a part of breakfast cereals; oat bran and flour are added to wheat bread or various bakery products because of its beneficial effect on human health. The Food and drug administration (FDA) accepted the oat bran as a food that can lower the risk of heart disease due to physiological effects of  $\beta$ -D-glucan (a structural component of cell walls in oat bran) on the mammalian digestive system which lowers serum cholesterol (FDA, 1997). In addition, it is exploited in the prevention of heart and vascular diseases and some cancer types, especially the

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cancers of digestive system. The spikes and panicles of these crops are attacked by fungi from *Fusarium* genus several times a year which are able to produce various types of mycotoxins contaminating grains during the pathogenesis. Several *Fusarium* spp. are widespread pathogens on small-grain cereals (soft and durum wheat, barley, oats, rye and triticale) all around the world, including all European cereal-growing areas. In the Slovak Republic (SR), the data about the occurrence of genus *Fusarium* on oats is not known as much as in wheat where *F. graminearum* Schwabe and *F. culmorum* (W. G. Sm.) Sacc are the most common. However, *F. poae* or *F. graminearum* have been predominating in the past few years (Sudyova and Slikova, 2011). Fungi *Fusarium* can produce different types of mycotoxins (Bottalico and Perrone, 2002). Trichothecenes is one of the major mycotoxin groups which was categorized into four types. Type A is mainly represented by T-2 and HT-2 toxin; type B by DON, nivalenol and their acetylated precursors; type C by crotoxin and baccharin; and type D by tratroxin and roridin. Research into the natural occurrence of mycotoxins has shown that DON is one of the most frequently found mycotoxins in foodstuffs (Schollenberger et al., 2005) and in feed (Streit et al., 2013). Uhling (2013) reported that 46 metabolites (all of fungal origin) were detected in barley, oats and wheat samples (these were collected during “worst-case” season with weather conditions favouring fungal infection in Southern Norway). The analyses confirmed high prevalence and relatively high concentrations of type-A and -B trichothecenes (deoxynivalenol and HT-2 toxin). Mycotoxin DON has been associated with human gastroenteritis, and in experimental animal models, acute DON poisoning causes emesis, whereas chronic low-dose exposure elicits anorexia, growth retardation, immunotoxicity as well as impaired reproduction and development resulting from maternal toxicity (Pestka, 2010). The toxicity of the mycotoxin has led many countries to set up regulations for its control in grains and food products that are intended for human or animal consumption. The maximum allowed limits for DON have been set by the European Commission in unprocessed wheat and food products. EC Regulation 1126/2007 applied to unprocessed cereals other than durum wheat, oats and maize is 1250 µg.kg<sup>-1</sup> and unprocessed durum wheat and oats is 1750 µg.kg<sup>-1</sup>.

The present paper reports the results of the survey on natural occurrence of DON in the samples of oat and wheat grains obtained after the harvest of oats and wheat grown in different localities of Slovakia.

## MATERIAL AND METHODS

The wheat and oat grains were obtained during the 2013 growing season from 9 locations (oat samples) and 89 locations always from 2 farmers' fields (wheat samples) of Slovakia (Fig. 1). The samples were collected from grains of wheat cultivars and oat cultivars which were stored by growers in halls or granaries immediately after harvesting. The incremental samples were mixed and put in paper bags and stored in cool place (cca. 2000 g). After grinding the full lot sample, a subsample (5 g) was taken for analysis. A commercial ELISA kit



was used to determine the DON concentration in the samples (Ridascreen Fast DON, RBiopharm, Darmstadt, Germany) with limit of detection < 0.2 mg.kg<sup>-1</sup> (ppm) and limit of quantification 0.2 mg.kg<sup>-1</sup> (ppm). The grain samples were ground (Ultra Centrifugal Mill, type ZM 100, Retsch, Haan, Germany) with sieve size 1.00 mm. Then 100 ml of distilled water was added to 5 g of each sample and the mixture filtered. The filtrate, in aliquots of 50 µl, measured. The absorbencies of the wells were determined photometrically at 450 nm (MRX II, Dynex Technologies, Chantilly, Virginia, USA) and the DON concentrations were calculated in mg.kg<sup>-1</sup> by Revelation Version 4.25 (Dynex Technologies). The data were evaluated by descriptive statistics (mean, median and max values). Statistical analysis was performed using SPSS software 11.5 (SPSS, Chicago, Illinois, USA) and the statistical significance levels were set at 95 % ( $p < 0.05$ ) and 99 % ( $p < 0.01$ ).

## RESULTS AND DISCUSSION

Wheat samples obtained after harvesting crops in 2013 contained on average 4.4 more mycotoxin DON content than oat samples. The number of analysed oat samples is low; however, they were collected in various localities all around the territory of Slovakia (Fig. 1). Growing season in Slovakia in 2013 was quite favourable for the development of fusaria producing DON as shown in the results of the occurrence of mycotoxin DON in samples (Tab. 1). Total precipitation in Slovakia in 2013 was 127 mm in May and 112 mm in June (mean monthly precipitation was calculated by data obtained from about 600 meteorological weather stations in Slovakia by the Slovak Hydrometeorological Institute (Lapin, 2015). These results correspond with previously published data where there was a positive relationship between rainfall and DON content in wheat. Previous data were obtained from samples taken from wheat cultivated between 2004 - 2006 (Slikova et al., 2008). High mean DON content (0.93 mg.kg<sup>-1</sup>) in wheat samples in 2010 (Slikova et al., 2013) probably related to precipitation (mean precipitation in Slovakia was 235 mm in May and 148 mm in June, Lapin, 2015). There was a different situation with the occurrence of DON in wheat samples in 2011 when mean DON content was 0.30 mg.kg<sup>-1</sup>, in May = 67 mm and June = 124 mm (Slikova et al., 2013). The weather was similar in 2012 when we found low mean DON content in samples (0.27 mg.kg<sup>-1</sup>; not published), precipitation was 46 mm in May and 103 mm in June (Lapin, 2015). Weather conditions during the flowering and ripening stages of wheat were critical for *Fusarium* head blight development. The study showed that high temperatures in central Poland in July and August in 2009 accompanied by high rainfall in July were responsible for high DON levels in wheat (Wiśniewska et al., 2014). The contamination of oat grain by mycotoxin DON in Slovakia was only detected after artificial infection of panicles by fusaria (Slikova et al., 2010). Research into natural occurrence of mycotoxin DON in oat samples obtained from the crops in 2013 in Slovakia showed that DON content was low (Tab. 1).

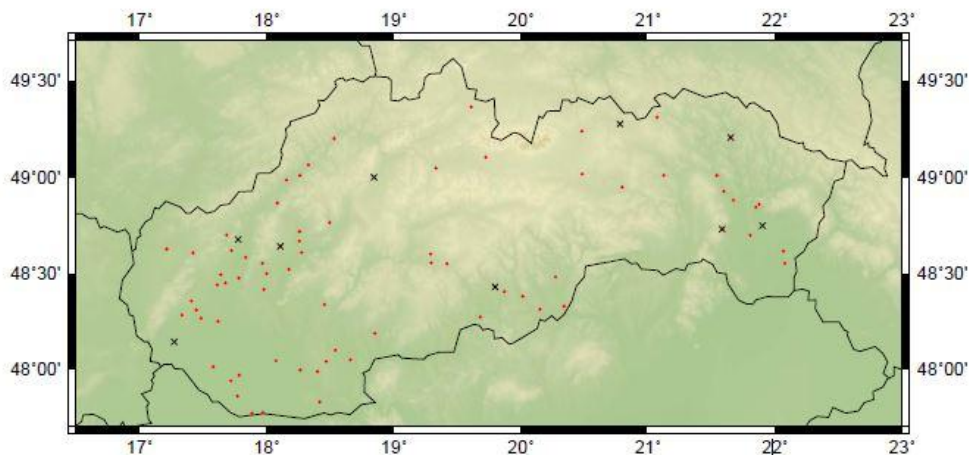


Figure 1. Location of sampling sites in the Slovak Republic from which oat samples (n = 9; cross) and wheat samples (n = 89; spot) were collected in 2013

Table 1. Deoxynivalenol, prevalence, median, mean and maximum detected amount of grain grown in Slovakia (2013)

Sample	N. samples	Positive samples (%)	Median (mg.kg <sup>-1</sup> )	Maximum (mg.kg <sup>-1</sup> )	Mean (mg.kg <sup>-1</sup> )
Wheat	178	82.0	0.34	5.10	0.74
Oats	10	30.0	0.20	0.49	0.17
Wheat + Oats	188	80.9	0.33	5.10	0.71

a Positive samples: mycotoxin concentration above detection limit > 0.2 mg.kg<sup>-1</sup>

ANOVA has revealed that don occurrence in wheat samples obtained from various localities in Slovakia was different (tab. 2). Mean don content lower than 0.2 mg.kg<sup>-1</sup> was found in samples obtained from 18 localities, the presence of mycotoxin ranging from 0.2 to 1.25 mg.kg<sup>-1</sup> was found in 146 localities and there was mean don content more than 1.25 mg.kg<sup>-1</sup> in samples from 14 localities. Two samples were obtained from every locality (i.e. From two fields) and their contamination within the locality was very similar in some cases. Excess contamination of both samples was detected in samples from 6 localities. The occurrence of at least one sample with excess don content was found in 16 localities. Don content in samples from these localities ranged from 0.33 to 5.10 mg.kg<sup>-1</sup>

Table 2. ANOVA of mycotoxin DON content

Source of variation	Sum of Squares	Degree of freedom	Mean Square	F-value	P-value
Location	137.166	88	1.559	3.516	0.000
Error	39.454	89	0.443		
Total	273.841	178			

## CONCLUSIONS

Nowadays, growers are well-informed about the occurrence of mycotoxin DON in the grains of cereals grown in EU countries. Results from analyzing samples of wheat and oat grain obtained from the crops in 2013 showed that the contamination in oat was several times lower than in wheat. Mean contamination of wheat samples relates to very favourable conditions for the development of diseases caused by fusaria in that year. The contamination of samples from one locality (two fields) was more or less the same. Significant differences in the contamination of samples in different localities were detected. This implies that mainly in years with favourable conditions for the development of Fusarium head blight, it would be suitable to choose such places for collecting samples which take into account the diversity of the country in monitoring the mycotoxin as in some localities both samples were without contamination and in some they were highly contaminated.

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## **EATING TODAY AND TOMORROW: EXPLORING INDIGENOUS FARMING SYSTEMS OF SMALLHOLDER ARABLE CROP FARMERS IN THE AGE OF CLIMATE CHANGE IN NIGERIA**

### **SUMMARY**

Indigenous farming systems have been able to sustain agriculture in Nigeria before the introduction of Western systems of farming which have brought changes to the farming systems and rural economy. This study assesses the use of indigenous farming systems by smallholder arable crop farmers with a view to providing sustainable rural economy. Quantitative data were collected with the aid of structured interview schedule from the farmers in derived savannah of Osun State, Nigeria. Descriptive statistics were used to summarise the data while multinomial logit, and farming system index were used to determine the sustainable indigenous farming systems that thrives during change in climate and their extent of use. Results showed that the mean age of farmers was  $48 \pm 5$  years. The mean farming experience was  $22 \pm 3$  years with more male farmers and extension contacts. Farming system index revealed that indigenous farming systems used by arable crop farmers include; different planting dates, planting of different varieties, multiple cropping, shifting cultivation, cereal and legume intercrop and mulching among others. Irrigation and zero tillage were the least practices among the farmers. Multinomial logit analysis showed that; Different planting dates, multiple cropping, mulching and shifting cultivation were positively significant with age of the farmers at  $P < .05$ . Also planting different varieties, multiple cropping and crop rotation were positively significant with their income at  $P < .05$ . The study concluded that farmers used different sustainable farming system that improves their rural economy to the advantage of their production.

**Keywords:** Smallholders, Farmers, Arable and Indigenous systems

### **INTRODUCTION**

Agriculture is the main source of livelihood for about 60 to 70 per cent of population of the region and also contributes substantially to the Gross Domestic Product (GDP) of the regions. Agriculture is the economic mainstay accounting for about 20-30 per cent of GDP in sub-Saharan Africa and representing up to 55 per cent of the total value of African export (Sokona and Denton, 2001). In fact,

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70 per cent of all Africans and nearly 90 per cent of their poor, work primarily in agriculture (World Bank, 2000). Agriculture in the region is still rain fed and anchored on smallholders; as a result it has been very difficult to cope with ever increasing population of the region.

Climate change is a challenge facing all countries across the globe. The Intergovernmental Panel on Climate Change IPCC (2007) defines the climate change as statistically significant variations in climate that persist for an extended period typically decades or longer. It includes shifts in the frequency and magnitude of sporadic weather events as well as the slow continuous rise in global mean surface temperature. The panel went further that climate change is known to be caused directly or indirectly by human. Zoellick (2009) stated that as the planet warms, rainfall patterns shift, and extreme events such as droughts, floods and forest fires become more frequent. UNFCCC (2007) reported that results in poor and unpredictable yields, thereby making farmers more vulnerable, particularly in Africa. However, sub-Sahara African countries are particularly disadvantaged because the region tops others among the poorest countries of the world.

Besides, Africa has recently experienced growing environmental degradation such as deforestation, desertification, declining soil productivity, loss of biodiversity and depletion of freshwater. Rosenzweig (2001) had predicted that “in a most fundamental way, climate change will bring change to agriculture wherever it is practiced.” Given Africa’s high dependence on agriculture as highest provider of labour, the effect of climate change could put millions of people at risk of poverty and hunger. Butt et al. (2005) also predicted future economic losses and increased risk of hunger due to climate change. The United Nations Intergovernmental Panel on Climate Change (IPCC) (2007) predicted that climate change could cause crop yields in some African countries to fall by 50 per cent between 2000 and 2020, seriously threatening the continent’s food security. Nigeria like other countries of sub-Saharan Africa is highly vulnerable to the effects and impacts of climate change (NEST, 2004; IPCC, 2007; Apata et al., 2009). The threats of climate change can be observed in both agriculture and non-agricultural socio-economic and infrastructural developments, agricultural production activities are generally more vulnerable to climate change than other sectors, (Kurukulasuriya et al., 2006). If the threats of climate change continue unchecked in Nigeria and Africa, the aim of poverty eradication or drastic reduction by the year 2020 will be a mirage. This is because food security is an important factor in poverty control.

Researchers have shown that Nigeria is already being plagued with diverse ecological problems, which have been directly linked to the on-going climate change (Odjugo, 2001). The increasing temperature and decreasing rainfall have led to frequent drought and desert encroachment. The effects of climate change is noticed everywhere, how to mitigate this effect should be the concern of everyone. Dabi et al. (2007) reported that many rural households in Nigeria typically have low capacity to adapt to climate variability because of very limited

financial, natural, physical, human, and social capital. Fluctuations in crop outputs over the years have been due mainly to fluctuations in weather and climate (Sekumade and Adesoji, 2009). However, smallholder arable crop farmers in Nigeria have employed different farming systems as strategies to mitigate climate change, which have enabled them to remain in production. These strategies are not known by many farmers in developing countries who are leaving farming for other enterprises. This study aims at filling this information gap by investigating into sustainable farming systems which serves as mitigation strategies of smallholder arable crop farmers in Nigeria. This will afford policy makers the advantage of providing friendly, affordable, and sustainable policy for the farmers, these are policies that are expected to assure food security for now and the future. With sustainable indigenous mitigation methods in practice, smallholder arable crop farmers would not suffer much from climate change. Therefore this study described the socio-economic characteristics of smallholder arable crop farmers in the study area, identified adaptation strategies employed by these farmers to mitigate climate change; and determined the extent of use of the strategies.

## **MATERIAL AND METHODS**

Multistage and proportionate random sampling techniques were employed. Purposive selection of 50% of the total Local Government Areas (LGAs) where arable crop farming was prominent was the second stage. The prominence was based on the records from the Ministry of Agriculture in each of the State. Incidentally, the LGAs were found in the derived Savannah zone of the States. This resulted to 24 LGAs (15 from Osun and nine from Ekiti) out of the 48 in the two States. The third stage involved random selection and interview of at least ten smallholder farmers who cultivates only arable crops. Structured interview schedule was used to collect information on socio-economic characteristics of respondents, adaptation strategies used to mitigate change in climate and the extent of utilization of the adaptation strategies. In all, total of 240 smallholder arable crop farmers were interviewed, 150 in Osun and 90 in Ekiti States.

### **Measurement of variables and Analytical Methods**

The dependent variable was adaptation strategies used to mitigate change in climate. This was measured by counting the number of adaptation strategies claimed to be used from the list of strategies among smallholder arable crop farmers. The list of adaptation strategies were standardised by asking sampled arable crop farmers in Southwestern Nigeria the adaptation strategies they employed to mitigate climate change. All the different strategies employed were included in the list. The list include different planting dates, planting different varieties, multiple cropping, crop rotation, shifting cultivation, mulching, irrigation and cultivation of cover crops.

Each adaptation strategy was scored one. If none of the strategies was adopted respondent was scored zero.

### Analytical Techniques

The dependent variable was adaptation strategies used by arable crop farmers. Multinomial Logit (MNL) regression model was used to determine the probability of usage of the adaptation strategies. In MNL model, if  $y$  denote a random variable taking on the values of  $\{1,2,\dots,J\}$   $J$  denotes a positive integer, and if  $x$  denote a set of conditioning variables. In this case,  $y$  denotes adaptation options and  $x$  contains respondents attributes like age, education etc.

Let  $\chi$  be a  $1 \times k$  vector with first element unity. The MNL model has response probabilities:

$$P(y = j | x) = \frac{\exp(x\beta_j)}{1 + \sum_{h=1}^J \exp(x\beta_h)}, J = 1 \dots$$

Where  $\beta_j$ ; is  $k \times 1$ ,  $j = 1 \dots j$

Kurukulasuriya and Mendelsohn (2006); Adesoji and Farinde (2010) used MNL model to analyse crops, livestock and fisheries, respectively on choice of respondent's adaptation to mitigate change in climate. MNL was used because it permits analysis of decisions across more than two categories, allowing the determination of choice probabilities for different strategies.

Parameter estimate of MNL model require that the probability of using a certain adaptation option (that is  $P_j | P_k$  is independent of the remaining probabilities).

$$U_j = \beta_j X_j + \epsilon_j \text{ and } U_k = \beta_k X_k + \epsilon_k.$$

$U_j$  and  $U_k$  are perceived utilities of adaptation options  $j$  and  $k$ , respectively,  $X_j$  is the vector of explanatory variables that influence the perceived desirability of the method,  $\beta_j$  and  $\beta_k$  are parameters to be estimated, and  $\epsilon_j$  and  $\epsilon_k$  are error term (Green, 2000). The parameter estimates of the MNL model provide the direction of the effect of the independent variables on the dependent (response) variables. Parameter estimate coefficient provides the actual magnitude of change or probabilities in SPSS. The marginal effects measures the expected change in probability of a particular choice being made with respect to a unit change in an independent variable from the mean (Koch,2007).

Adaptive Strategies Use Index (ASUI): ASUI was used to access the extent of use of the different climate change adaptive strategies by arable crop farmers. In analyzing the extent of use of any of the options by arable crop farmers, an Adaptive Strategy Index (ASI) was developed by ranking. The extent of use of the ASI was then expressed using a four-point scale with the scoring order 3, 2, 1 and 0 for regularly used, occasionally used, rarely used and not used, respectively. To obtain the ASI score, Islam and Kashem (1999), Adesoji and Farinde (2010) were modified and adopted.

Where: ASUI = Adaptive Strategies Use Index

$N_1$  = Number of arable crop farmers using a particular ASI regularly

$N_2$  = Number of arable crop farmers using a particular ASI occasionally

$N_3$  = Number of arable crop farmers using a particular ASI rarely



$N_4$  = Number of arable crop farmers not using any of the adaptive strategies.

The ASUI was used in rank order to reflect the relative position of each of the ASI in terms of their use. The extent of use of the ASI was then obtained for sampled arable crop farmers in the study area.  $ASUI = N_1 \times X_1 + N_2 \times X_2 + N_3 \times X_3 + N_4 \times X_4$ .

## RESULTS AND DISCUSSION

Results in Table 1 show that the mean age of arable crop farmers was  $48 \pm 4$  years. This indicates that they were still within their active production age. Only 30 per cent fell under 40 years, which could be regarded as youth. About 44 per cent was between the age brackets of 41 and 60 years. Majority (85.8%) of arable crop farmers were male and married. This shows that males were more involved in farming than their female counterparts. Farming could be described as a family enterprise thus members of the family assists on the farm to reduce labour cost. About 48 per cent of arable crop farmers spent between one and six years in school (Primary school level). Also 35.4 per cent had no formal education while only 16.7 per cent spent between 13 and 18 years in formal education institution, which correspond to tertiary education. It could be said that arable crop farmers were not well educated. The mean farming experience of the arable crop farmers was  $24 \pm 4$  years. Information from agricultural extension personnel was generally low for the farmers. This shows that most of the farmers could either acquire information on their farming activities through their neighbours and farmer friends.

Results in Table 2 show the parameter estimate for marginal effect (coefficient) of Multinomial Logit that measures the expected change in probability of adopting a particular strategy of mitigating climate change by arable crop farmers. Age, for example was positively significant to adaptation of planting at different dates, multiple cropping, shifting cultivation, and mulching. This indicates that increase in age of respondents by 1 year would influence the choice of the particular adaptation measures. For example increase in the age of the respondents by 1 year would influence the probability to choose planting at different date by .040 at 5 per cent confidence level. For multiple cropping (.013), Shifting cultivation (.826) However, the probability of chosen irrigation was negatively significant, this shows that as the age of respondents increases, the probability of chosen irrigation to mitigate climate change decreases. This shows that younger farmers would prefer the use of irrigation to mitigate climate change. The coefficient of Multinomial Log of preference was -.062, significant at 1 per cent level of confidence. Years of farming experience of arable crop farmers was found to influence the probability of choice of planting at different date. If year of farming experience increases by 1 year, planting at different dates would increase by .005 units, also planting different varieties would increase by .024 units and probability of chosen shifting cultivation would increase by .167 units. All the adaptation strategies were positively significant at different levels.

Table 1. Distribution of arable crop farmers by Socio-economic characteristics (N = 240)

Variable	Frequency	Percentage	Mean and standard deviation
<b>Age</b>			
< - 20	12	5	
21-40	60	25	48±4
41-60	106	44,7	
61-80	62	25,83	
<b>Gender</b>			
Male	206	85,83	
Female	34	14,17	
<b>Farming experience (years)</b>			
1-10	50	20,83	
11-20	46	19,7	24±4
21-30	60	25	
31-40	28	11,67	
41-50	48	20,00	
> - 50	08	3,33	
<b>Marital status</b>			
Single	36	15,00	
Married	202	84,17	
Divorced	02	0,83	
<b>Household size</b>			
1-3	124	51,7	
4-6	90	37,5	3±2
7-9	18	7	
10-12	0,8	3,3	
<b>Farm size (Ha)</b>			
< 3	166	69,17	
3 - 5	64	26,66	
> 5	10	14,17	
<b>*Information sources</b>			
Extension agents	74	30,83	
Other farmers	144	60	
Other farmers	84	35,0	

\*Multiple responses possible

Household size of arable crop farmers was also found to influence the probability to choose crop rotation and mulching as adaptation strategies for mitigating climate change. The coefficients of Multinomial log are 0.884 and .085 both positively significant at 1 per cent confidence level. This indicates that an increase in the household size by one would cause the probability of arable crop farmers to choose crop rotation and mulching to increase by 0.884 and .085, respectively. The parameter estimate for income was positive for all the adaptation strategies. This shows that income is essential in the choice of adaptation strategy to mitigate climate change. Income from arable crops also influenced the probability of choosing planting different varieties of crops, multiple cropping and crop rotation as strategies for mitigating change in climate. Multinomial log increase in income from arable crop of N1 would cause an increase in the probability of chosen planting different varieties of arable crops, multiple cropping and crop rotation by .936, .081 and .083, respectively.

Farm size also influenced the probability of chosen different planting dates, multiple cropping and mulching as adaptation strategies to mitigate climate change. An increase in the size of farm by 1 hectare would cause the probability of chosen planting at different dates, multiple cropping, and mulching to increase by .452, .213, and -.526, respectively. Planting crops at different dates and multiple cropping were positively significant while mulching was negatively significant at 1 per cent level. This shows that mulching favours small sized farms while arable crop farmers with large sized farms would prefer planting of crops at different dates and multiple cropping as adaptation strategies to mitigate climate change. Years spent in schools also influenced the probability of chosen different planting date, planting different varieties of crop, multiple cropping, crop rotation, shifting cultivation and cultivation of cover crops as adaptation strategies of mitigating climate change by arable crop farmers. All the strategies were positively significant. This shows that an increase in years of schooling by 1 year would increase the probability of chosen any of the adaptation strategies mentioned.

Results in Table 3 show frequency of usage of the adaptation strategies of arable crop farmers using ASUI method. The table revealed that planting at different dates was the most frequently used adaptation strategy among the arable crop farmers. This was closely followed by planting different varieties of crop. The third mostly used of the strategies was multiple cropping. All the three showed that some of the crops planted either due to wrong timing; non-resistant varieties or even a particular crop that could not withstand the stress of the climate would wither away. The fourth one with ASUI percentage of 13.4 was planting of cover crops. This is an adaptation strategy that would conserve soil moisture. This is also related to mulching which is the fifth frequently used strategy. Shifting cultivation is the sixth and it is followed by another related farming method, crop rotation. The last of the methods that was frequently used is irrigation. Irrigation is expensive might be the reason while it was not frequently used by arable crop farmers who might not be cultivating large hectareage. The mean farm size of the respondents was 2.7 Ha which shows they were small holder farmers.

Table 2. Parameter estimate and marginal effects from multinomial logit on climate change adaptation for arable crop farmers

Explanatory variables	Diff. planting dates		Planting different varieties		Multiple cropping		Crop rotation		Shifting cultivation		Mulching		Irrigation		Cultivation of cover crop	
	Coeff	Sig	Coeff	Sig	Coeff	Sig	Coeff	Sig	Coeff	Sig	Coeff	Sig	Coeff	Sig	Coeff	Sig
Age	.040**	.044	-.013	.508	.013***	.005	.362	.332	.826***	.000	.081**	.024	-.062***	.000	-.355	.362
Farming experience (Yrs)	.005*	.101	.024**	.051	.026	.842	-.602***	.013	.167**	.0395	.202	.431	.165	.170	.452**	.044
Household size	-.224	.343	.622	.494	.083*	.082	.884***	.002	.936	.243	.085***	.004	.427	.186	.094	.396
Income from arable farming	.864	.311	.936*	.092	.081***	.003	.083**	.020	.663	.362	.101	.943	.223	.874	.160	.895
Farm size	.452**	.045	.987	.684	.213***	.005	.021	.936	.312	.872	-.526***	.001	.532	.556	-.504	.586
Information source	.000	.633	.010	.969	.000	.999	.000	.966	.000	.999	.000	.887	.002	.966	.004	.899
Extension contact	.299	.300	-0.325	.257	.309	.272	.298*	.091	.409	.338	-0.029	.313	.301	.411	.150	.781
N Years of Schooling	.631*	.081	.399*	.100	.055**	.044	.051***	.008	.801*	.088	.411	.821	-.361	.555	.080***	.003

Source: Field survey, 2014 \*\*\*1%, \*\*5%, \*10%

Table 3. Rank Order of Climate Change Adaptation Strategies of Arable Crop Farmers by Frequency of Usage

Adaptation strategies	Not used 0	Rarely used 1	Occasionally used 2	Regularly used 3	ASUI	Percentage of respondents $\frac{ASUI}{\sum ASUI \times 100}$	Rank
Different planting dates	3	2	8	93	297	15.5	1
Planting different.							
Varieties	6	6	8	91	295	15.4	2
Multiple cropping	10	12	17	78	280	14.6	3
Crop rotation	47	45	29	29	190	9.9	7
Shifting cultivation	23	18	21	61	243	12.7	6
Mulching	8	14	58	39	247	12.9	5
Irrigation	87	71	9	7	110	5.7	8
Cultivation of cover crop	15	21	31	58	$\frac{257}{\Sigma=1919}$	13.4	4

Source: Field survey, 2014 \*\*\*1%; \*\*5%; \*10%

## CONCLUSIONS

Arable crop farmers respond to climate variability and change by employing simple and indigenous methods to mitigate its effects. The methods of the mitigation did not involve any foreign technology thus sustainable. These technologies were common among small holder farmers. The study also revealed that parameters like age of respondents, farming experience which is also a function of age, education measured in years of schooling are very important variables to be considered by policy makers, when planning climate mitigation programmes for arable crop farmers. Farm size and income are also important for arable crop farmers.

It could therefore be recommended that:

- Awareness about climate change should be raised among farmers and empowerment programmes should include sustainable methods of climate change mitigation;
- Adaptable crops that are resistant to harsh climatic conditions should be developed.
- Calendar of work should be developed by extension experts so that farmers could know the appropriate time to plant their crops.

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## **THE IMPORTANCE OF APPLICATION OF URBAN ECOLOGY PRINCIPLES TO URBAN REGENERATION OF PUBLIC OPEN SPACES – PARKS OF NOVI GRAD IN PODGORICA**

### **SUMMARY**

The rapid growth of modern urban environments is accompanied by degradation processes causing the need and application of urban ecology principles in urban regeneration of public open spaces. The public open spaces of functionalist residential areas built in the second half of 20th century nowadays represent the inherited spaces shaped over the decades of their use, often without specific purpose, and characterized by buildings and urban complexes built according to the standards of the time, different socio-economic, technical and technological conditions, but with the continuity of green open spaces organized as parks or linear green areas that still represent a special quality of these parts of the city.

Learning about the role and importance of park areas on a concrete example of Novi Grad in Podgorica, as well as about the role of the city in creating its identity, importance and experience for urban parks users, then the principles of urban ecology, along with the application of the ecological planning models provide further strategic guidelines for urban regeneration which can be incorporated in the planning documents and thus improve the relationship between the built environment and landscapes, all in order to create environmentally sustainable and self-sustaining space within the urban tissue.

**Keywords:** public open spaces, urban regeneration, urban ecology, urban parks, sustainability

### **INTRODUCTION**

The concept of landscape represents a part of nature, an element of landscape architecture, a selected unit that requires the observer's view, imagination and empathy, but as the segment of urban environment it becomes something that people interpret, model and create for their use. The landscape seems to stand opposite to contemporary city model, industrial production and technology that make it difficult to perform the play of harmonious relationship between man and nature.

As for the public open spaces and urban parks, the analysis of the possibility to integrate human activities and nature we are bound to and by, has been performed. Or, in other words, the question is what one can incorporate in

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the concept of landscape. Certain locations can be reformulated, planned and designed as a landscape. The openness of the landscape concept that can be made even in the broader city area leads to not only the cultural landscape concept, but also to the aesthetic landscape, along with the society capability to draw attention to certain elements, through the authenticity and recognizability.

In today's developed countries` cities that have similar principles of spatial organization, where the borders between the city and suburbs are disappearing, the significance of park is increasing as it highlights the cultural identity, the inherited natural characteristics and its connections to forests. The management of the integration of space, conservation, education and sustainability aims at establishing the overall quality of space.

The inconsistent content of the planning documents and the current situation in terms of the lack of ecological urbanism and sustainability indicators in the execution and implementation phase are the results of unsustainable urban design in social, economic and environmental aspects. Avoiding the traditional trend of urban planning and management that pay little attention to the environment, determining the effective indicators of sustainability and ecological urbanism is an important step towards the possibility of urban space regeneration.

Evaluation of existing systems of public open spaces - park areas of Novi grad shows the need for urban regeneration and aims at setting goals and standards for the design of future parks, and thus the development of the community.

**The importance and the role of parks.** The content and the role of a park as a public space is also visible in ecology, aesthetics, sociology and sports and recreation. The variety of different user groups` interests and needs are reflected in ways the space is used. Park planning and design, a selection of style, target groups and the context are the elements and requirements facing the urban planners today. The area of today's park is a product of the urbanized, industrialized and democratic society. It represents a cultural environment used to express and explores the nature and significance of public open spaces of the city, a meeting place of people from different social classes, backgrounds and ages; a place for unobtrusive observers and active participants in a wide range of activities. Entertainment, relaxation, play, refreshment are just some forms of recreation in contemporary parks that were also present in 19th century traditional parks, the archetypal green oasis, surrounded by the densely built neighborhoods. Parks cannot survive without public monitoring and cultural affirmation. The problem arises when these conditions are not met, as, then, the essential difference between parks and open space disappear.

**Urban ecology.** The complex relations between man and nature conditioned by economic development, infrastructure and bio-geo-physical elements within the urban environment indicate the multidisciplinary character of



urban ecology studies which determines its specific position within the group of scientific disciplines dealing with the environment.

A number of causal relationships determine the human environment in an urban area, with social influences prevailing and modifying the effects of a geobiosphere. An ecosystem represents a coherence of a biotope (a habitat) and biocenosis as a specific community of plant and animal populations. The changes of biotope through the construction of various facilities or through intensive agricultural activity lead to changes in the relative stability of the ecosystem. The anthropogenic processes as a possible consequence of the built environment, seriously encumber the natural components, causing a change of tolerance and dynamic balance.

Marina Alberti points out that cities are hybrid phenomena driven simultaneously by people and biophysical processes (Alberti, 2008), and the urban ecology is the study of the ways that humans and ecological systems evolve together in urbanizing areas.

Through urban environmental planning, designers help in mitigating the human impact on natural environment by harmonizing human activity with the activity of other living organisms in the given environment. The concept of observing human settlements as ecosystems appeared in the fourth century BC when Hippocrates argued on the effects of air, water and a place of living on human health while Vitruvius in the fifth chapter of the book "On architecture" refers to the importance of building orientation toward the sun, the winds, and with particular regard to climate. Frederick Olmsted and Lewis Mumford promote the importance of understanding the nature and the complex interaction between nature and human beings and their products, whether they are: buildings, cities or landscapes. Kevin Lynch (1984) describes the city as part of nature considering the role of nature from a more scientific point of view. Peter Calthorpe, and later David Owen, when dealing with the environment planning, incorporate sustainability as a defining objective in many environmental efforts.

White (2002) agrees that the ecological city is the one that provide acceptable standards of living for its residents, without exhausting its ecosystems and biogeochemical cycles it is dependent on. The idea of sustainability is an important element of the ecological park, which implies the possibility that the park offers its best in present, without compromising the future needs.

Inclusion of urban ecology as the main component of urban planning implies understanding the nature of cities, integration of humans and other living beings in the ecosystem, the causes of climate change and the need to use renewable energy sources through the use of elements from nature. In the process of city planning, urban ecology explores the human activities that cause the changes of natural or artificial elements, through defining the spatial characteristics of environmental conflicts (load, degradation, pollution), as well as social and psychological reactions (resistance, tolerance). All the above mentioned constitutes the basis for developing the guidelines for space urban revitalization that should be harmonized with other scientific disciplines.

## MATERIAL AND METHODS

The general scientific method used in the scientific research is analytical and synthetic method, while the basic method of case study provides critical research and describe the studied phenomenon - the need of setting environmental standards and standards of environmental sustainability and urban planning in an appropriate spatial context.

In the part of the research that implies the establishing of theoretical basis of the topic and the problem and formulating the analytical research apparatus, the method of critical analysis of theoretical sources will be applied. Along with the method of case study, the method of scientific analysis of planning documentation and critical analysis of texts that are indirectly related to the research subject and problem. In this research, the grounded theory method will be used as well, as one of the qualitative approach methods that includes constant comparison and theoretical sampling of the research materials, particularly applicable in the ecological urbanism.

The final part of the study contains the organization and evaluation of the information obtained by interviewing the citizens about the use and opportunities to use public spaces in Novi Grad neighbourhoods. The survey was conducted in the autumn of 2015, and involved 86 residents of Novi Grad neighbourhood. The paper also contains the results of a survey on the assessment of the current situation and future needs that was published in the Spatial urban plan (SUP) for the capital of Podgorica - up to 2025. (SUP, 2014.). This survey included 120 citizens and 10 urban planners and architects.

In order to evaluate the existing system of park areas and set goals and standards for planning the future green areas within the city districts, the analyses of the urban complex of Novi Grad, part of the urban core of Podgorica located on the right bank of the River Moraca, was carried out.

## RESULTS AND DISCUSSION

**Spatial and functional aspects of Novi Grad.** The geostrategic position of Podgorica, at the confluence of the Moraca and Ribnica rivers had a crucial influence on the formation and morphological characteristics of the urban matrix of Stara Varos, Nova Varos and Novi Grad (Fig.1). Nova Varos and Novi Grad have similar geometric models developed on an orthogonal system of blocks, in contrast to the organic grid of the Stara Varos. The difference in size and organization of the blocks of orthogonal grids indicates the various stages of formation: Novi Grad, the functionalist organization of free-standing buildings on the open agricultural land has been built for 30 years, in the second half of the last century. Moraca river bed separates Novi grad from the rest of the city, but it is well connected by pedestrian and boulevard bridges with the city center which is in the walking distance, so the strategic location of this urban settlement is very favourable and attractive.

Novi Grad did not use properly the potentials of the green belt along the river, except as a landscaped area around the buildings for public use, without

continuity and the access to the river bank, or as open unplanned green zones. The revision of the General Urban Plan in 1990. recognized a landscaped green river belts with "special landscape and recreational forms" that are connected to the park green zones of Novi Grad and Nova Varos making the "the green sleeves (green street, alleys, green spaces in the blocks) and to the forest parks of the hills Gorica, Ljubovic and Malo brdo.

Urban green spaces of Novi Grad are classified as:

- Park greenery;
- Linear greenery (avenues and hard landscaping);
- Greenery of central activities;
- Greenery of pedestrian areas;
- Residential building blocks greenery.

In the basic underlying principles of the revision of General urban plan from 1990 it is stated that "it is necessary that parks as basic elements of traditional space design become more present in future urban planning, primarily in the narrow urban area, and that the concept of urban extensions and construction on new areas should ensure a norm of 3m<sup>2</sup> per an inhabitant (MP 1990)". The revision of the General urban plan for Novi Grad in 1990 prescribe the arrangement of the attractive river banks which would allow social and functional integration of all parts of the city area into a unique whole.

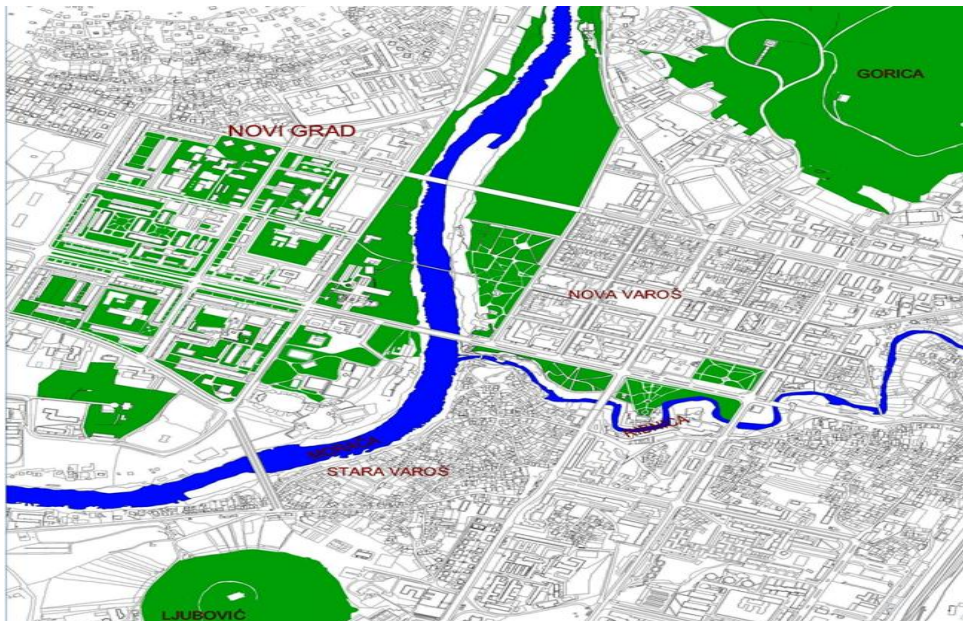


Figure 1, The position of park areas in Podgorica - Novi grad

The necessity of urban reaffirmation of open areas has been recognized in planning documents of Novi Grad. Detailed urban plans from 2006 and 2011

allowed construction on certain unoccupied locations within the blocks, thereby increasing density within the existing and added street network. Moreover, the introduction of tertiary activities in new buildings and in the ground floors of the existing buildings, with the activation of the central functions in contact zones causes an increase of passive transport: parked cars occupying open green areas within blocks, degrading the space created in accordance with the standards of the time in which this part of the city was founded. With a lack of maintenance and inadequate management of green areas, and the very obvious decay of urbanity, it becomes clear that urban regeneration of these important spaces is necessary.

Guidelines for environmental protection prescribed in Spatial urban plan of Podgorica (SUP, 2014.) recognize the need to preserve the environment by identifying the need for determining measures to protect the land from the "pollution by harmful substances (pesticides, heavy metals deposition from air pollution, the effects of acid rain, traffic impacts, untreated industrial water and wastewater from the roadway, waste, accidents, etc.); changes in physical, chemical, biological, microbiological and other characteristics of the soil (a decrease of humus content, soil compaction), permanent conversion (legal and illegal construction of buildings, infrastructure, exploitation of mineral resources), soil erosion (due to inadequate agricultural practices and forestry practices, the consequences of forest fire). (SUP, 2014).

One of the priority measures of soil protection was "development, or updating, and implementation of an integrated program for formation of protective forest plantations in accordance with the measures and programs of other sectors. The realization of other biotechnical measures on reducing the intensity of erosion (care for vegetation cover, maintaining of terraces, etc.)"; As a permanent measure, it is stated: "To perform regulatory measures of use of perennial crops instead of annual plants, as well as the obligation to maintain anti-erosion forests on slopes, and the like."

On the issue of guidelines for landscape design of space, the Landscape Plan has set a target of "effective optimization of space use that ensures the greatest possible protection of natural and cultural values, thus creating the concept of "sustainable development". The plan can also determine development of detailed studies of landscape that include the analysis of a small area or location through:

- identification of landscape elements;
- recognition of significant landscape elements, landscapes and views;
- evaluation of landscape elements;
- vulnerability assessment;
- suitability assessment.

From the standpoint of landscape planning and according to the Spatial urban plan, the term protection of nature refers to activities which "directly or indirectly assist the maintenance and improvement of plants and animals, as well

the protection and improvement their basis of life in the whole area" The very nature protection tends to:

- genetic variation in populations of animals and plants;
- species diversity within the biocenosis;
- biologically diverse landscape;
- long-term care of complex visual images or landscape images.

The planned concept of landscaping of Podgorica takes and develops the concept of the green system of the city set in the General urban plan in 1990, amended by the newly planned green areas taken from the current detailed urban plans.

When creating Spatial urban plan of Podgorica (SUP, 2014), a survey was conducted among citizens and professionals with the aim to assess the current situation and future needs. As stated in Spatial urban plan, it is important that the opinion of citizens and experts perceptions are almost identical to the results of the analytical part, especially in identification of the problem and quality in the area of Podgorica.

Some of the answers to the question "How will Podgorica look like by 2025?" were:

- a modern city in the environment, it is necessary to restore the soul of the city, enrich the public spaces of the city-monuments, fountains, cedars, green oasis-city gardens and alleys, pay more attention to greenery and parks (irrigation systems), to provide the citizens better city life, no vandalism and crime;
- to raise public awareness on environmental issues by enabling citizens to engage actively in environmental protection (to introduce a decree on the arrangement of green areas in peripheral parts of the city, on the improvement of city public spaces, continually educate people and raise environmental awareness);

Some answers to the question "What are the qualities of Podgorica today?" are:

- geographical location and climate;
- the wealth of water and greenery;
- The question "What are the biggest problems in Podgorica today?" was answered as follows:
  - preservation of greenery and municipal sanitation services;
  - water supply and sewage and storm water channeling;
  - We will also mention several responses to the question "In your opinion, what is of the most important precondition for quality of life in the city/suburban area?"

- urban planning in line with the needs of the city (consistent implementation of urban plans);
- healthy environment;
- well-designed and eventful city public space (parks, squares, pedestrian streets);

According to the survey results, most citizens of Podgorica believe it will be a modern city with nice and, in terms of their content, interesting public spaces (parks, green oasis, squares, bicycle and pedestrian paths), with a renewed cultural heritage and identity of urban spaces that make the "soul of the city". That is why it is necessary "to protect the environment and provide a higher quality of life in the city." (SUP, 2014). It is evident that respondents recognized the importance and necessity of preserving and activating the city green areas, as well as raising environmental awareness.

In an independent survey of 86 residents of Novi Grad neighbourhoods, of different age and gender, conducted in autumn 2015 (according to data from the Detailed urban plan of Novi Grad from 2012, there were 16,035 inhabitants in this part of the city, but there is no official data on the number of adult inhabitants) by asking questions about their perceptions and attitudes on the use or possible use of public spaces of Novi Grad neighbourhoods, we got the answers that indicate the importance of public space in the social life of the neighbourhood.

The survey results point to the problem of urbanity, and only:

- 2,6% organize and maintain the space around their buildings;
- 13,9% most often socialize with neighbours in the neighbourhood open space;
- 51,8% of respondents never spend their leisure time in a neighbourhood public, and
- 31,7% of respondents spend some time in the space around their buildings ones or twice a day.
- In most cases, the open spaces near their buildings are considered:
  - 39,1% as a public urban space,
  - 22,6% of respondents think it belongs to the residents of the surrounding buildings.
  - 38,3% of respondents think it belongs to all the residents of Novi Grad neighbourhood.

When it comes to free response questions, predominantly expressed is the need for the higher level of organization and maintenance of park areas (87% of respondents mentioned one or both of these problems).

When answering the question of whether they think that living in this part of the city is a privilege, 83% of respondents gave a positive answer to this question, emphasizing the advantages of the position of the city in relation to the functions and comfort that provide large greenery areas.

The conclusion is that there is a presumed relationship between public space, territorial practice and the space design. It is important to take into account the identified differences in perception and attitudes of respondents in relation to the use and importance of park areas not only for the residents of this part of the city, but also for the city as a whole.

### **Models for implementing ecological planning**

Interviewing the residents of Novi Grad neighbourhoods and all potential users and stakeholders, as well as professionals in the field of architecture/urban planning and landscape architecture, bringing stakeholders to the location and consideration of their views, opinions and recommendations represent an important tool for urban planners.

In the planning literature we can find many examples of research models that are useful in analysing the specific processes of urban design and that are applicable to this specific case – parks in Novi Grad, and thus to a complete park area in Podgorica.

Steiner proposed ecological planning process consisting of 11 steps (Palazzo and Steiner, 2011):

1. Problem and/or opportunity identification
2. Goal establishment
3. Regional-level inventory and analysis
4. Local-level inventory and analysis
5. Detailed studies
6. Planning concept
7. Landscape plan
8. Education and citizen involvement
9. Detailed designs
10. Plan and design implementation
11. Administration

The ecological planning model synthesizes other processes of regional and landscape planning. Its main references are the ecological methods for design and planning formulated since the 1960s by Ian McHarg (Palazzo and Steiner, 2011), which formulated the idea of linking environmental information through ecological knowledge to design and planning decisions by what McHarg called the “layer-cake model.” (Fig.2)

Planning involves managing land use in cities, agriculture areas and forests, in terms of process. The planning and management of natural resources can be achieved by using the principles of a stewardship defined as concern for

the planet, counting on human and individual responsibility in relation to the common good.

According to Sexton (Sexton et al. (1999); Palazzo, Steiner: 2011), the process can be implemented in seven steps:

1. Identify the problem, decision makers, their authorities, the stakeholders, and the decision-making process.
2. Define the problem and refine the objectives.
3. Develop alternative actions to achieve the objectives.
4. Compare each alternative with the objective.
5. Choose a preferred alternative.
6. Implement the chosen alternative.
7. Monitor and evaluate.

The application of the ecosystem approach is mainly used in the implementation of the development, conservation, restoration and rehabilitation, which refers to the urbanized and natural/rural areas. Ecological landscape planning was applied in a similar manner in urban environment. In Europe, the implementation has been focused on environmental problems arising from the rapid intensification of land use that creates extreme competitiveness between agriculture, forestry, industry and urban development, which is understandable given the dominance of the human impact on land and landscapes of Europe. By contrast, in North America the focus is on the planning of the network of habitats and wildlife conservation in rural and natural areas, with special emphasis on the conservation of biological diversity and the sustainable use of land. (Ndubisi, 2002).

Ecological planning emerged in North America during the 1960s through the pioneering studies of McHarg (1969) and others, and then evolved in 1980s.

Steiner (Palazzo and Steiner; 2011) provides ecological planning model and a list of elements, from regional to local, to be inventoried in the design process:

- Regional climate - temperature and precipitation;
- Geology-geological maps to evaluate the suitability of an area as a construction site;
- Terrain - physiography (elevation and slope);
- Water - water budget (precipitation, uses, and groundwater), hydrological cycle, flooding areas, water quality, hydrologic system, water supply, and sewage treatment systems
- Soils - characteristics, soil survey and soil capability classification (the survey particularly helps with understanding land uses and land values for specific activities)
- Microclimate - ventilation, solar radiation, albedo, and temperatures



- Vegetation - plant communities; rare, endangered, or threatened plants; native and disturbance adaptive plants
- Wildlife - species; habitat values; habitat of rare, endangered, or threatened species
- Existing land use and land users - the physical arrangement of space utilized by humans, ownership (public and private), settlement patterns, buildings, and open space types.

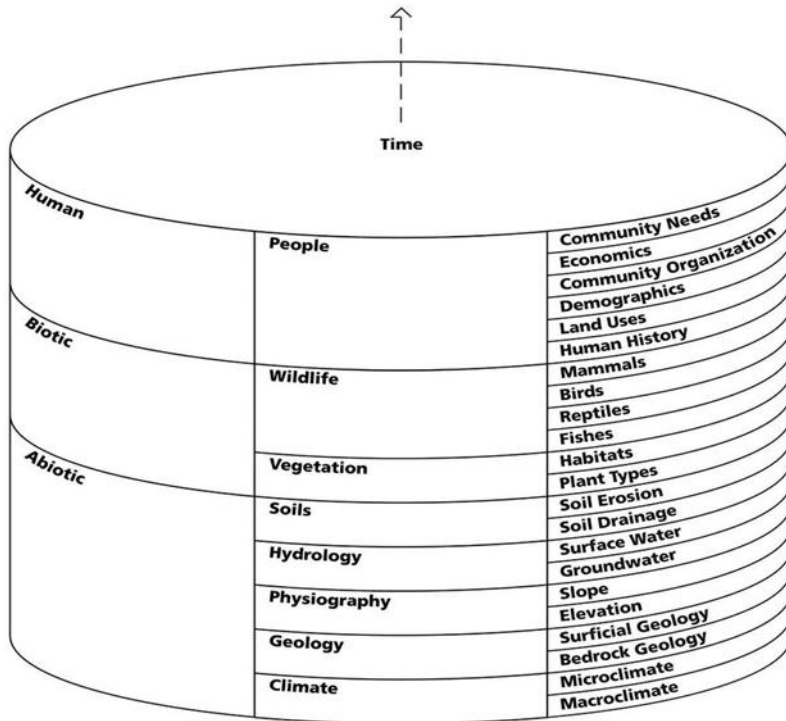


Figure 2. Layer-cake model. (Palazzo and Steiner 2011)

The **ecological footprint** concept that was developed by Wackernagel and Rees (Palazzo and Steiner, 2011) involves measuring the consequences of human actions, especially in urban uses, on ecosystems. The analysis compares the resources needed to support the project with nature’s ability to regenerate those resources and to produce ecosystem services. As a result, the designer could estimate demand for water, food, and energy as well as waste and carbon produced by the project. The ecological footprint measures the impact of consumption and subsequent waste discharge by converting impact variables into the single unit of land. This includes land appropriated by fossil energy use, the built environment, gardens, crop land, pasture, managed forest and land of limited availability, including untouched forests and non-productive areas. This

concept could be applied at various scales, but at the urban level, this approach can be used to calculate the equivalent amount of land consumed in order for a city to function.

Presented survey results shows the differences in perception and attitudes of respondents of Podgorica's district Novi Grad in relation to the use and importance of park areas, but also indicate the overall importance of educations of citizens about urban ecology and the consequences of human actions on ecosystems.

Those models of ecological planning incorporated in planning documents, with education and active participation of citizens and all stakeholders can significantly contribute in terms of evaluating the existing system park areas, determining the needs of urban regeneration of open public spaces and setting goals and standards for the future green areas within the city districts.

### CONCLUSIONS

The specific character of the location determines the prevailing conditions and directs the ecological approach in urban planning. The tendency of integration of natural and cultural characteristics in the self-sustainable system will support the desire to promote the location condition.

Urban environmental development is important for the sustainability development of an urban environment and it should be considered at all stages of urban planning and design. The main research results are as follows:

- To present a suitable method for the evaluation of the development strategy from the environmental point of view.
- To prepare the indicators and factors for the development of the urban scale evaluation in order to achieve a form of sustainability.
- Method of population participation in the evaluation may lead to easier environmental problems detection.
- Knowledge of all environment characteristics as a guiding principle in urban regeneration of space defines a strong sense of place and identity.

The existing awareness of the role and importance of green spaces in the urban environment and recognition of the need for urban regeneration in the light of modern technological progress and social change, imposes the application of the principle of an effective urban ecology model.

With the aim of finding the guidelines for the urban revitalization of public open spaces and park areas, further investigation and identification of the citizens preferred modes of use is needed, along with constant research on the effects of such uses to the existing ecosystem.

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**FRACTAL ANALYSIS OF SPATIAL VARIABILITY OF ORGANIC  
CARBON IN ANTHROPOGENIC SOILS. CASE STUDY:  
KASTEBA BAY, CROATIA**

**SUMMARY**

Soil organic carbon (SOC) is a key variable of the soil, agriculture and whole environment. The spatial variation of SOC is controlled by many spatially independent processes and their complex interactions which operate on its own discrete scale. Understanding the causes and characteristics of SOC spatial variation can help knowing how to measure it and to design better site-specific management strategy or precision farming. The objectives of the research are to characterize spatial variability of SOC in the anthropogenic soils and identify the principal factors that determine its spatial pattern. In the total of 206 top-soil samples (0-30 cm) collected on a 4 960 ha of the anthropogenic soils at the Kastela Bay, Croatia, SOC was analyzed. The mean SOC content was 22.19 g C kg<sup>-1</sup> and ranged between 7.75 and 40,87 g C kg<sup>-1</sup>. The SOC has shown a multifractal behavior characterized with partial spatial independence over a scale of 5 000 m separated with break point. Estimated fractal parameters for SOC, derived by variogram method, for maximum distance between point pair of 21 000 m, were: fractal dimension (D) 1.92, ordinate intercept - gamma ( $\gamma$ ) 1.09 and associated coefficient of determination ( $R^2$ ) 0.50. The very high fractal D value, high gamma ( $\gamma$ ) value and low  $R^2$  indicated a very small spatial continuity of SOC. This has shown that short-range effects prevail over long-range ones in affecting the overall complexity of distribution and that variations of SOC at small scale are significant. The results of research showed that spatial variability of SOC, at selected scale, probably is related with the differences of parent material, composition of the colluvial deposits and its spatial periodicity as well as differences and repeatability of topography.

**Keywords:** soil organic carbon, fractal dimension, break point.

**INTRODUCTION**

Soil organic carbon (SOC) content and its spatial pattern are an important issue in the context of soil productivity, soil degradation risks (Lal, 2004), biological diversification and climate changes (EC, 2006). Therefore, the issues of the spatial variability and amount of SOC were a frequent subject of interest of numerous studies which have focused on different aspects of SOC spatial pattern.

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However, despite of its importance an understanding of the spatial variability of SOC has remained poor. Agricultural soils typically have a lower SOC contents than natural. The forecasted climate change will likely result in further reductions in SOC. This is especially so in the Mediterranean area which is the most sensible ones to climate change (IPCC, 2007) and where the SOC content in soils is generally low (Jones et al., 2005). Therefore study of the spatial variation of SOC, particular in typical Mediterranean area is vitally important for the sustainable land management and preservation of soil quality and whole environment. Also, we hope that better understanding of the causes of SOC variation can help to advance field investigations, improve sampling design and interpolation procedures for mapping.

The spatial pattern of SOC is controlled by many different factors which may operate independently or in combination with other factors over a wide range of spatial and temporal scales with different intensities. Generally, parent material and climate are assumed to define large-scale patterns of SOC content (McLauchlan, 2006), while other factors, like topography and human impact, define the SOC variation at smaller scales (Dendoncker et al.; 2004; Sleutel et al., 2007; Schulp et al., 2008). Therefore the spatial pattern of SOC is very complex and more complicated than landform and other environmental matrices.

The objective of this paper is to establish characteristics of spatial variability of the organic carbon in the anthropogenic soils by using fractal theory. According to fractal theory introduced by Mandelbrot (1967) fractals are defined as objects with structures partially correlated at all spatial scales with a scale-dependent self-similarity. The fractal dimension (D) is a single and constant parameter applicable to all scales. Mathematically, fractal is a self-similar object which is exactly similar to a part of itself. In the real world many objects are only statistically self-similar natural features which often show self-similarity or scale invariance in more scales and a distinct fractal dimension for each particular scale.

The soils are fractal because increase of the scale resulting in an increase of quantity of details. We used fractal dimension (D), as a measure of the complexity of the SOC spatial pattern. The fractal analysis has been shown as a widely used technique for spatial analysis of the landscape and natural processes (Burrough, 1981;1983a,b; Mark and Aronson, 1984; Goodchild and Mark, 1987; Krummel et al., 1987; DeCola, 1991; Milne, 1988). We expect that the results of the study contribute to a identification and better understanding of the factors and processes that determine the spatial pattern of SOC.

## **MATERIAL AND METHODS**

### **Study area**

Kastela Bay is situated in the Middle Adriatic, centred on 43°33'26" N; 16°21'55" W. The research area covers total of 4 960 hectares and occupies a narrow zone between towns of Split and Trogir, steep slopes of mountain Kozjak in the north and the urbanized area along the coast in the south. The study area

had mean annual precipitation of 1 062 mm and mean air temperature of 15.9°C. According to Köppen climate classification (1918) climate of study area is classified as Mediterranean with hot summer (Csa).

Geologically, study area was built of Eocene Flysch marls and sandstones, sometimes a sedimentary sequence consisting of coarse conglomerates or breccia's (Marinčić et al. 1971). Flysch sediments are partly covered by Quaternary colluvial skeletal and non-skeletal deposits thickness of 20(30) cm to several meters. Colluvium is a loose, unconsolidated sediment composed of the various deposits (soil mixed with rock fragments of various sizes and quantities). Elevation of the location varies from 10-305 m above sea level and continues with a massive of mountain Kozjak 779 m above sea level.

The topography is characterized by huge relief differences and a variety of terrain slope. The most common are gently sloping terrain (slope 2-5%) and very gently sloping terrain (slope 1-2%) covering 34.3 % and 22.7% of total area, respectively. Sloping terrain (5-10%) occupies 18.4% of area, while 17.0% of area are nearly flat terrain (0-1%). The strongly sloping terrain (10-15%) and very strongly sloping terrain (15-30%) covering the smallest area, 5.1 and 2.5 % respectively. Described relief characteristics and a watertight geological base with a wide elevation interval made this area vulnerable to erosion. Therefore terracing is a basic measure for soil protection in this typical torrential area.

According to the Croatian Soil Classification System (Škorić et al., 1975) soils of the study area are classified as Rigosol, non-skeletal silty clay loam, strongly calcareous, medium deep and deep formed on Flysch sediments and Rigosol, skeletal clay loam, slightly to moderately calcareous, medium deep and deep formed on Quaternary colluvium. According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014) investigated soils we classified as Terric Anthrosols (Siltic/Clayic/Loamic) and Terric Anthrosols (Clayic/Loamic, Skeletic). Current agriculture is characterized by the small, mixed and dislocated parcels of the olive groves, vineyards, orchards of Mediterranean species, ploughed and abandoned land.

### **Soil data set**

Point (pedon) data on soil organic carbon (SOC) which consists of 206 point observations from the topsoil (0–30 cm) were derived from Kastela Bay Soil Databases (Miloš 1992, 2002). The soil samples were collected randomly with a sample distance of approximately 500 meters. Maximum distance between point pars was 21 057 meters. The SOC content was determined by Kotzman method (JDPZ, 1966).

### **Measurement of fractal dimension**

The fractal dimension (D) represent geometry of fractal pattern. The variogram method in the estimation of the fractal dimension was used, because of its applicability to dataset with irregular grid of point data set. The semivariance  $\gamma$  (h) for soil properties at distance “h” or lag h is defined in equation:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} (Z_{x+h} - Z_x)^2$$

where,

$\gamma(h)$  is the semivariance at lag „h“, refers to interval between neighboring sampling points

$Z_{(x)}$  and  $Z_{(x+h)}$  – values of the variable (soil property) at location  $x_i$  and  $x_{i+h}$ , respectively and

$N(h)$  is the number of pairs considered

The fractal dimension was calculated from the slope of a double logarithmic plot of the semivariance,  $\gamma(h)$ , versus the lag distance  $h$  by fitting a straight line by the method of last squares. The calculation of  $D$  was made on the following relationship (Burrough 1981):

$$2g_h = h^b$$

where,

$b = 4-2D$  (the slope of the log-log plot variogram)

$D = 2 - b/2$  (Hausdorff-Besicovitch statistic)

For a linear fractal function, the  $D$  dimension falls between 1 and 2 (Burrough 1981). Variables with strict spatial dependence at all scales,  $b=2$  have a dimension  $D = 1$ . Conversely, those with complete spatial independence,  $b=0$  have the fractal dimension  $D = 2$ .

### Determination of break point

In this study we calculated fractal dimension ( $D$ ) for the soil carbon content ( $g C kg^{-1}$ ) for the maximum point pairs distance (all lag  $h$ ) and for the first linear segment of the log–log variogram defined with break point. The break point (BP) defined break distance (BD) within which the log-log variogram can be fitted by a line provided that the coefficient of determination as a goodness-of-fit measure  $R^2 > 0.90$  (Klinkenberg 1992). The additionally fractal parameters was gamma ( $g$ ) defined as the intercept of the best-fitting line with the ordinate (Klinkenberg and Goodchild, 1992). In addition to these following requirement for the definition of break-point was the minimum of 100 point pairs at a distance with goodness-of-fit measure  $R^2 > 0.90$ .

## RESULTS AND DISCUSSION

### Descriptive statistics of SOC

The mean SOC content was  $22.19 g C kg^{-1}$  and ranged between  $7.75$  and  $40.87 g C kg^{-1}$ . The variability of SOC expressed by the coefficient of variation was  $30.58 \%$ . The histogram for SOC data shows nearly symmetrical (Skewness  $0.34$ ) and slightly flatter distribution (Curtosis  $-0.39$ ), Figure 1.



About two-thirds of investigated soils (65.5 %) have medium SOC content (20-60 g C kg<sup>-1</sup>). Low SOC contents (10-20 g C kg<sup>-1</sup>) were determined in 33.5% of samples, while only 1% of the investigated soils have reached a very low SOC content of 10 g C kg<sup>-1</sup>. This low value is proposed by the EU Soil Bureau as the value below which soils are considered to be particularly vulnerable to enhanced degradation, due to a lower aggregate stability (Jones et al, 2004). Loveland and Webb (2003) proposed a higher SOC threshold (20 g C kg<sup>-1</sup>) below which soil quality can be largely decreased. However, soil degradation thresholds can depend on soil and climatic conditions.

Our data fit into averages for SOC in topsoils of Europe. According to Europe Map of top-soil organic carbon (0-30 cm) 13% of area has very low content of SOC (< 1%), 32% low (1-2%), 45% medium (2-6%) and 5% high (>6%) content of SOC (Jones et al. 2003). However, in southern Europe almost three-quarters (i.e. 74.6%) of the soils have top-soils containing very low ( $\leq 1\%$ ) or low ( $\leq 2\%$ ) amounts of SOC. Less than a quarter (24.6%) of southern European top-soils contain medium to high (>2%) amounts of SOC (Zdruli et al, 2004).

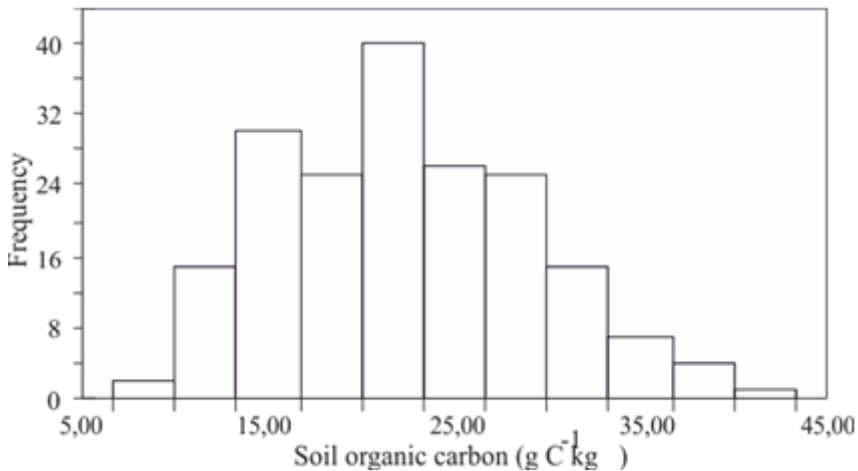


Figure 1. The histogram for top-soil organic carbon

### Fractal parameters of soil organic carbon content

The fractal parameters for SOC (fractal dimension -D, gamma value - $\gamma$ , break-point -BP, standard error - SE and coefficient of determination -R<sup>2</sup>) calculated over range of 21 000 m with distance of 500 m as interval lag -h are given in Table 1 and Figure 2. The log-log variogram form shows a great discontinuity at origin (high gamma value) then, gradually increase with separation and having achieved sill at the distance of 5 000 m (Figure 2). After that, it decreases to a distance of 7 500 m, and then increased again to distance of 21 000 m. Described variogram model illustrates nonmonotonic growth of the semivariance with distance and exhibits a periodic semi-variation structures

known as a hole effect, indicating a multifractal behavior of SOC. The hole effect expressed in shape of semi-variogram, may be caused by the confounded effects of a periodic variation of parent material and colluvial deposits, as well as spatial repetition of topography.

Approximation of this variogram form with straight line resulted in a high intercept gamma ( $\gamma$ ) of 1.09, a high standard error (SE) 0.329, a low associated coefficient of determination ( $R^2$ ) 0.50 and consequently very high fractal D value (Table 1). The overall high fractal D value for SOC in our study was in good agreement with other studies which found high fractal dimensions for soil parameters (Burrough, 1981, 1983a; Culling, 1986). This results show a very small spatial continuity of SOC content and indicate that short-range effects of variations of SOC dominate. The domination of the short-range effects of variations indicates that its content in anthropogenic soils of Kastela Bay varies as a result of the various forming factors and processes acting at different spatial scales. The reasons for the observed spatial variability of SOC, at selected scale, mainly can be related with the differences of parent material, composition of the colluvial deposits and its spatial periodicity as well as differences and repeatability of topography.

In order to estimate the maximum distance at which the spatial correlation or scale independence of SOC can be applied, we have singled out first linear regression segment - break distance (BD) on the log-log plot of the semivariogram. The break- point or scale break defines point or border between correlated and uncorrelated values and provides information about the spatial organization of SOC pattern. The results have shown that SOC point values are correlated and scale independent over a scale of 5 000 metres (Table 1). This result is consistent with research of Burrough (1981) which pointed out that soils may exhibit self similarity over only a limited range of scales. Fractal parameters for estimated break distance (first linear segment with  $R^2 > 90$ ) were: fractal value (D) 1.81, ordinate intercept - gamma ( $\gamma$ ) 0.51 and associated coefficient of determination ( $R^2$ ) 0.96 (Table 1).

Table 1. Fractal dimension (D), ordinate intercept - gamma ( $\gamma$ ), standard error (SE) and associated coefficient of determination ( $R^2$ ) of the SOC (g C kg<sup>-1</sup>) for maximum distance between point par (all lag h) and for break distance (BD).

Statistics	All lag <b>-h</b>	Break-distance (BD)
	21 000 m	5 000 m
Fractal D	1.92	1.81
Gamma ( $\gamma$ )	1.09	0.51
$R^2$	0.50	0.96
Standard error (SE)	0.329	0.122

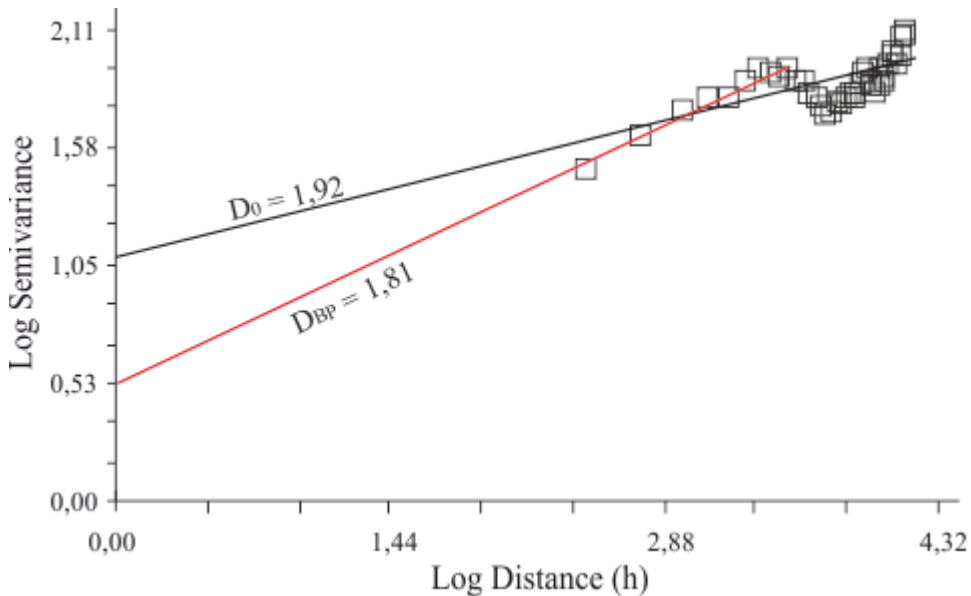


Figure 2. Log-log plot of the empirical semivariogram for soc, fractal dimension for the regression line estimated for the maximum distance between point pair ( $D_0$ ) and for first linear segment of the log-log plot defined with break-point ( $D_{BP}$ )

### CONCLUSIONS

Estimated fractal parameters for top-soil organic carbon (SOC) in investigated anthropogenic soils, including high fractal  $D$  value, high value of gamma ( $\gamma$ ) and low coefficient of determination ( $R^2$ ), have shown domination of short-range variations, implying its very small spatial continuity.

The log-log semi-variogram model of SOC has shown a multifractal behavior characterized with hole effect. We found that the SOC point values are correlated and scale independent over 5 000 metres separated with break point. The established fractal parameters indicated that SOC pattern is mainly related with differences of parent material, composition of the colluvial deposits and its spatial periodicity, as well as, differences and repeatability of topography.

This study showed that fractal analysis can be useful tool in studying SOC spatial variability and identification of soil forming factors which have caused it.

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## **ECONOMIC DEVELOPMENT OF AGRICULTURE IN MONTENEGRO IN THE PERIOD FROM 1878-1914**

### **SUMMARY**

Following the Congress of Berlin (1878), Montenegro managed to meet all the requirements for rapid economic and overall social development upon being granted diplomatic recognition and significant territorial expansion, including fertile plains, towns and boroughs, and a coastline.

Significant expansion of arable land enabled more intensive cultivation of agricultural crops, although Montenegrin agriculture still faced the challenge of extension.

It can be gathered that industrial development of Montenegro in 1878-1914 in the sphere of food industry was hindered by numerous factors, despite the incentives provided by the government. There was a lack of ongoing capital investments, mainly present in the trading area, and industrial enterprises operated with small capacities and insufficient resources.

**Keywords:** agriculture, development, enterprises.

### **INTRODUCTION**

Numerous initiatives were taken from the governmental level for improving and advancing the state of agriculture (agronomy, livestock farming, and pomiculture), which implied introducing regulations, forming social organisations, forming educational institutions and educating the staff, engaging international experts, providing financial aid from the state budget, and incentivising remarkable growers in the field of agricultural production. (Marović, 2006: 277).

In 1880, the first Institute for the Dairy Industry was founded, along with the first technical school for agriculture, as a result of a growing need for experts in the area of agriculture, which was the main driver of the livelihood of the population (Kaluderović, 1910: 338). Prince Nikola I decreed that every soldier was to plant vines, and every officer was to plant olives, in addition to which the most distinguished workers would be exempt of taxation. (Pedeset godina na prestolu Crne Gore, Cetinje 1910: 223). Numerous exhibitions and courses on cultivation of fruit were organised, and aid was provided for mitigating the impact of vermin and plant diseases. The first agricultural associations were

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

formed in Zeta and Nikšić, in line with the regulations articulated by the Department of National Economy, founded in 1902, within the Ministry of Internal Affairs. The department was in charge of conducting analyses of soil in particular areas for more precise allocation of ground to crop cultivation. This resulted in replacing the cultivation of corn with barley in Banjani, Jezera and the Nikšić Nahiya. Planting activities were also organised, as evidenced by the chestnut-tree planting action in 1889, when 10,000 seedlings were provided (Marović, 2006: 278).

The country allocated grants from the budget to support the development of agriculture, while educating the people through manuals and information packages, with the aim of enhancing agricultural production. Awards and premiums were given to the best producers in agriculture, and to the finest cattle breeders. Students interested in these fields received scholarships for studying abroad.

It can be inferred that the state invested considerable effort to improve the circumstances, but despite those efforts, the majority of country folk did not change their methods of cultivation. The primitive methods and simple tools further hindered the development of agriculture. Scarcity of areas of arable land and its dispersion, along with a low level of utilisation of mechanisation acted as additional contributors.

The objective of this research was to determine of economic development and position of agriculture in Montenegro in the period from 1878-1914.

## MATERIAL AND METHODS

The aim of the paper was the analysis of economic development of agriculture in Montenegro in the period from 1878-1914. All the available literature about the studied area related to the agriculture- livestock farming and plant production), social organisations, educational institutions, agricultural cooperatives, trade and food industry were studied. The data were provided by the different sources including available official data.

## RESULTS AND DISCUSSION

Data on how impeded agricultural production was is rich both in terms of harvests and the cultivation methods. We will rely on data from 1910 to illustrate this in the context of the sowing structure and crop yields per arable land 22,934 *ralo*<sup>2</sup> of land was covered in wheat, with a total harvest of 2,820,922 kg of crops, and an average of 123 kg per *ralo*; 77,414 *ralo* were covered in corn, with a total harvest of 16,088,982 kg, i.e. 207.83 kg per *ralo*; barley encompassed a surface of 14,328 *ralo*, with a harvest of 3,100,693 kg of crops, i.e. 216.40 kg per *ralo*; and rye covered 8,460 *ralo*, with 1,450,073 kg of crops, or an average of 172.36 kg per *ralo*. In addition to these grains, millet, buckwheat, spelt and oats were

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<sup>2</sup> *ralo* is an old Montenegrin unit of measure, still in existence and use at present. It is equivalent to 1820 square metres.



sown on 4,742 ralo, with a harvest of 791,714 kg of crops. As for vegetables, potato covered 16,714 ralo of land, with 13,858,458 kg of crops, or 829.15 kg per ralo; cabbage covered a total of 1,852 ralo, with 2,250,482 kg of crops, an average of 1,233.14 kg per ralo; beans covered 1,771 ralo, with a harvest of 188,857 kg of crops, or an average of 106.63 kg per ralo; and onions covered 318 ralo, with a total harvest of 271.23 kg per ralo. With regards to industrial crops, tobacco was cultivated on a surface of 1,125 ralo, with a total harvest of 180.41 kg. Hay covered a surface of 217,604 ralo, resulting in a harvest of 72,031,824 kg of crops, or 331.02 kg per ralo. Clover covered 394 ralo, and yielded in 327,210 kg of crops, or 830.48 kg per ralo. There was a total of 4,388,828 vines, used for the production of 1,696,767 litres of wine, and 206,645 litres of brandy, or an average of 0.377 litres of wine produced per vine, and 0.047 litres of brandy per vine (DACG, MUD, 1911, 70734; Branislav Marović, 2006: 276).

Data from this period indicates that in 1914, Montenegro had 160,075 plum trees, 15,075 apple trees, 20,750 pear trees, 59,100 chestnut trees, 80,030 fig trees, 7,900 walnut trees, and 18,310 trees categorised as 'various fruits' (Kaluderović, 1910: 330).

Within the period considered, livestock farming was the leading branch that produced a market surplus, and essentially generated the livelihood of the population.

Data illustrating the quantity of livestock indicates fluctuation, as livestock was affected by disease, and a great number of head of livestock was commandeered during the Balkan Wars (201,208 head of sheep and 36,619 head of cattle). (Đurović, 1960: 98). The livestock of Montenegro comprised 635,488 head in 1880, at the start of the analysed period, and 952,489 head in 1914. (Marović, 1998). Given that pastures represented the largest areas of land, sheep and goat comprised the majority.

A significant suggestion that contributed to the improvement of livestock farming was forwarded by a Dalmatian, Ilija Beara, who worked as a professor in the Lyceum and the Seminary in Cetinje. This suggestion was first submitted in 1878 to the Minister of Internal Affairs, Mašo Vrbica. Particular emphasis was placed on livestock nutrition, as food was scarce. In order to increase the production of food, he advocated creating "artificial meadows", i.e. planting Alfalfa, which "is the most productive, appropriate, and drought-resistant food for the livestock." Beara proposed forming a dairy institute which would collect greater amounts of milk, and importing a cheese production unit from Switzerland. Thus, the state would use its own capital to form institutes which would help develop the farming skills of the country folk, and increase the production of livestock food and fertiliser. Furthermore, he considered that the Montenegrin cheese was fit for European quality standards, and as such, it could easily be targeted at European markets. (Marović, 1998: 285).

Prince Nikola most likely took these specific suggestions and general recommendations into account when he decided to form the Institute for the Dairy Industry in Nikšić in 1880. The institute was a "practical centre for

communicating smart farming of livestock and its utilisation”, and its headquarters were located by the Trebjesa Hill, with 100 cows and several bulls - “both domestic and foreign breeds. An expert from Switzerland acted as head of the institute, Dr L. Švalej, who approached “livestock farming from a scientific and experiential viewpoint, which is why the process made great progress” (Pedeset godina na prestolu Crne Gore, 1910: 220).

Another famous scientist from that period, Dr Leopold Adametz, a professor of livestock farming at the University of Krakow, and later on, a professor at the High School of Agriculture in Vienna, also provided suggestions on methods of improving the area of livestock farming. Following a series of visits and studies of livestock in Montenegro, Adametz submitted his report to Prince Nikola in 1895. He suggested forming a centre for the advancement of livestock farming in the central region of Montenegro, with the aim of forming a pure breed from a selection of the Montafon and Oberinntal breeds. These breeds could provide Montenegrin farmers with sufficient amounts of milk, wool and meat. Additionally, the breeds were more robust, so Adametz concluded that mixing other breeds of sheep would not be profitable “when the country already has wonderful material, which can easily be transformed into absolute perfection. Adametz proposed a cross-selection with the Hungarian pig breed Mangalica, a sturdy, resilient and fertile breed. He recommended farming goats only in areas that were not suitable for other domestic animals” (Marović, 1998: 287).

The Department of National Economy formed a Livestock Institute in 1901, with an Agricultural Unit by the Čadalica Hill in Nikšić, as a type of experimental centre for developing livestock farming. (Marović, 1998: 292).

Traditional folk medicine was used in treating the animals. As the need for a more professional approach to this issue emerged, a Medical Department was formed within the Ministry of Internal Affairs in 1879, which included a veterinary centre that was annexed by the newly formed Department for National Economy in 1902. Petar Plamenac, a veterinarian who studied in Vienna, was appointed head of the department in 1906. (Marović, 1998: 295-296).

Fishing experienced a gradual development towards a more modern approach. Smoked bleak, smoked carp, eel, mullet and the nase were quite common. Stevan Lukačević was a trader who exported not only fish, but also the scales of bleak, used for producing pearl essence. (Franetović, 1960: 490). In 1884-85, the catch of bleak was 400 miliars, according to the data presented by Rovinski, who further asserts that the income from fish sales in the pool of the Skadar Lake was around 400,000 forint. (Rovinski, Marović, 1998: 299).

Fishing was also popular among the people of the Bay of Kotor, which was not part of Montenegro at the time. At the beginning of the 20<sup>th</sup> century, fishing was the main source of livelihood for 500 families in this area, and more than 1000 families practiced fishing on an occasional, non-commercial basis (Radusinović, 1978: 97).

Viticulture and the production of wine and brandy were incentivised with governmental activities, such as the aforementioned decree issued by Prince

Nikola. In 1909, a central plant nursery for resistant American vines was created, occupying 20 morgen of land in the village Sotonići. 31,560 vine roots were added to existing vineyards, so vines covered a total surface of 600 hectares in 1914 (Kaluderović, 1927: 330). A part of the coastline which was under Austro-Hungarian occupation at the time had 1,072 hectares of vineyards (Radusinović, 1978: 104).

As in other agricultural fields, traditional methods of planting and processing were dominant in viniculture, so the Department of National Economy granted scholarships for studying abroad to a small number of pupils.

Following the Congress of Berlin, when Montenegro got its coastline, the country received 132,147 olive trees, and several olive mills, which were used for producing olive oil. The number of olive trees grew to 150,000 at the beginning of the 20<sup>th</sup> century, and a total of 1,558,662 kg of olive oil was exported in 1905-1910, contributing to an income of 1,070,793 perper (Marović, 1998: 304).

Beekeeping in Montenegro could not be developed at a sufficient level, and the production of honey stagnated, despite the efforts invested in education. Based on taxation protocols noted in the relevant literature, the number of beehives fluctuated in the following manner:

Table 1. Number of Beehives in Montenegro (1883-1913)

YEAR	Beehives
1883	31.191
1890	19.736
1895	32.734
1900	38.363
1905	20.487
1910	38.922
1913	19.250

Source: Branislav Marović, „Stočarstvo Crne Gore 1860-1953“, Podgorica 1998.

Oscillations are evident, which confirms that beekeeping was heavily affected by various diseases, as well as grazing, and keeping conditions (Marović, 1998: 305).

There were several attempts of forming agricultural cooperatives. The first such attempt, in 1904, resulted in forming 6 cooperatives in Zeta, 1 in Piperi, and 1 in Bratonožići, with a total of over 100 members. The number of cooperatives grew to 12 by the end of the year. They were mixed-type organisations, credit-purchasing, with unlimited liability, dealing mostly with the procurement of agricultural tools, seeds, and other essentials. By the end of 1914, there were over 30 cooperatives in the country. They did not receive financial aid or subsidies from the government, which only provided moral support to the members, encouraging the idea of cooperation, and contributing with general directions. (Marović, 1998: 307-308).

In the period covered by this paper (1878-1914), industrial production in the sphere of food was in its incipient stages, but even the very first few industrial objects made a significant contribution to the overall development of Montenegro. The first brewery, “Onogošt” was opened in 1896 in Nikšić, by Vuko Krivokapić. Initially, the brewery could not cater for the market in Nikšić alone, but a few years in the production, it covered the needs of Podgorica, as well as other cities. Bread yeast was also produced, and the brewery was exempt from taxation for a period of 10 years. In 1912, it produced 3,700 hectolitres of beer, which induced innovations and modern production methods, so new capital investment models were in demand (Đurović, 1959: 83). In 1907, the brewery was turned into a “consortium” of 13 traders from Nikšić, who invested 210,000 perper for the procurement of new industrial machines, construction of a new well and storage areas (Đurović, 1959: 83). A few years later (1909), the brewery grew into a joint-stock company, with a capital of 250,000 perper, shared into 2,500 stocks, with a 30 years horizon (Đurović, 1959: 83).

In 1911, another brewery, “Trebjesa” was founded in the form of a joint stock company. It utilised advanced equipment, and it had greater production capacities. The company’s basic capital was 500,000 perper, shared into 100 perper stocks, with a 50 years horizon. The brewery operated well, with growing profits per annum, and growing production per annum – starting from 1,500 hectolitres when it was first founded, to 7,500 hectolitres in 1912. (Đurović, 1959: 94-96).

A beer factory was opened in 1999, in Pljevlja, which operated as a private company of the family Šećerović, although it was built with a shared capital. The capacity of the brewery was 2000 hectolitres of beer per annum, and the main consumers were soldiers from the Austro-Hungarian garrison in Pljevlja. The beer was also sold in surrounding towns and boroughs. The brewery operated until 1908, after which the production was renewed, but on a temporary basis and with smaller capacities (Marović, 2006: 444).

The first hydraulic oil production unit with iron presses, which could produce far more olive oil than the old, traditional production units with small capacities, was started in Bar, by duke Mašo Vrbica in 1888. A similar industrial enterprise for oil refinement, with an olive press mill was set up in Ullcinj. (Marović, 2006: 305). In Bijela, the brothers Mardešić from the island of Vis opened a canned fish factory in 1907, with the capacity of producing up to 240 tons of canned fish, although the actual production levels were never that high. The excellent quality and price of products contributed to their overall appeal. Consequently, they were exported to various countries, and salt fish was exported to Austria on an ongoing basis (Marović, 2006: 308).

The Tobacco Monopoly factory in Podgorica was arguably the most significant industrial institution in this timeframe, and it was handed over to an Italian stock company. Italian companies invested in construction projects in Podgorica and Bar in 1903 and 1904, and by the end of 1905, the total invested capital reached 2,820,000 perper (Đurović, 1960: 384). In 1905, the turnover was

364,270 perper, and pure profits neared 150,000 perper. In 1907, the Italian capital invested in Tobacco Monopoly reached 3,500,000 coronas (Marović, 2006). The factory produced around 180,000 kg of fine-cut tobacco, and 24,000 units of cigarettes. 95% of the sales was fine-cut tobacco, and 5% was cigarettes (Marović, 2006: 387).

It can be gathered that industrial development of Montenegro in 1878-1914 in the sphere of food industry was hindered by numerous factors, despite the incentives provided by the government. There was a lack of ongoing capital investments, mainly present in the trading area, and industrial enterprises operated with small capacities and insufficient resources.

## CONCLUSIONS

Following the Congress of Berlin (1878), Montenegro managed to meet all the requirements for rapid economic and overall social development upon being granted diplomatic recognition and significant territorial expansion, including fertile plains, towns and boroughs, and a coastline.

Numerous initiatives were taken from the governmental level for improving and advancing the state of agriculture. The country allocated grants from the budget to support the development of agriculture, while educating the people through manuals and information packages, with the aim of enhancing agricultural production.

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The first hydraulic oil production unit with iron presses was started in Bar. A similar industrial enterprise for oil refinement was set up in Ullcinj. In Bijela is opened a canned fish factory in 1907, with the capacity of producing up to 240 tons of canned fish. The excellent quality and price of products contributed to their overall appeal. Consequently, they were exported to various countries, and salt fish was exported to Austria on an ongoing basis.

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Industrial development of Montenegro in 1878-1914 in the sphere of food industry was hindered by numerous factors, despite the incentives provided by the government. There was a lack of ongoing capital investments, mainly present in the trading area, and industrial enterprises operated with small capacities and insufficient resources.

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## **REGIONAL DEMOGRAPHIC PROBLEMS AND THEIR IMPACT ON THE DEVELOPMENT OF AGRICULTURE IN MONTENEGRO**

### **SUMMARY**

The level of development of particular regions is a result of geographical position, which has a great influence on vicinity or isolation in relation to the economic centres. Balanced regional development is one of the most important topics in recent years. Role of the state in the processes of the regional development is being more emphasized, as well as the need for establishing a legal framework and institutions that will deal with regional development in the future.

Three regions are classified due to the Regional Development Strategy of Montenegro: Northern, Central and Southern region. The basic characteristic of regional development of Montenegro is its disproportion. Undeveloped areas of Montenegro are characterized by unfavourable demographic and economic situation. It is expressed in depopulation of the Northern region and high concentration of population and economy in the Central and Southern region. Concentration of population is particularly evident in the municipality of Podgorica. Such trends have negative consequences in the economic, spatial and social areas. Rural areas of Montenegro remains empty, while rural areas in Europe are becoming increasingly important alternative for living and working in relation to the of cities.

The paper analyzes the processes of depopulation of certain regions in Montenegro and their impact on the development of agriculture. In the observed inter-census period (1960-2010), a digressive trend of population in the northern region was evident, which resulted in a lagging of the development of not only agricultural, but also other activities. Factors causing uneven regional development are: migratory movements, peripheral situation of rural areas in the Northern region, deagrarisation, lack of quality infrastructure and similar. A comparative analysis of demographic changes, migration and regional mobility, as well as agricultural development and changes in the agrarian structure are applied within this paper. Carried out analysis indicates that strategically important areas became depopulated in the observed period, remaining natural resources unexploited. At the same time, in developed centres there was an excessive concentration of population and economy, which led to negative consequences in the economic, social and spatial sphere.

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With the aim of improve regional image of Montenegro in the future, it is necessary to work on the revitalization of the Northern region, in order to prevent its further depopulation.

**Key words:** regional problems, depopulation, deagrarisation, agriculture

## INTRODUCTION

Regional development in general, and particularly in terms of rural development, is not observed as an integral part of the overall socio-economic development. Over decades, the problem of Regional Development has been marginalized and often analyzed as a separate aspect of the overall development. Regional differences were observed mainly in terms of development, so their economic development, social, and particularly demographic specifics were ignored (Milanović *et al.*, 2010).

With the arrival of spatial planning in the modern world, and the need for directing of development, organization and development of geographic space, regionalization is emerging as the most adequate way of politically territorial and spatially functional organization (Vojković, 2003). Regionalization is a complex procedure of great social and development significance.

The idea that sees the regionalism as a two-way concept has remained since the arrival of regional concept until today: the primary objective of regionalism is to be found in the final product integration of the region (Vojković, 2003). Region does exist (Odum, 1952), but it firstly implies to the region as an integral part of the whole.

Increased interest in issues of regionalization was developed in parallel with the raise of awareness about the necessity of global, and therefore harmonized regional development. Establishing of regions, in accordance with the current socio-economic development, should be a framework for regional development policy and a basis for overcoming of the consequences of previous haphazard and uneven spatial and socio-economic development, i.e., a prerequisite for regional harmonized development of the country, and therefore a tool of effective economic policy (Papić, 1987, Petrović, 1957).

Rural development should respond to regional needs, enhance endogenous development, to be launched by regional development policies, as well as to be focused on sustainable development and implement the regional capital (Đorđević and Panić, 2004, Petrović 2009). The main functions of the regional administration should be twofold: on the one hand to strengthen economic identity, motivation and social capital and on the other hand to manage programmes, technical support and monitoring (Stojić Karanović, 2002). Region must be comprehensive natural geographic, anthrop-geographical, economic, historical, ethno-cultural and civilization entity, which that way in its complete individuality, can manifest its advantages in the best manner, but it also must be in line with its environment (Vojković, 2003). Human resources are the main driver of economic development because the productivity relies on them (Zjalić,



2009; Rakić, 2006). Human resources imply knowledge, experience, creativity, innovation and ability of individuals directed to the improvement of society, and therefore it is necessary to constantly invest in them (Đerčan, Bubalo Živković and Lukić, 2010 a). Balanced regional development includes an economic development of rural areas, which means the education of the rural population in technical, technological, cultural and environmental terms. Reaffirmation of underdeveloped regions would have favourable economic and demographic consequences, because it would employ young people, in order to, at least partially, prevent emigration from these areas (Gligorijević and Stefanović, 2009).

Revitalization of underdeveloped areas is one of the key factors in the process of accession of Montenegro to the European Union. By means of EUROSTAT, European Union has stated the criteria for administrative territorial organization of European countries through the so-called NUTS system (Nomenclature des unites territoriales statistiques). This categorization insists on the territorial levels of the hierarchical structure of management and unification of the region by size (Stojkov, 2000). The established nomenclature starts from the population size of the territory and suggests five levels of organization of territorial units: NUTS 1 - the state or territorial unit with 4-5 million residents; NUTS 2-level of macro-region with 1-4 million residents; NUTS 3 - level of the district or region with 100,000 to 1 million residents; NUTS 4 - with 10,000 - 100,000 residents and NUTS 5 -level settlement under 10,000 residents. When it comes to the statistical division of Montenegro, according to the Eurostat criteria on division of the country on spatial units for statistics, Montenegro is observed as one region (Regional Development Strategy of Montenegro, 2010-2014). Regardless the fact that Montenegro is considered as one region, there are significant differences in the level of development of municipalities, and thus the areas to which they belong.

The Regional Development Strategy of Montenegro 2010-2014 classified regions: Northern, Central and Coastal region. According to the geographical features, the Northern region is consisted of the following municipalities: Andrijevića, Berane, Bijelo Polje, Mojkovac, Kolašin, Plav, Pljevlja, Plužine, Rožaje, Šavnik, Žabljak; Central region: Podgorica, Danilovgrad, Nikšić and Cetinje; Coastal region: Bar, Budva, Herceg Novi, Tivat, Kotor and Ulcinj. It should be noted that in the meantime, Petnjica and Gusinje gained the status of municipalities, and their geographical position belongs to the Northern region.

Region must have sufficient size in order to be able to control the appropriate level of its own economic destiny (Derić, Atanacković, 2000). According to the OECD methodology, three regions of Montenegro (Northern, Central and Southern) were observed and the Northern region is consisted of 13 municipalities and it is predominantly rural (59.7% of the population lives in rural areas), while the Coastal (41.7%) and Central (20.4%) belong to the

transition. Agricultural production may not be the only function of rural areas, but there could be a number of other activities that will enhance the growth of the rural economy and the impact on reducing the gap between urban and rural areas.

The aim of this paper is to analyze the demographic changes and socio-economic structure of the population by regions, as well as the analysis of changes in the agrarian structure in the period 1965-2010.

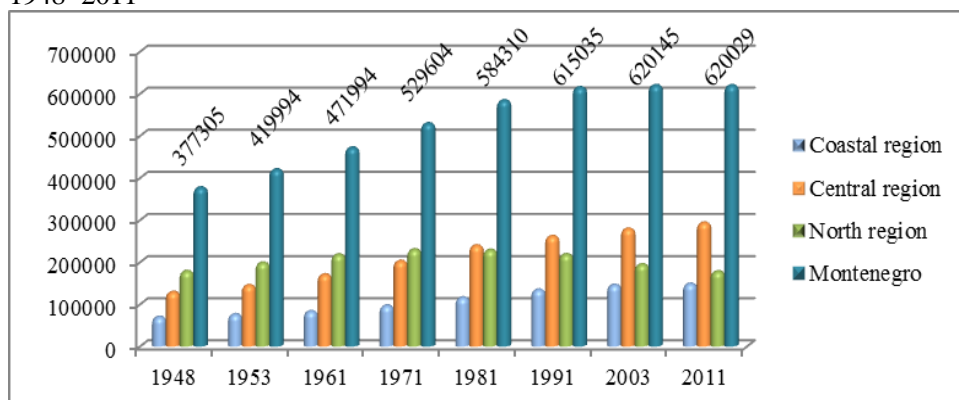
### **MATERIAL AND METHOD**

This paper analyzes the causes of regional and demographic problems in Montenegro and their impact on the development of agriculture and rural areas. The analysis was conducted for the period 1965 - 2010, when agricultural census was carried out in Montenegro. During the research for this paper the work we used the official data of the Statistical Office of Montenegro (MONSTAT), as well as scientific and professional papers that dealt with this issue. Data of the Agricultural Census of Agriculture in Montenegro and Population Census for the period between 1948 and 2010 were used for this paper's analysis. In order to display the data, the statistical tables and graphs were used. Using the relative numbers of structure, it is shown the share of the population of some regions in the total population of Montenegro. Dynamic statistical analysis, namely, the method of calculation of basic and chain indices were used, as well as the methods of descriptive statistics. The paper uses the methods of research at the table "desk research" and methods of comparison. The paper aims to draw attention to the causes of uneven regional development in Montenegro, as well as the consequences of which are reflected primarily in the abandonment of rural areas and neglect of agricultural production.

### **RESULTS AND DISCUSSION**

During the entire twentieth century, Montenegro was typical emigration area. Weak economic development, as well as the severe war damages caused the mass exodus of the population. After the Second World War, the net migration balance was negative in each inter-census period. The value of net migration rate was the highest in the period of 1953-1961 (-7.2 ‰), while the highest annual average (negative) balance was recorded in the inter-census period of 1981-1991 (MONSTAT, 2008). The biggest emigration wave from Montenegro emerged immediately after the end of the Second World War. This is the period of implementation of agrarian reform and colonization of fertile areas, primarily of Vojvodina and Slavonia. Colonization has largely been covered by the population of passive areas (Đurđev, 1995). 5,500 households with a total 31,000 of colonists moved out from Montenegro, which represented 8.2% of the total population in Montenegro (1948). It is characteristic that emigration more than immigration was evident in Montenegro (Kalezić, 1978). Figure 1 shows the movements trends of Montenegrin population by region in the period of 1948 -2011.

Figure 1. Movement trends of Montenegrin population by region in the period of 1948 -2011



Source: *Demographic trends in Montenegro from the middle of 20<sup>th</sup> century and perspective until 2050, MONSTAT, 2008*

Total population in Montenegro recorded a constant growth in the period from 1948 to 2011. Total population growth is uneven when it is observed by regions. Table 1 shows total population trends in the regions using the base and chain indices, as well as the percentage share of population of particular regions in the total population of Montenegro.

Table 1. Total population trends by regions, percentage share (%), base and chain indices

Year	REGIONS								
	COASTAL			CENTRAL			NORTH		
	Participation in total population (%)	Base index	Chain index	Participation in total population (%)	Base index	Chain index	Participation in total population (%)	Base index	Chain index
1948	18,5	100	-	34,2	100	-	47,3	100	-
1953	18,1	97,84	97,84	34,6	101,17	101,17	47,3	100	100
1961	17,7	95,68	97,79	36,1	105,56	104,34	46,2	97,67	97,67
1971	18,3	98,92	103,39	38,3	111,99	106,09	43,4	91,75	93,94
1981	19,8	107,03	108,20	41,0	119,88	107,05	39,2	82,88	90,32
1991	21,9	118,38	110,61	42,6	124,56	103,90	35,5	75,05	90,56
2003	23,5	127,03	107,31	45,1	131,87	105,87	31,4	66,38	88,45
2011	24,0	129,73	102,13	47,3	138,30	104,88	28,7	60,68	91,40

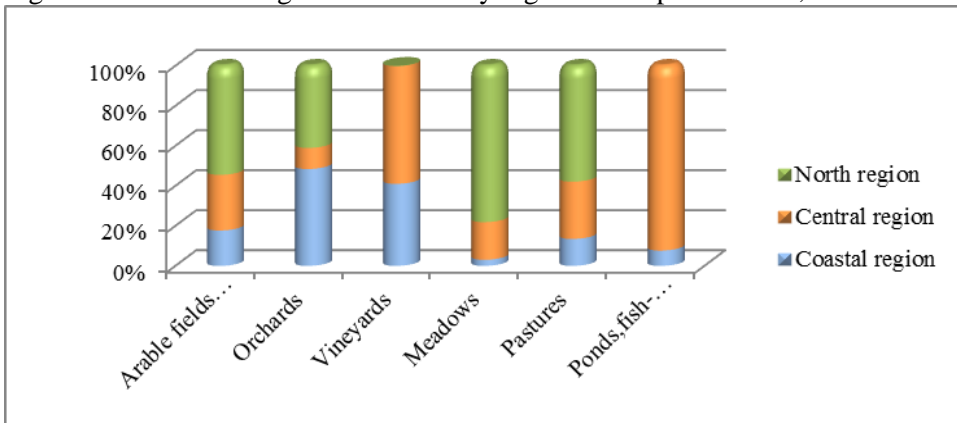
Source: *Calculation of the corresponding author according to the data, MONSTAT (2008)*

Data shown in Table 2 indicate that since the Census of 1981, the share of the population of the Southern and Central regions rose in comparison to the total population of Montenegro and the share of the population of the Northern region had declined. Calculated base indexes indicate that in 2011, in comparison to the based year of 1948, population in the Coastal region increased by 29.73%, the

Central by 38.30%, while in the North it was reduced by 39.92%. Chain indices show the changes from one census to another (Despotović *et al.*, 2015).

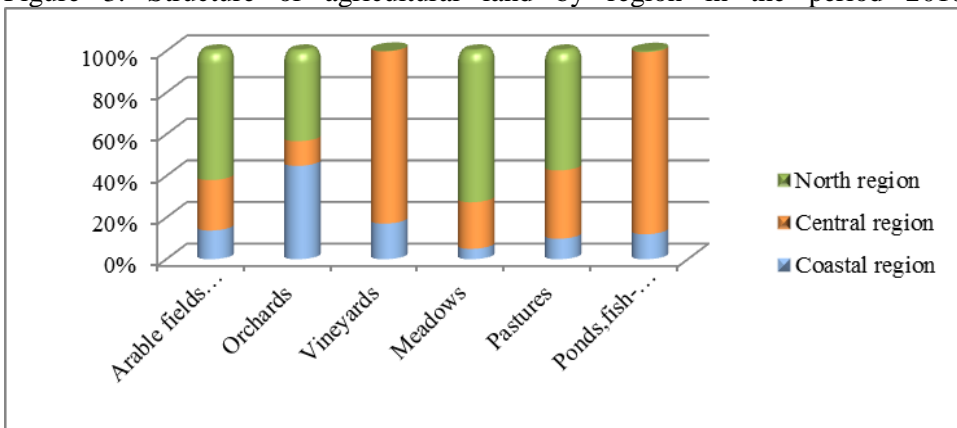
The decline trend in the population of the Northern region caused a weaker pace of development of agriculture. Regarding to that, in the period from 1965 to 2010 there was a change in the structure of agricultural land at the level of the entire territory of Montenegro, as well as in the surveyed regions. As one of the underlying principles of regionalization, natural conditions and land are considered in terms of demo-geographic regionalization, because they are a link between population and the environment, which determines the type of activity of the population, agricultural production and food security of the population of a given region (Vojković, 2003). In this regard, Figure 2 depicts the structure of agricultural land by region in the period 1965-2010.

Figure 2. Structure of agricultural land by region in the period 1965, ha



Source: Calculation of the corresponding author according to the statistical data, *Statistical Review-Agriculture 1947-1965*

Figure 3. Structure of agricultural land by region in the period 2010



Source: Calculation of the corresponding author according to the statistical data, *Statistical Year Book, 2012*

Table 2. Indices of agricultural land structure by regions, 1965-2010 ha

	Indices of agricultural land structure by regions (1965 -2010), ha						
	Total	Arable fields and gardens	Orchards	Vineyards	Meadows	Pastures	Ponds, fish-ponds, reeds
	2010/1965	2010/1965	2010/1965	2010/1965	2010/1965	2010/1965	2010/1965
Montenegro	88,21	69,33	134,79	311,63	112,58	82,47	106,34
North region	87,62	78,81	142,22	200	104,59	81,57	-
Central region	98,16	59,54	151,22	438	133,95	94,54	100,69
Coastal region	69,82	54,15	124,91	130,10	185,29	60,29	167,36

Source: Calculation of the corresponding author according to the statistical data, 1965 and 2010

Movement trend of entire Montenegrin population by region for the period of 1948-2011, indicates that there was an evident intense internal migration. Northern region recorded a continuous decline in population, while the Central and Southern region recorded a significant increase in the population. If we observe the structure of agricultural land, it is seen that the area of the Northern region accounts to about 60% of the total agricultural land in Montenegro, the Central region 29,70% and Southern 9.8%. The uneven distribution of the population, compared to the structure of agricultural land, has contributed to the neglect of agricultural production. Indices of structure of agricultural areas in Montenegro indicate that in the period 1965-2010, total agricultural area decreased by about 11.27%, arable fields and gardens are at the top, whose reduction is by 30.67%. If it is observed by the regions, total agricultural land was reduced the most in the Coastal region (30.18%), followed by the North (11.79%) and in the Central region - only by 1.84%. Arable land and garden areas recorded the largest drop in the Coastal region (45.85%), Central (40.46%) and the North (21.19%). Orchards areas recorded growth in all three regions, with the largest growth recorded in the Central region (51.22%), and lowest in the Coastal region (24.91%). In the period after the Second World War II, Montenegro had high agrarian density in relation to the Yugoslav average (Vukčević, 1963). Due to the post-war migration of the population, there was a radical change in the structure of village and youth population movement from rural to urban areas. Compared to other republics of the former Yugoslavia, in the period between 1953 and 1971, Montenegro recorded the highest growth of the urban population in relative terms (Vujošević, 1990). From the aspect of regionalization, the population must be seen in a wider context than purely demographic, and therefore through the analyzing of historical trends of population development, displacement and territorial organization of the population (Vojković, 2003). To set the population in the regional context means exactly to observe the population as an autonomous bio-social, and geographical system (Radovanović, 1988). The integrity of the system and its demographic

distortion is very easy to be traced through the change of a single demographic characteristic: e.g. declining birthrate is reflected on the changes in age and sex structure, and it is followed by renewal of the workforce and this creates a circular chain of other conditionality (Vojković, 2003). Table 3 depicts regional structure of population of Montenegro form the period of 1961-2003.

Table 3. Regional structure of Montenegrin population, 1961-2003

	1961	1971	1981	1991	2003
North region	46,19	43,44	39,19	37,17	33,01
Central region	36,13	38,28	41,00	42,56	43,30
Coastal region	17,68	18,28	19,81	20,27	23,69
Total	100	100	100	100	100

Source: Sectoral Study 4.9. Spatial Plan of Montenegro until 2020

Analysis of regional structure of the population of Montenegro for the period of 1961-2003 shows that in 1961 the population of the Northern region participated with 46.19% in the total population of Montenegro, but it was constantly declining, and in 2003 it was 33.01%. In the observed period, the Central and Southern region period recorded a constant growth of the population, therefore in 2003 the Central region had a share of 43.30%, Southern 23.69% of the total population of Montenegro. The highest growth was acquired in the Central region, which had the smallest decline in total agricultural area (1.4%) in the period of 1965-2010.

Demographic changes occurred in the period 1965-2010 had an impact on the further development of family holdings in Montenegro by regions. The main characteristic of holdings is their fragmentation and low productivity. Meadows and pastures (87.50%) have the largest share in the structure of agricultural land, while other categories of land account for about 12.50%. Table 4 depicts the family agricultural holdings by size of the class type of utilized agricultural land in the period of 1960-2010.

In the period between two agricultural censuses (1960-2010) there were significant changes in the structure of agricultural holdings<sup>2</sup>. According to the last Agricultural Census, total number of households decreased by about 25% compared to the Census of 1960. According to the Census of 2010, the highest share goes to the households of size from 0.10 to 0.50 ha (31.6%) and a very small number of households with 100 or more hectares (0.87%). Comparing the results of Census of 1960 and 201, it could be seen that in 1960, the largest share accounts to the holdings size of 1-2 ha (18.39%), while the share of households larger than 10 ha were at the level of 13.55%. Analysis of the data indicates a significant change in the number of households by type of using. In the period of fifty years, the share of households increased to 2 ha and it was 55.25%, while

<sup>2</sup> According to the methodology, Agricultural Census conducted in 1960 did not include households without land, holdings of less than 0.1 ha, holdings of 5-6 ha, but holdings of 5-8 ha. Also, larger households are treated only as holdings of over 10 ha.

according to the Census of 1960, this share was at the level of 38%. The share of households in size from 2.1 to 10 ha, according to the Census of 2010, decreased by approximately 27% compared to 1960. Their share was at a level of 48% (1960), while according to the Census of 2010, it was about 21%. Similar changes occurred in all regions.

The structure of agricultural holdings, according the agricultural labour force (1960-2010) is different. Households with 1-2 members, observed in absolute terms, are about equal, but their number is 0.5% higher in 2010 compared to 1960. If we compare the other categories of households, it can be seen that they were significantly more numerous in 1960 and that the number declined by about 50% in 2010.

Table 4. Family agricultural holdings by size of the class type of utilized agricultural land in the period of 1960-2010

Family holdings by the size of class type of utilized agricultural land	1960	2010
no land	-	581
< 0,10 ha	-	2.514
0,10<0,50 ha	5.899	15.418
0,50< 1,00 ha	6.900	8.465
1,00< 2,00 ha	11.939	8.865
2,00 < 3,00	8.643	4.076
3,00< 4,00 ha	6.362	2.256
4,00< 5,00 ha	4.586	1.287
5,00< 6,00 ha	8.506	1.056
6,00< 8,00 ha		1.066
8,00<10,00 ha	3.285	588
10,00<15,00 ha	8.798	814
15,00<20,00 ha	-	342
20,00< 30,00 ha	-	323
30,00 < 50,00 ha	-	312
50,00< 100 ha	-	436
100 ha and more	-	425
<b>TOTAL</b>	<b>64.918</b>	<b>48.824</b>

Source: Agricultural Census for 1960 and 2010

Today, the modern family household in Montenegro is "old". This is supported by the fact that 65.7% of total workforce force in the households is 45 years old. Share of male labour force amounts to 60.41%. Common for male and female workers is that the largest share account to group of people who are 65 years old or more. The share of the workforce under 24 is only 6.83 (Agricultural Census, 2010). Problems of age structure are also present in the European Union where on 9 farmers over the age of 55 "goes" one younger than 35 (Jelić *et al.*).

On the whole, demographic and regional problems in Montenegro can be expressed through the following characteristics:

- after the Second World War, the growth has continued of the total population of Montenegro, with a tendency of stagnation in recent years
- ageing of population
- reducing the number of households
- depopulation of the Northern region and rural areas
- reduction of total agricultural area and by regions
- reducing of the number of family households members
- deruralization
- deagrarisation

These regional and demographic problems had influenced on depopulation of rural areas, which was negatively reflected on the overall development of the agricultural and food industry in the period after the Second World War. Demographic depopulation of the Northern region is a limitation for the total and balanced regional development of Montenegro. Evident migration dynamics in spatial and vertical sense, conditioned by human activities, is an important indicator of physical functional connections, which are established in the region and between regions (Vojković, 2003).

## CONCLUSION

Problems of regional divisions of some areas are multiple and complex. Balanced regional development is the basis for rapid economic growth and development of any society. The European Union expects that regionalization is implemented in accordance with its criteria, in order to ensure compatibility of the territorial organization of the Member States.

In the post-war period, demographic problems occurred in Montenegro, as a result of evident internal migration, which caused uneven regional development. More specifically, there is a dislocation of the population from North to Central and Southern region. Rural areas are still empty and they are characterized by low population density. As a direct consequence of these processes, there is a lag in development of agricultural production. The share of agricultural population has decreased by about 74% in the last seventy years. Uneven regional development is reflected in the fact that the Northern region covers more than 50% of the national territory, but it is inhabited by only a third of the population. The largest migratory movements are in the direction from the



Northern region to Podgorica, as well as from the Northern to Coastal region, especially in the last ten years.

The consequences of this imbalance of regional development are reflected in the reduction of the number of family agricultural households, reducing the total agricultural area by regions, depopulation of rural areas, as well as reducing the number of members of the agricultural family agricultural households. These phenomena indicate that any violation of the system affects the demographic changes in the context of agricultural activities, available workforce, etc., and this creates a further circular chain of causality.

In the future it is necessary to work due to even overall territorial development and rural areas. This can be achieved by measures which will be aimed at developing the rural economy, as well as on improving the quality of life not only in the Northern region, but also in the Central and Southern.

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## **WATER USE EFFICIENCY AND YIELD-DEPENDENCES FOR CANOLA (*Brassica napus*, L.) UNDER IRRIGATION**

### **SUMMARY**

Worsened water availability conditions caused by the recent processes of climate warming evoke the attention of the scientists to the efficiency of the water use by crops. A useful tool for successful yield and water management is the yield-water relationship. The goal of the paper is to study the interrelations between water, water use efficiency and yield of canola and to calibrate some yield-water dependencies which can be recommended for prediction of the irrigation water amounts and the yield. A moderately early canola hybrid (*Brassica napus*, L.) was studied for its sensitivity and response to water. A field experiment in Sofia region, Bulgaria, was conducted. Three levels of soil moisture conditions in a chromic luvisols were tested: rain-fed; deficit moisture, managed by 50% deficit irrigation; and normal moisture conditions, managed by full irrigation at a refill point 80% of field capacity. The data from the experiment was processed by analysis of variance and regression analysis. The results show that soil moisture level has statistically significant impact on the yield accumulation. It contributed to increasing the seed yield from a minimum 1.319 Mg/ha at  $ET=189.0$  mm under rain-fed conditions to a maximum of 4.889 Mg/ha at  $ET=310.0$  mm under normal moisture conditions. The maximum irrigation water use efficiency in the experiment was  $1.78 \text{ kg/m}^3$  at an irrigation depth of 94 mm,  $ET=268.5$  mm and seed yield - 4.189 Mg/ha. The maximum water use efficiency occurs earlier than the maximum yield. By managing 12% less (than needed) seasonal evapotranspiration, the yield losses were only 6%. Elasticity (sensitivity of the crop to water) can be used as an indicator for the critical range of the seasonal evapotranspiration, in which the water use efficiency and the yield are maximal ( $0 \leq EWP \leq 1$ ). The yield response factor  $K_y$  of FAO linear function was established as 1.52. The parameters of the local Davidov equations were calibrated as  $a=3.53$  and  $k=1.58$  for the single-power equation and  $q=2.39$  and  $r=13.63$  for the two-power equation. Davidov equations

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

are recommended with priority for forecasting of canola yields on the base of the seasonal crop evapotranspiration.

**Keywords:** canola, irrigation, water use efficiency, elasticity, FAO yield response factor, Davidov's equations, Bulgaria

## INTRODUCTION

Worsened water availability conditions caused by the recent processes of climate warming evoke the attention of the scientists to the efficiency of the water use by crops. In the countries, where the water resources are insufficient and unevenly distributed over the territory, yield is predicted by means of models that guarantee high water use efficiency, for the purpose of obtaining economically acceptable production results. A useful tool for successful yield and water management in these models is the yield-water relationship, written down in different analytical forms with calibrated coefficients for particular crops and environments. The yield-water dependences describe the impact of water on the yield accumulation at different water supply levels. Some authors have the opinion that when crop suffers of an evenly distributed throughout the growing season water deficit, the yield is a linear function of the evapotranspiration (Jensen, 1968; Stewart et al., 1973; Doorenbos, Kassam, 1979; Tzakiris, 1981; Varlev, Popova., 1999, Varlev, 2004). Downey, 1972 notices that the plants grown in an open field are not exposed to constant water deficit because the evapotranspiration after rain or an irrigation application is the maximum. The water stress starts when the soil starts to dry. The dynamics of the soil water regime is a good reason to think that the yield losses are not a simple function of the evapotranspiration. The results from testing the impact of a proviso regular throughout the growing season water deficit have revealed so far that the yield losses are dependent on the weather conditions. There are many authors who think that the yield-water dependence is a power function, which can be represented by a series of two-incurvation curves with an inflection point. These authors are convinced that except the meteorological peculiarities of the individual years, the yield-water dependence reflects the biological characteristics of the crops (Pare, Olivier, 1969; Davidov, 1982, 2004; Mate, 2001; Zhukov, Davidov, 2003). Stewart et al. (1973) have established that the reduction of the yield is not proportionate to the reduction of the water given by irrigation. Consistent with that, Zhivkov, (1994, 1995) has obtained 4-10%; 9-15%, 14-25 and 51% reduction of corn yield by 20, 40, 60, and 75% reduction of the irrigation depth. By applying 50% irrigation water deficit the yield reduction of corn on haplic chernozems is around 5-6% (Rafailov et al., 1998).

The goal of the paper is to study the interrelations between water, water use efficiency and yield of canola and to calibrate some yield-water dependencies which can be recommended for prediction of the irrigation water amounts and the yield in the temperate continental climate conditions of Bulgaria.

## MATERIAL AND METHODS

A field experiment with irrigation of winter canola was carried out in Sofia region, Bulgaria (42.6° N, 550 m a.s.l.) during three growing seasons 2010-2011, 2011-2012 and 2012-2013. The climate of the region is temperate-continental. The average annual temperature of the site is 10.3°C. The place is one of the most humid in the country. The average annual precipitation is 610 mm (Geography of Bulgaria, 2002). The rainfall totals of the period March-July in 2011, 2012 and 2013 were 132.8, 174.6 and 187.6 mm respectively.

Table 1. Probability of exceedance

Year	March- July	March- April	April- June	June- July
Rainfalls				
2011	85.0	100.0	100.0	20.0
2012	92.5	97.5	80.0	95.0
2013	60.0	80.0	35.0	30.0
Air Temperature				
2011	75.0	72.5	85.0	50.0
2012	10.0	50.0	12.5	2.5
2013	30.0	50.0	17.5	45.0
Vapour pressure deficit				
2011	35.0	60.0	55.0	25.0
2012	5.0	12.5	5.0	5.0
2013	52.5	65.0	22.5	65.0

As to the 50-year (1974-2013) rainfall probability of exceedance, the period March-July in 2011 and 2012 was dry and average in 2013. Analogously, this period is featured cool in 2011 and warm in 2012 and 2013. As to vapour pressure deficit, it was dry in 2011 and 2012 and average in 2013 (Table 1).

The experiment was put in a randomized complete block design in three replications. Irrigation was in three levels: rain-fed, 50% deficit irrigation, and full irrigation at a refill point 80% of field capacity ( $RP_{80}$ ). The canola variety Triangle, which is popular on Bulgarian market, has been tested. The soil was

chromic luvisols with total water content  $TWC=327$  mm, available water content  $AWC=165$  mm, and bulk density  $\alpha=1.5$  g/cm<sup>3</sup>. Land preparation, fertilizers and weed control were applied according to the standard agricultural practices in the region. Sowing was done each year in the period 25-30<sup>th</sup> September.

The irrigation application depth at  $RP_{80}$  was  $m=60$  mm and was calculated as:

$$m = 10H\alpha(\beta_{FC} - \beta_{RP_{80}})$$

where  $\beta$  is the moisture percentage by weight (Kostyakov, 1951). The depth of root expansion was adopted as  $H=1.0$  m. The soil water content in the root zone was estimated in each 10 days by the soil sampling method.

The 10-day crop evapotranspiration was calculated by the water balance equation:

$$ET_c = W_{i-1} - W_i + m + R$$

where  $ET_c$ —10-day actual crop evapotranspiration, mm;  $W_{i-1}$ —soil water content in the 1-m soil layer on the first day of the 10-day period, mm;  $W_i$ —soil water content in the 1-m soil layer on the tenth day of the 10-day period, mm;  $R$ —the 10-day effective rainfall total, mm. The spring-summer evapotranspiration totals were calculated through summarizing the 10-day values.

Variance analysis was applied to the yield results. Regression analysis was applied to establish the impact of the irrigation depth on the irrigation water use efficiency (*Irr.WUE*).

FAO yield-evapotranspiration dependence

$$Y_{def}^i / Y_{max} = 1 - K_y (1 - ET_{def}^i / ET_{max}) \quad (\text{Doorenbos, Kassam, 1979}) \text{ and}$$

Davidov power equations

$$Y_{def}^i / Y_{max} = 1 - a (1 - ET_{def}^i / ET_{max})^k \text{ and}$$

$$Y_{def}^i / Y_{max} = \left( 1 - (1 - ET_{def}^i / ET_{max})^q \right)^r \quad (\text{Davidov, 1982, 2004}) \text{ were calibrated,}$$

where:  $Y_{def}^i$  - yield under irrigation deficit in plot  $i$ , Mg/ha;  $Y_{max}$  - yield under full irrigation, Mg/ha;  $ET_{def}^i$  - actual crop evapotranspiration at  $Y_{def}^i$ , mm;  $ET_{max}$  - maximum evapotranspiration in the experiment, mm;  $a$  - parameter;  $q$  - power index that reflects the impact of the water supply on the yield ( $q < 1$ ),  $r$  - power index that reflects the sensibility of the crop to the water deficit;  $k$  - power index;  $K_y$  - yield factor (=const. for a certain crop).

Interrelations of yield, evapotranspiration, and water use efficiency were established from a marginal analysis of the water production functions (Liu et al., 2002).

## RESULTS AND DISCUSSION

Following the dynamics of the meteorological conditions, in 2011 was given one irrigation application and in 2012 and 2013 - two irrigation applications.

Table 2. Results from the field experiment

Year	Variants	Irrigation depth, mm	ET, mm	Seed yield, Mg/ha
2011	Raifed	-	189.8	1.319
	50% irrigation deficit	30	228.2	3.894+++
	Full irrigation	60	260.0	4.889+++
2012	Raifed	-	220.6	2.833
	50% irrigation deficit	60	255.3	3.838+
	Full irrigation	120	272.5	4.797+++
2013	Raifed	-	260.0	2.517
	50% irrigation deficit	60	296.0	4.123+++
	Full irrigation	120	310.0	4.737+++

+significant at P=5%; ++significant at P=1%; +++significant at P=0.1%

The results in Table 2 show significant impact of irrigation on the yield. The seed yield under rain-fed conditions varies from 1.319 to 2.833 Mg/ha. The yield under 50% deficit irrigation varies from 3.838 to 4.123 Mg/ha and the yield increase insignificant at probability P=5% and P=0.1%. The yield under full irrigation is considerably higher – from 4.737 to 4.889 Mg/ha and is significant at P=0.1%. These results confirm the results, obtained in different parts of the world. As to Istanbuloglu et al. (2010) and their review, the seed yield of canola, obtained in rain-fed and irrigation conditions, varies from 1.0 to 5.3 Mg/ha. Alberta

Agriculture (1980) reported for 1.0-2.6 Mg/ha seed yields without irrigation, which were considered good, and 3.2-4.0 Mg/ha under full irrigation. As to North (2010), the yield in rain-fed conditions in Australia also tended to be 1.7-1.8 Mg/ha, while the best on-farm yields were 1.8-3.6 Mg/ha. The yields from the experimental fields were as high as 3.8-5.2 Mg/ha. The yield of irrigated winter canola in Nebraska, USA was reported to be  $\approx 3.0$  Mg/ha (Aiken, Lamm, 2006).

Crop sensibility to water was evident from the relation  $ET$  increase – yield-increase. It is seen in Table 2 that the actual evapotranspiration under rain-fed conditions is in the range of 189.8-260.0 mm, in conditions of 50% irrigation deficit – 228.2-296.0 mm, and under full irrigation – 260.0-310.0 mm. By giving 50% of the necessary irrigational water in 2012-2013,  $ET$  increased with 14-16% while yield increased with 35-64%. By giving the whole needed amount of irrigational water,  $ET$  increased with 19-24% and the yield - with 69-88%. In 2011, depending on the meteorological conditions, an increase of 20% and 37% of  $ET$  caused unproportioned double and triple yield increase respectively. These results are evidence for different efficiency of the irrigational water in the range of the irrigation depth (Fig. 1). It is seen that the irrigation water use efficiency ( $Irr.WUE$ ), as dependent on the irrigation depth, increases in a polynomial law, and the approximation of the data has high coefficient of determination  $R^2=0.67$ .

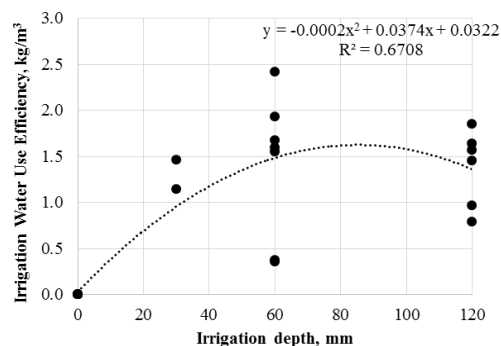


Figure 1. Impact of the irrigation depth on the irrigation water use efficiency

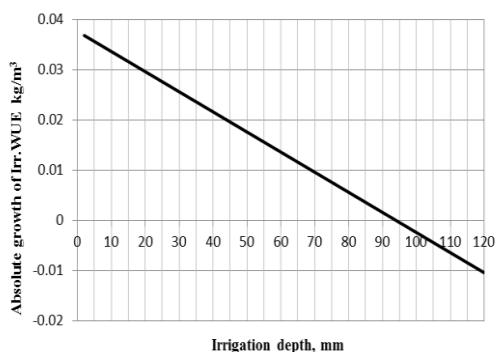


Figure 2. Rate of change of the Irrigation WUE

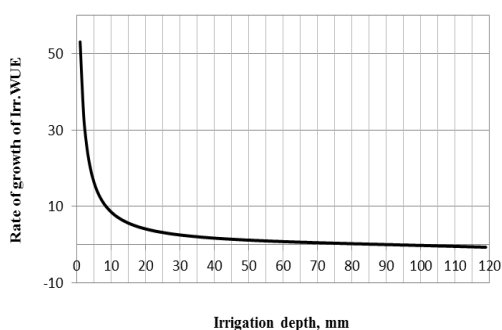
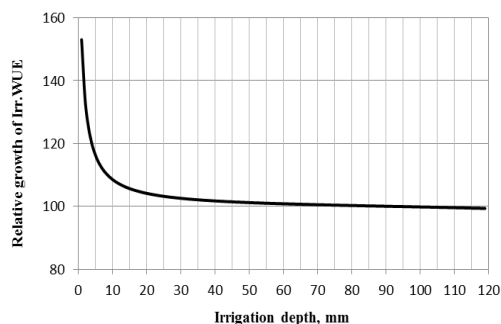


Figure 3. Relative growth (a) and rate of growth (b) of IrrWUE

The absolute growth on a chain basis is  $\text{const.} = -0.0004$ , the average is  $0.0186 \text{ kg/m}^3$  (Fig. 2). The line of the absolute growth crosses the x-axis at the point when the relative growth is 100% (Fig 3a) and the rate of growth is zero (Fig.3b). This point is the point of  $\text{Irr. WUE} = 1.78 \text{ kg/m}^3$ . It is seen that  $\text{Irr. WUE}$  is maximum at irrigation depth  $M=94 \text{ mm}$ . According to the quadric approximation, the relative growth and the rate of growth are greatest in the range approximately 0-30 mm of the irrigation depth, after which they sharply drop.

The results of the yield losses caused by the deficit of irrigation water make it possible to assess the nature of the “yield - irrigation depth” relationship in terms of its proportionality and to provide practical advice for the farmers for managing of the irrigation scheduling. The dependence of the yield losses on the irrigation depth in the experiment is linear and inversely proportional. The coefficient of proportionality is  $a = -0.4943$  (Fig. 4). The relative yield losses when maintaining 50% deficit irrigation are 15-30% and when without irrigation are 30-50%. Our results correspond to those reported by Fanaei et al. (2009) for 54-82% relative yield losses in different drought conditions in comparison with optimum moisture conditions. As to them, drought is one of the strongest abiotic stress factors for the development and productivity of canola.

The issued up to here yield response to the irrigation water has only local meaning and is useful for local water management, confirmed by Hexem and Heady (1978). This relationship is influenced by the geographical location, especially by the weather. It is hardly applied for irrigation system management outside of the soil and climatic conditions for which this relationship is established. One of the more relevant and widely used relationships is that of the yield to evapotranspiration. It is universal and is bound mostly to the crop biology, to its sensitivity to water and ability to use water efficiently. After a thorough review of the research work in this field Vaux and Pruitt (1983) have concluded that crop yield is a linear function of the evapotranspiration. Their follower in Bulgaria is Varlev (2004). Dooreboos & Kassam (1979) have introduced the crop factor  $K_y$  to describe the relationship between the yield loss and the deficit of evapotranspiration. On Fig. 4 is shown the yield-evapotranspiration relationship for canola, which is based on the data of the experiment. As a result of a regression analysis, the value of the crop factor was established as  $K_y = 1.52$ , with a high coefficient of determination  $R^2 = 0.73$ . The value of the crop factor, i.e. the slope of the straight line to x-axis shows great sensitivity of canola to water. It reveals that a small deficit of evapotranspiration can cause high reduction of the yield:

$$Y_{def}^i / Y_{max} = 1 - 1.52 \left( 1 - ET_{def}^i / ET_{max} \right).$$

The marginal approach to calculation of  $K_y$  (Liu et al., 2002) is based on the calculation of the marginal (maximum) water use efficiency ( $MWUE$ ). The interrelations between the yield ( $Y$ ), the seasonal evapotranspiration ( $ET$ ) and  $WUE$  based on the elasticity ( $EWP$ ) were studied. Elasticity is treated as the sensitivity of the crop to water in yield accumulation. Elasticity is limited by the maximum water



use efficiency (*MWUE*), which is the first derivative of the yield-evapotranspiration function, and can be expressed as:

$$EWP = MWUE/WUE = (dY/Y)/(dET/ET).$$

It is evident that elasticity is different in different parts of the yield-evapotranspiration function.

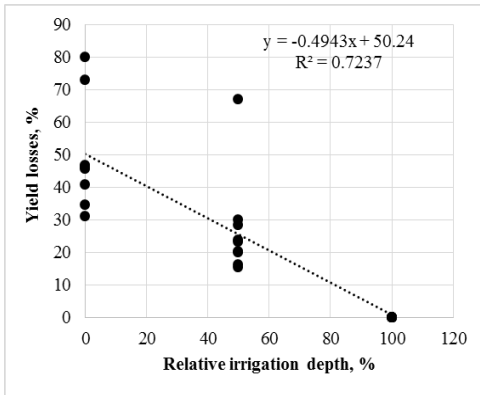


Figure 4. Relative yield losses, %

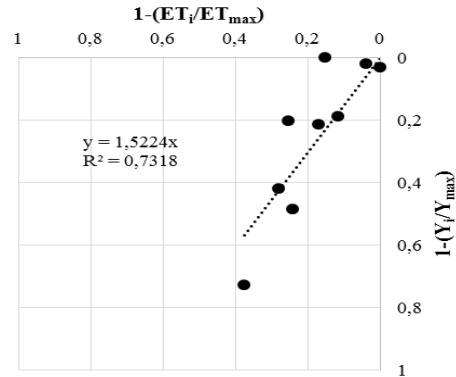


Figure 5. The “yield-evapotranspiration” dependence in FAO methodology

If a linear approximation is considered (Fig. 6ab), then:  $Y = a_1 + b_1 ET$ ,  $MWUE = \text{const.} = 2.451 \cdot WUE$  is calculated as  $WUE = Y/ET = a_1/ET + b_1$  and performs a parabola with an asymptote  $MWUE = b_1$  (when  $a \neq 0$ ) (Fig. 6b), and  $EWP$  is calculated as  $EWP = b_1 ET / (a_1 + b_1 ET)$  (Fig 6c).

For  $EWP > 0$ ,  $WUE$  increases. In this linear relationship  $Y_{\text{max}} = a_1 + b_1 / ET_{\text{max}}$ , i.e. the crop accumulates maximum yield at maximum evapotranspiration.

$$\text{Then } 1 - Y/Y_{\text{max}} = b_1 ET / (a_1 + b_1 ET) (1 - ET/ET_{\text{max}})$$

$$\text{and } K_y = b_1 ET / (a_1 + b_1 ET) = 1.52.$$

The crop yield response factor can be calculated directly from the linear expression for  $EWP$  by substituting  $ET$  with  $ET_{\text{max}}$ .

In case of a quadric approximation (Fig. 7), then:

$$Y = a_2 + b_2 ET + c_2 ET^2,$$

$$WUE = a_2 / ET + b_2 + c_2 ET,$$

$$MWUE = b_2 + 2c_2 ET,$$

$$EWP = b_2 + 2c_2 ET / (a_2 + b_2 ET + c_2 ET^2).$$

It is seen on Fig. 7b, that with the increase of  $ET$ ,  $MWUE$  decreases linearly; the dependence  $WUE-ET$  is a parabola that reaches maximum at  $ET = \sqrt{a_2/c_2}$ , then decreases;  $Y$  reaches maximum at  $ET = -b_2/2c_2$ ;  $MWUE$  occurs before the maximum yield. Assuming that the yield  $Y$  is maximal at maximum evapotranspiration, then:

$$1 - Y/Y_{\max} = -c_2 ET_{\max}^2 / Y_{\max} (1 - ET/ET_{\max})^2 \text{ and}$$

$$K_y = -c_2 ET_{\max}^2 / Y_{\max} .$$

The yield response factor in the quadratic equation is  $K_y = 4.69$ .

The results obtained show that if the objective of the procedure is to obtain a maximum yield ( $Y_{\max}=4.444$  Mg/ha), the evapotranspiration should be maximum ( $ET_{\max}=310.0$  mm).  $ET_{\max}$  is with 12% higher than  $ET$  at  $MWUE$  (268.5 mm), while  $Y_{\max}$  is with 6% higher than  $Y$  at  $MWUE$ .

There are two typical  $EWP$  values, proceeding from the quadric approximation. One of them is  $EWP=1$  that indicates  $MWUE$ . The other one is  $EWP=0$  that indicates  $Y_{\max}$  and  $ET_{\max}$ .

Calculation of  $EWP$  can be useful for indication of the critical range of the seasonal evapotranspiration around which the productivity and the yield would be maximal ( $0 \leq EWP \leq 1$ ). Further, this information can be used for predicting the needed water amounts for irrigation according to the seasonal weather and precipitation forecasts.

Some local researchers like Davidov (1982, 2004) have the opinion that the yield-evapotranspiration relationship has more complicated nature. The calibrated parameters of two Davidov equations for canola are as follows:  $a=3.53$  and  $k=1.58$  in the single-power equation

$$Y_{def}^i / Y_{\max} = 1 - 3.53 \left( 1 - ET_{def}^i / ET_{\max} \right)^{1.58} \text{ (Fig. 8) and}$$

$$q=2.39 \text{ and } r=13.63$$

in the two-power equation

$$Y_{def}^i / Y_{\max} = \left( 1 - \left( 1 - ET_{def}^i / ET_{\max} \right)^{2.39} \right)^{13.63} \text{ (Fig. 9).}$$

Both approximations have very high coefficients of determination, which in turn are much higher than that of FAO linear approximation. The results indicate that the Davidov functions approximate the experimental data more accurately, compared with the quadric function either.

All the calibrated equations can be used for yield prediction for canola but with priority given to Davidov equations.



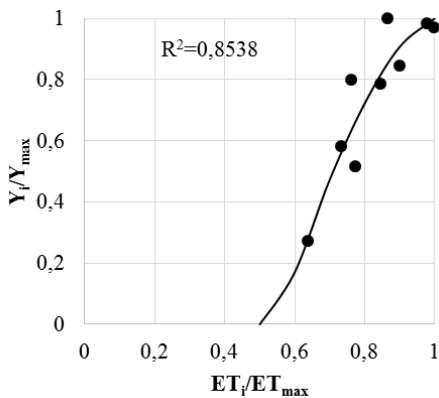


Figure 8. Davidov single-power approximation

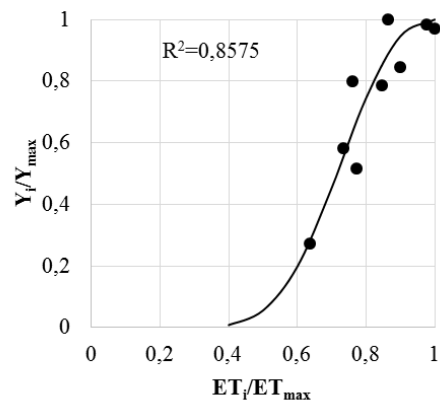


Figure 9. Davidov two-power approximation

## CONCLUSIONS

1. Irrigation has statistically significant impact on canola yield. In a three-year period, it contributes for seed yield increase from a minimum of 1.319 Mg/ha under rain-fed conditions to a maximum of 4.889 Mg/ha under full irrigation.

2. Water use efficiency occurs not with the maximum yield but a bit earlier. The maximum irrigation water use efficiency in the experiment was obtained at an irrigation depth of 94 mm and was 1.78 kg/m<sup>3</sup>. By managing 12% less (than needed) seasonal evapotranspiration, the yield losses can be only 6%.

3. Elasticity is an indicator for the critical range of the seasonal evapotranspiration, in which the water use efficiency and the yield are maximal ( $0 \leq EWP \leq 1$ ). This information is useful for prediction of the irrigation water amounts according to the seasonal weather and precipitation forecasts.

4. The yield response factor in FAO linear function is  $K_y=1.52$ . It indicates canola high sensitivity to water. The parameters of Davidov single-power equation are  $a=3.53$  and  $k=1.58$ . The parameters of Davidov two-power equation are  $q=2.39$  and  $r=13.63$ . Davidov approximations are more accurate than those of FAO linear equation and the quadric function. They are recommended with priority for forecasting canola yields on the base of the seasonal evapotranspiration.

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## SOIL MOISTURE DYNAMICS IN DIFFERENT IRRIGATION REGIMES OF TOMATO CROP

### SUMMARY

The main purpose of this research was to determine the soil moisture dynamics in different irrigation regimes of tomato crop grown in Skopje region. In addition, one of the goals of this research was to determine the influence of the soil moisture dynamics on tomato yields. For this purpose, a field research was conducted in two seasons in the period from May to September with tomato crop, hybrid Optima, grown near the Faculty of Agricultural Sciences and Food in Skopje. Five different irrigation and fertilization regimes were performed during the investigation. The first three of them were drip fertigated in every 2, 4 and 6 days, respectively (B1, B2 and B3), the fourth one was drip irrigated with conventional application of fertilizers (Ø1), while the last one was furrow irrigated with conventional application of fertilizers (Ø2). It can be seen from the results from the two year investigation that best conditions regarding the content of soil moisture, as well as highest yields were obtained at the treatments B1 and B2, which is a result of the continuous maintenance of easily available moisture in the soil over 80% of the field capacity (FC). Higher soil moisture oscillations were noticed at B3 treatment, which is a result of the irrigation interval, due to which it has produced lower average yields of 10.07 t/ha when compared to B2 in the first year, i.e. 18.46 t/ha with B1 in the second year. Our results have shown that in addition to the continuous procurement with water, the yields were highly affected by the continuous procurement with mineral nutrition, which is especially obvious when compared treatments B2 with Ø1. Most of the time during the vegetation period, the soil moisture in the control treatment Ø2 was under the 80% of FC. Despite having good irrigation interval of 7 days, such strong stresses, together with the method of applying fertilizers is one of the crucial factors that caused lower yields when compared to the treatments irrigated with the drip irrigation system.

**Keywords:** drip irrigation, furrow irrigation, drip fertigation, soil moisture, tomato yield.

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

## INTRODUCTION

Tomato is a crop that is greatly affected by the lack of soil moisture. According to Doorenbos et al., (1986), the long-term deficit of water can sometimes limit the yields to a great extent in such a manner that the subsequent irrigation with higher quantities of water will not be able to remedy such losses. According to the same authors, the tomato has highest need for water in the blooming period, and this period is especially important for uniformed blooming, which then results in even more uniformed ripening of fruits. Excessive irrigation in this period is not recommended since it makes flowers fall off and reduces the binding of fruits. Some authors (Tanaskovik 2005; Petrevska 1999) point out that the need for higher soil moisture at the tomato is owed to the fact that it develops higher vegetative mass, produces high yields, and its root system has low suction power. Hence its need for maintenance of soil moisture within 80 to 95% of the field water capacity (FC). Iljovski and Cukaliev (1994) distinguish two sub-periods within the vegetation period of the tomato, in terms of satisfying the soil moisture as follows: 70-80% of FC in the first and 80%-85% of FC in the second sub-period, counting from the day of fructification and ripening of fruits, this crop has higher demands of soil moisture. According to Vučić (1976), the mass appearance of the first fruits is a sign that the soil moisture should increase and be maintained continuously at high level over 70-80% of FC. According to Bošnjak (1999), the technical minimum or lower point of easily available water (LPAW) of the soil up to the production of the first fruits should extend within 70% of FC, and the same can be increased to 80% of FC after this period. Stanley and Maynard (1990) have determined that the growth of tomato reduces when 60% of the available water from the active rhizosphere is consumed by the plants, which approximately corresponds to 80% of FC.

The soil moisture represents a dynamic value that constantly varies. According to some authors (Tanaskovik et al, 2009; Evett, 2007; Cukaliev, 1996), the soil moisture depends of the inflow and outflow of water in the soil, the ecological conditions, and the cultivation practices, especially the irrigation. The knowledge of the soil moisture dynamics is one of the ways to find out the content of soil moisture, i.e. the easily available water required for the crop. The need to determine this parameter comes primarily from the needs for determination of irrigation application rate and the time of application of water in agricultural production. According to Tanaskovik et al., (2013), one of the most important issues in irrigation practice is irrigation scheduling of agricultural crops. According to the same authors, the optimum irrigation regime of the agricultural crops implies maintenance of the soil moisture within the range of medium to easily available water. In case of oscillation from the optimum irrigation regime, mostly in the direction of hardly available to vary hardly available water, the plants suffer stress due to lack of water. The lack of available water, more or less, affects the yields and the quality, and thus, the price of the products.



There are many methods in the agricultural practice for proper irrigation scheduling, which are usually divided into three groups according to the method of determination: soil-based (soil moisture monitoring), plant-based (plant condition monitoring) and climate-based (climate characteristics monitoring) methods (Tanaskovik and Cukaliev, 2013). None of these methods alone is sufficient for accurate determination of irrigation application rate and the time of application of water. The crops, the biological characteristics, the expected yields and the water-physical properties of soil, as well as the climate parameters have always been taken into account. On the other side, soil moisture monitoring method still represents the best and the most accurate manner to determine irrigation application rate and the time of application of water. According to many authors (Tanaskovik et al., 2013; Evett, 2007; Warrick and Or, 2007), the thermo-gravimetric method is the most accurate method and serves for calibration of all other methods used for monitoring the soil moisture dynamics and determination of the proper irrigation regime.

Hence, the main purpose of this research was monitoring of the soil moisture dynamics during the application of different irrigation regimes in tomato crop, as well as determination of the effect of the irrigation regimes on tomato yields.

## MATERIAL AND METHODS

The field experiment was conducted on Fluvisol soil type (WRB 2015) with a tomato crop, hybrid Optima, near the Faculty of Agricultural Sciences and Food in Skopje (42° 00' N, 21° 27' E), Macedonia. In order to monitor the soil moisture dynamics, at 60 cm depth, the following parameters were examined: bulk density with Kopecki cylinders with volume of 100 cm<sup>3</sup> (Iljovski and Cukaliev, 2002); FC (ICARDA, 2001); soil moisture retention at 15 bars (a value near the permanent wilting point – PWP) with Pressure plate extractor according to Richards (Townend et al., 2001; ICARDA, 2001); and technical minimum or lower point of easily available water with an increase of 20% of the value obtained with water extraction at 15 bars (Iljovski and Cukaliev, 2002). The results from the water-physical properties of the soil are shown in Table 1.

Table 1. Water-physical properties of the soil

Layer cm	Bulk density	Permanent wilting point (PWP)			Lower point of easily available water (LPAW)			Field capacity (FC)			Maximum irrigation application rate
		cm	g/cm <sup>3</sup>	mass% vol% m <sup>3</sup> /ha	mass% vol% m <sup>3</sup> /ha	mass% vol% m <sup>3</sup> /ha	mass% vol% m <sup>3</sup> /ha	mass% vol% m <sup>3</sup> /ha	mass% vol% m <sup>3</sup> /ha		
0-20	1,50	10,93	16,40	328,00	13,12	19,68	393,60	20,56	30,84	616,80	321,00
20-40	1,60	9,82	15,71	314,20	11,78	18,85	377,00	20,15	32,24	644,80	333,80
40-60	1,45	7,59	11,01	220,20	9,10	13,19	263,80	19,20	27,84	556,80	340,80
0-60				862,20			1034,40			1818,40	784,00

FC in the root system development zone up to 60 cm, is amounting to 1818.40 m<sup>3</sup>/ha, and the capacity of water obtained with retention at 15 bars (a value near the PWP) is amounting to 862.20 m<sup>3</sup>/ha, the LPAW is 1034.40 m<sup>3</sup>/ha. The content of available water capacity (AWC) is 956.2 m<sup>3</sup>/ha which indicates that the soil is provided with goods quantity of water. The maximum irrigation application rate is amounting to 784.00 m<sup>3</sup>/ha.

The agrochemical properties of the soil (ICARDA, 2001) are shown in Table 2. In the layer from 0 to 60 cm where most of the root mass of the tomato is developed, the soil pH was 7.5, and the procurement with nutrients was as follows: 2.40 mg/100 g soil easily available nitrogen (N), 19 mg/100 g soil easily available phosphorus (P<sub>2</sub>O<sub>5</sub>) and 18 mg/100g soil easily available potassium (K<sub>2</sub>O).

Table 2. Soil chemical characteristics of the experimental field

Layer cm	CaCO <sub>3</sub> %	Organic matter %	pH		Available N mg/100 g soil	Available forms mg/100 g soil	
			H <sub>2</sub> O	KCl		P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
0-20	3,24	0,90	8,00	7,00	2,80	33,46	30,44
20-40	3,80	0,84	8,10	6,90	2,07	12,03	14,42
40-60	3,59	0,56	8,10	7,00	2,41	12,03	9,21

According to the literature data for the region, tomato planted in an open field in similar condition yields up to 80 t/ha (Tanaskovik, 2011). Tomato crop nutrient uptake for an 80 t/ha harvest are approximately: N 260 kg/ha, P<sub>2</sub>O<sub>5</sub> 160 kg/ha and K<sub>2</sub>O 320 kg/ha. The application of the fertilizer for the treatments was done in two portions (before planting and during the growing season), which is a common practice in Macedonia. For all treatments, the first portions of the fertilizers were applied before the planting. The rest quantity of the fertilizers needed for achieving the targeted yield were applied through the fertigation system in the drip fertigation treatments (Table 3), and by conventional fertilizer application in the control treatments (divided into two portions, given at the flowering stage and at fruit formation). All investigated treatments have received the same quantity of fertilizers but by different methods of application (Table 2). All treatments were provided with equal quantity of fertilizes, but in different manner and interval of applying water and fertilizers.

Table 3. Type and amount of fertilizers in drip fertigation

N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O			
268	164	320	kg/ha	N:P:K	
50	50	50	330 kg/ha	15:15:15	before replanting
/	93	60	179 kg/ha	0:52:10	drip fertigation
21	21	210	525 kg/ha	4:4:40	drip fertigation
197	/	/	428 kg/ha	46:0:0	drip fertigation

Note: same amounts and quantity of fertilizers were used in the control treatments Ø1 and Ø2 (spread in 2 portions)

The treatments were set up according to the randomized block system depending on daily evapotranspiration rate and the irrigation and fertilization regime:

Treatment 1 (B1). Drip irrigation according to daily evapotranspiration with fertigation in every 2 days

Treatment 2 (B2). Drip irrigation according to daily evapotranspiration with fertigation in every 4 days

Treatment 3 (B3). Drip irrigation according to daily evapotranspiration with fertigation in every 6 days

Treatment 4 (Ø1). Drip irrigation according to daily evapotranspiration in every four days and conventional fertilization (spreading of fertilizer on soil) (control 1)

Treatment 5 (Ø2). Furrow irrigation according to daily evapotranspiration in every seven days and conventional fertilization (spreading of fertilizer on soil) (control 2)

The fourth and the fifth treatments represent a comparison (control).

The size of each plot (replication) was 7.2 m<sup>2</sup> (18 plants in 0.8 m spacing between the rows and 0.5 m plant spacing in the row). Each plot (replication) was designed with three rows of crop. There were six plants in each row.

In general, the treatments were set according to the daily evapotranspiration, in accordance with the research purposes. The calculations of the necessary amounts of water during the vegetation for all treatments, monthly and daily (Table 4) were made according to the modified Penman-Monteith method (FAO, Irrigation and Drainage Paper 56), i.e. with application of the CROPWAT computer program, using crop coefficient ( $K_c$ ) and the stage length adjusted for local condition by the Faculty of Agricultural Sciences and Food in Skopje. Since the drip irrigation was applied only to a part of the total surface, the daily evapotranspiration of the drip irrigation treatments was 20% decreased (coefficient of the coverage). The furrow irrigation treatment (Ø2) received the full irrigation rate.

Table 4. Daily and monthly crop water requirements for tomato crop for the Skopje region

Months	V	VI	VII	VIII	IX
mm/day	2	4	6	5.0	3
mm/monthly	62	120	186	155	90

The monitoring of soil moisture dynamics was based on a thermo-gravimetric method by taking soil samples during the vegetation to a depth of 60 cm. According to the thermo-gravimetric method, samples were taken at every 20 cm depth, from each treatment separately with 3 repetitions. The samples in our experiment were taken according to recommendations made by Bošnjak (1999), thus for the treatments that were irrigated with drip irrigation, samples

were taken from the wetted soil moisture profile, while in the control treatment with furrow irrigation samples were taken from the middle of the furrow. The results for the yield were subjected to statistical analysis of variance and means were compared using the least significant difference (LSD) at the 5% level of probability ( $P < 0.05$ ) test.

## RESULTS AND DISCUSSION

**Meteorological conditions during the research.** The tomato crop needs a lot of heat during the whole growing period. If temperature is below  $15^{\circ}\text{C}$  the flowering stops and if temperature drops below  $10^{\circ}\text{C}$  the growth stops. The optimal temperature for growing tomato is  $18\text{-}25^{\circ}\text{C}$  during the day time and  $15\text{-}16^{\circ}\text{C}$  during the night. The average seasonal temperature for the experimental site (average in the growing period) was  $22.2^{\circ}\text{C}$  and  $20.5^{\circ}\text{C}$  respectively (Table 5). At temperatures higher than  $25^{\circ}\text{C}$  life processes in tomatoes decrease, and at temperatures above  $30^{\circ}\text{C}$  they completely stop. During the most intensive fructification period (June-August) the average temperatures over the two experimental seasons were within the optimum values.

Table 5. Monthly average air temperature in  $^{\circ}\text{C}$  and precipitation in mm in Skopje region, during the tomato vegetation

	First year	Second year	First year	Second year
Month	Average air temperature ( $^{\circ}\text{C}$ )	Average air temperature ( $^{\circ}\text{C}$ )	Precipitation (mm)	Precipitation (mm)
V	18.1	15.3	69.0	42.3
VI	23.8	21.3	62.3	55.2
VII	25.2	24.1	2.3	61.4
VIII	26.2	23.0	11.5	16.1
IX	17.7	18.8	/	14.7
Total/Aver.	22.2	20.5	145.1	189.7

The most critical period for tomatoes in terms of moisture is during flowering and fruit formation, and this period is in line with the relatively high temperatures in Skopje region, i.e. with a period of low rainfall, as can be seen in Table 5. Data presented in the Table 5 shows that the second year was very humid with a lot of rainfalls during the growing season (250.3 mm) which is rather unusual for the Skopje region and the major vegetable production regions in Macedonia. May and June of the first year had slightly higher rainfall totals in comparison with the remainder of the growing period. In the period of most active yielding there was a severe shortage of water coupled with very high temperatures, and thus fertigation in the first year had much higher effect on the measured parameters.

**Soil moisture dynamics in different irrigation regimes of tomato crop and effects on the yield.** The time period of soil sampling in our research depended on evapotranspiration, the irrigation application rate and the measurement method that was used.

Tanaskovik (2009) has pointed out that, with frequent irrigation and use of small irrigation application rates which are applied with a proper technique of drip irrigation, samples can be taken quite often, every 2 to 4 days. The same recommendation was used in our research.

The precipitation are shown as the sum of rainfall in the period between two irrigation intervals reduced by 30% or 50% depending on the month of the vegetation period. However, it should be emphasized that rainfall were calculated as part of the irrigation application rate.

It means that, if the rainfall amount were greater than the daily evapotranspiration rate, then irrigation interval was delay, i.e. if the rainfall amount was lower than the daily evapotranspiration rate, the irrigation interval was not changed and the irrigation was carried out as if there had been no rainfall.

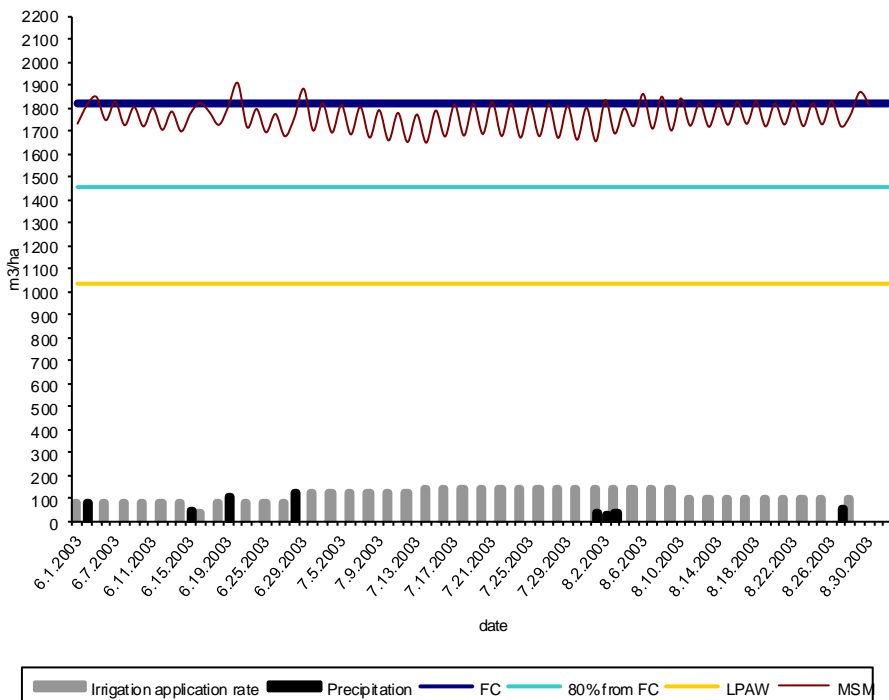


Figure 1. Soil moisture dynamics in treatment B1

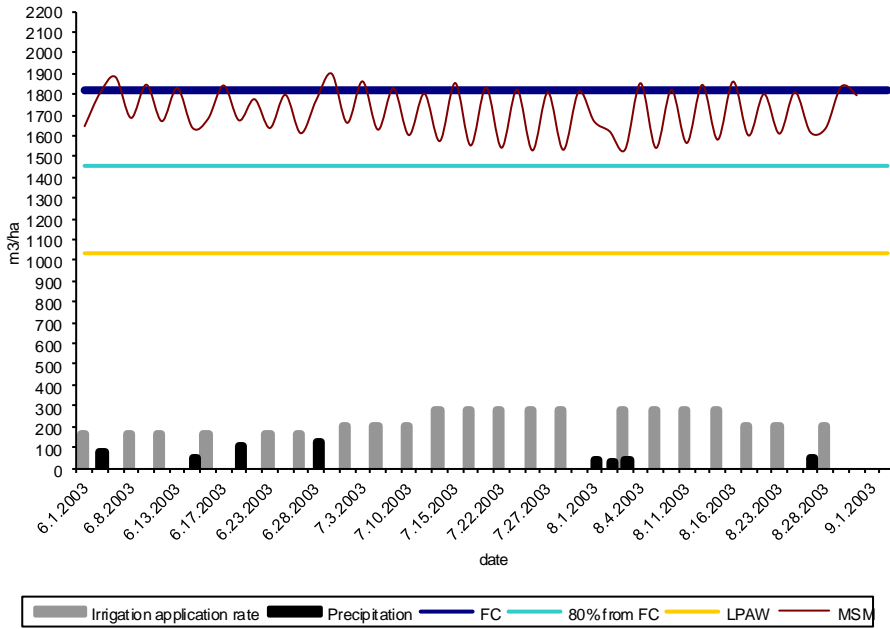


Figure 2. Soil moisture dynamics in treatment B2 and Ø1

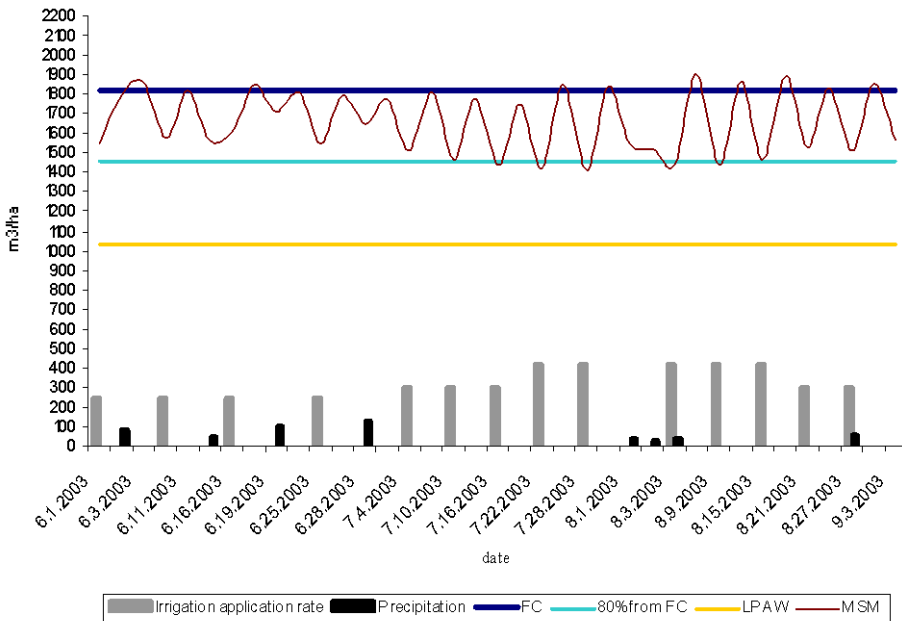


Figure 3. Soil moisture dynamics in treatment B3

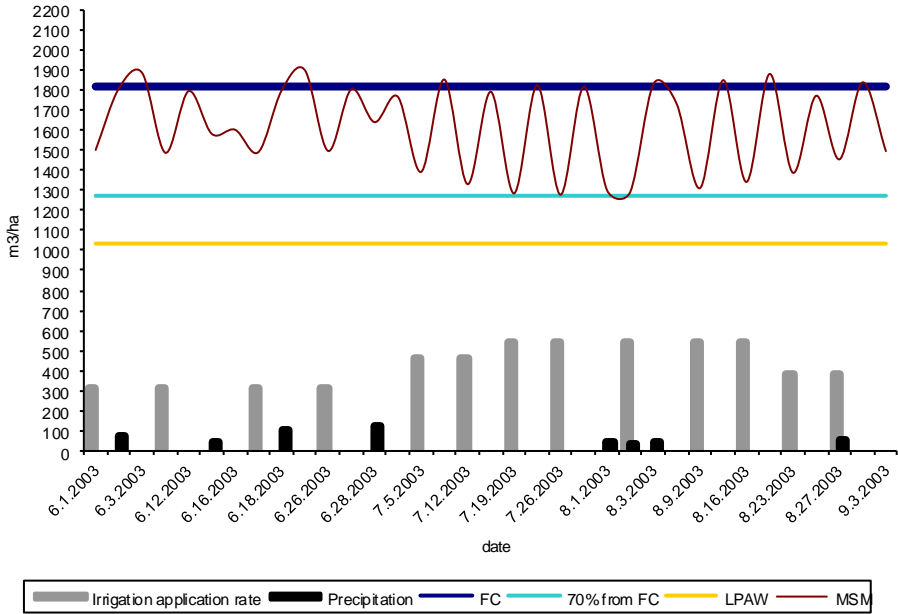


Figure 4. Soil moisture dynamics in treatment Ø2

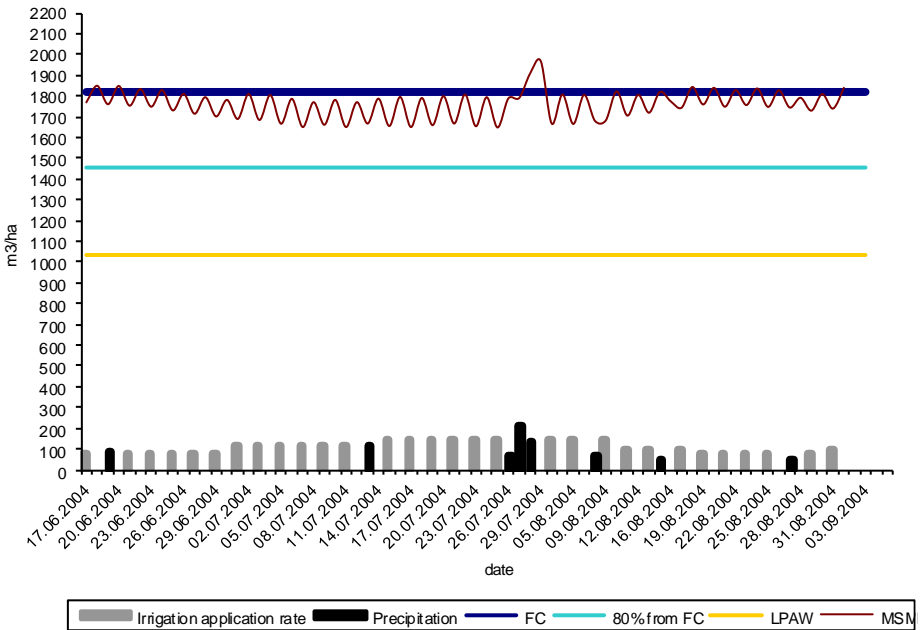


Figure 5. Soil moisture dynamics in treatment B1

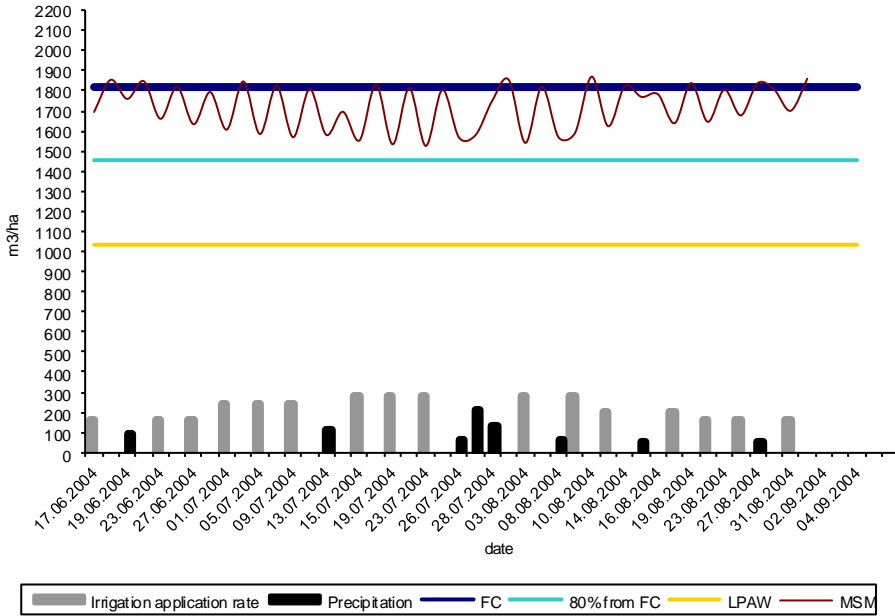


Figure 6. Soil moisture dynamics in treatment B2 and Ø1

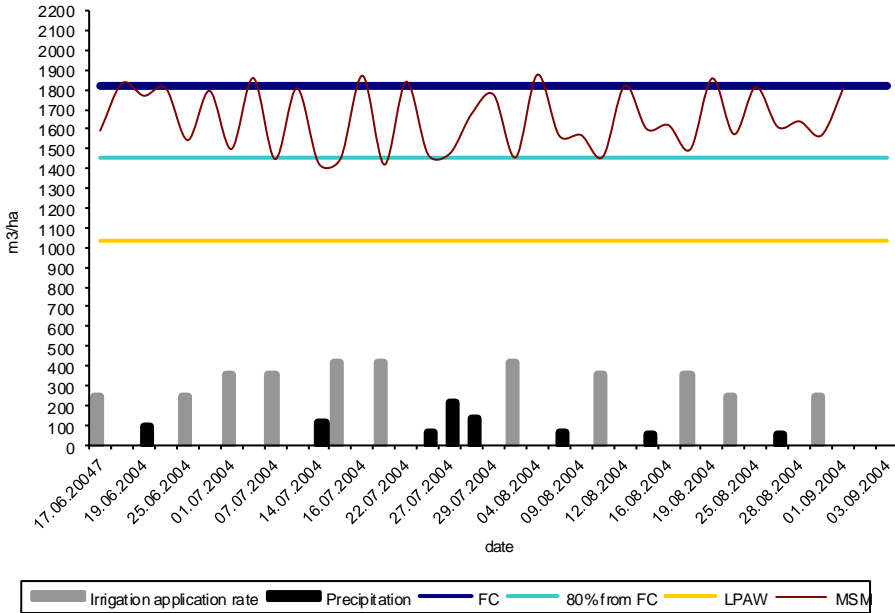


Figure 7. Soil moisture dynamics in treatment B3



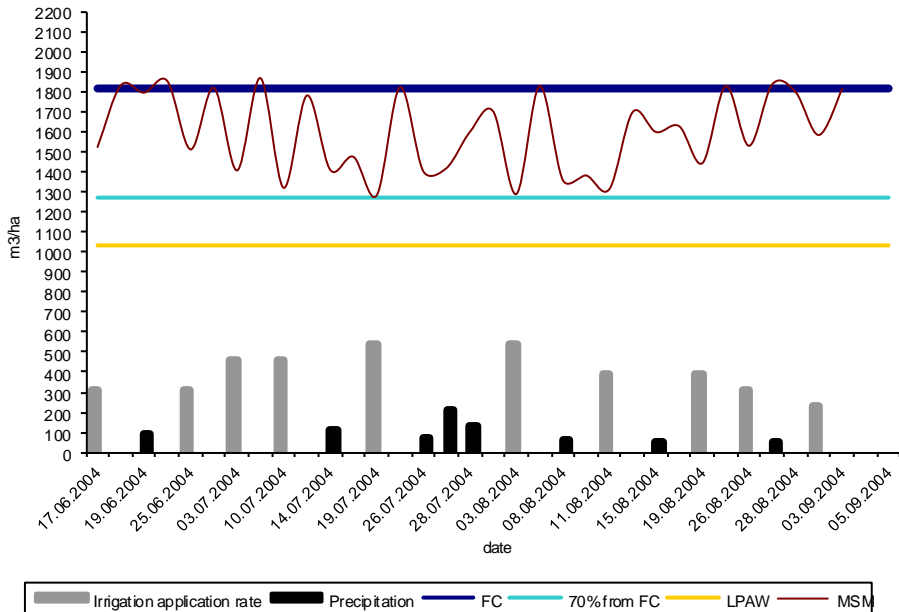


Figure 8. Soil moisture dynamics in treatment Ø2

In the first year, the climate conditions were more favourable for the realization of the experiment compared to the 2<sup>nd</sup> year. Namely, in both years of investigation at the beginning of vegetation there had been frequent rainfall, which allowed for longer maintenance of soil moisture content (MSM) within FC for all treatments. At the beginning of June the 1st year, higher temperatures and less frequent rainfall indicated a need to start the irrigation, whereby the irrigation regime started earlier by 17 days compared to the 2nd year and this was the result of rainfall followed by relatively low temperatures, atypical for this time of year. Despite these differences, figure 1 to 8 show that the best conditions in terms of soil moisture were noted in the treatments B1 and B2, because the MSM did not drop below the lower limit of 80% of FC during the all period of vegetation, which is determined by many authors as a lower limit of easily available water in which tomato does not tolerate stress (Tanaskovik, 2005; Iljovski and Cukaliev 1994; Stanley and Maynard 1990). In treatment B1 the soil moisture content dropped the lowest to 91% of FC in the 1st year, i.e. 90.6% in the 2nd year, while in treatments B2 and Ø1 in both years MSM fluctuations were noted of up to 84% of FC. This positive feature of treatments B1, B2 and Ø1 is in favour of the smaller water losses to evaporation with drip irrigation, which is the result of the proper irrigation regime and interval, i.e. the use of small and precise irrigation application rates. Generally, the treatments B1 and B2, as well as the Ø1, show small fluctuations in MSM despite the high temperatures in most of the vegetation period, which indicates regular and

continuous procurement of water in the most critical periods of growth and development i.e., in the phase of mass blooming and fructification. In addition to this, Vučić (1976) points out that the most critical period for tomato in terms of water is the period between binding and growth of the fruit. The author has noted that since this period is long, and harvesting of tomatoes is successive, it needs several applications of water, but with shorter intervals. The treatment B3, in both years of investigation has shown stronger fluctuations, especially during fructification when the MSM dropped 5 times below the value of 80% of FC in the 1st year, and 4 times in the 2nd year. Such fluctuations in treatment B3 are the result of the irrigation interval, which in this case was six days, and which according to the results proved to be less practical in intensely high temperatures. Figure 4 and 8 show that the MSM dynamics in treatment Ø2, in most of the vegetation period was below 80% of FC i.e., in several occasions it came very close to the limit of 70% of FC. If the manner of applying the fertilizers is not considered, then such powerful stresses in furrow irrigation treatment Ø2, especially during fructification are one of the crucial factors for decreasing the yield compared with drip irrigation treatments. In studies performed with tomato (hybrid Carla) by Tanaskovik (2005) and pepper (2009), the author noted that drip irrigation has high effect due to the maintenance of soil moisture content at optimal level or within the range from 80 to 95% of FC. In this manner, the deficit of soil moisture is constantly compensated and a high effect of irrigation is achieved, while with the interaction of water and mineral nutrition through the irrigation system (drip fertigation) the production potential is also affected.

Table 6. Tomato Yield in the 1st year and the 2nd year, in t/ha

The 1st year					
	B1	B2	B3	Ø1	Ø2
Yield (t/ha)	146,40 <sup>a</sup>	147,80 <sup>a</sup>	137,73 <sup>b</sup>	123,87 <sup>c</sup>	106,93 <sup>d</sup>
Comparison with Ø1 in %	118,2	119,3	111,2	100	
Comparison with Ø2 in %	136,9	138,2	128,8	115,8	100
The 2nd Year					
	B1	B2	B3	Ø1	Ø2
Yield (t/ha)	126,65 <sup>a</sup>	119,72 <sup>a</sup>	108,89 <sup>b</sup>	99,18 <sup>c</sup>	93,61 <sup>cd</sup>
Comparison with Ø1 in %	127,7	120,7	109,8	100	
Comparison with Ø2 in %	135,3	127,9	116,3	105,6	100

\*Values in rows followed by the same letter are not significantly different at the 0.05 probability level

The results of the yield shown in Table 6, show the differences between the years, which can be interpreted with the diametrically opposite climate conditions in the 1st year and the 2nd year and the differences in the start of the irrigation regime (about 17 days), as noted above. Despite this, in the two years of investigation the treatment B1 and B2 showed the highest yields as a result of continuous procurement of water, as well as continuous procurement of nutrients in the short irrigation interval. Both treatments showed statistically significant yield compared with the control treatments. Although the treatment B3 had a

lower average yield of 10.07 t/ha compared to B2 in the 1st year, i.e. of 17.76 t/ha to B1 in the 2nd year, which is a result of the longer irrigation interval, however, it showed a statistically significant yield compared to the control treatment Ø1 and Ø2. Expressed in relative values, the two-year averages of drip fertigation treatments compared to the Ø1 indicate that the B1 realized higher yield by 23.95%, the B2 by 20%, while the B3 by 10.5%, respectively. The comparison made between drip fertigation treatments and the control treatment Ø2, are much more pronounced. Thus, the B1 showed a yield higher by 36.1%, B2 by 33.1%, while the B3 had a higher yield by 22.5%. And the treatment Ø1, as a result of the applied irrigation technique and the irrigation interval showed a yield higher by 10.7% compared with Ø2. The smaller yield in Ø2 in our study is due to the continuous water stress, as observed also in the research by other authors (Dalla Costa and Gianquinto 2002; Burt et al., 1998), as well as the inadequate water and nutrient procurement (Tanaskovik et al., 2014; Wiertz and Lenz 1987). Our results have shown that in addition to the continuous procurement with water, the yields were highly affected by the continuous provision with mineral nutrition by drip fertigation. Yield difference between treatments with identical irrigation frequency of four days (B2 and Ø1) confirms that, if in the growing season portion of the fertilizer is applied through the drip irrigation system (B2); the yield is around 20% higher than that obtained by conventional spreading of similar fertilizer quantity (Ø1). Other authors have noted similar results in their research in tomato crops and other vegetables (Tanaskovik et al., 2011; Halitligil et al. 2002; Zuraiqi et al. 2002; Aleanter et al., 1999; Papadopoulos 1996).

## CONCLUSIONS

During the two years of investigation, the best conditions regarding the soil moisture content, as well as the best yield was demonstrated by the treatments B1 and B2, because the soil moisture did not drop below of 80% of FC during the all period of vegetation, i.e. the plants were continuously provided with easily available water. The treatment B3 showed slightly higher fluctuations in soil moisture content as a result of the irrigation interval. Thus, the B3 showed lower average yield of 10.07 t/ha compared to B2 in 2003, i.e. of 17.76 t/ha to B1 in the second year, but the yield was statistically higher compared with the Ø1 and Ø2, as a result of the simultaneous application of nutrients through the system. Yield difference between treatments with identical irrigation frequency of four days (B2 and Ø1) confirms that, if in the growing season portion of the fertilizer is applied through the drip irrigation system (B2); the yield is around 20% higher than that obtained by conventional spreading of similar fertilizer quantity (Ø1). Most of the time during the vegetation period, the soil moisture content in the control treatment Ø2 was under the 80% of FC. Despite having good irrigation interval of 7 days, such strong stresses, together with the method of applying fertilizers is one of the crucial factors that caused lower yields when compared to the treatments irrigated with the drip irrigation system.

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