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CONTENT

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Gordan S. KARAMAN [1]

NEW DATA OF THE SUBTERRANEAN SPECIES *NIPHARGUS RHODI* S. KARAMAN, 1950 (FAM. NIPHARGIDAE) IN RHODOS ISLAND, GREECE (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 296)

SUMMARY

The partially known species *Niphargus rhodi* S. Karaman, 1950b (Amphipoda, fam. Niphargidae) was described based on one female from the subterranean waters of Rhodos Island ["(spring on the Propheta Mt. (?Elias?)" (? = Profitis Ilias)], and later only mentioned by Pesce & Maggi (1983) for several localities of this island accompanied sometimes with amphipods *Bogidiella longiflagellum* S. Kar. 1959 or *Medigidiella chappuisi* (Ruffo, 1952).

This species, discovered and collected in numerous localities on Rhodos Island by various scientists, is now redescribed and figured, and its variability and relation to other members of genus *Niphargus* of Greece and some adjacent regions is discussed.

Keywords: taxonomy, redescription, *Niphargus rhodi*, Rhodos Island, Greece, subterranean.

INTRODUCTION

The freshwater fauna of Amphipoda in Greece has been studied for long time by various scientist and expedition (C. Bou, S. Karaman, S. Ruffo, A. Vigna-Taglianti, J. Stoch, G. Karaman, etc.) and numerous taxa were discovered and described belonging to various Amphipoda families [Ingolfiellidae, Bogidiellidae, Crangonictidae, Salentinellidae, Gammaridae, Hadziidae, Niphargidae, etc.]. Among them the most numerous taxa belong to the family Niphargidae (genera *Niphargus* Schiödte, 1849, *Exniphargus* G. Karaman, 2016b, *Niphargobatoides* G. Karaman, 2016b*)*, i. e. to the genus *Niphargus* (nearly 15 taxa), almost all highly endemic.

Despite the fact that the subterranean fauna of the family Niphargidae in Greece was studied since 1934, when S. Karaman described some new taxa [*Niphargus adei* from Samothrake island and *N. graecus* from Akrokorinth in continental Greece], this fauna in Greece is still only partially known, and we can expect discovery of other new taxa from this region.

Regarding Rhodos Island (Aegean Sea), S. Karaman described (1950b) new species *Niphargus* rhodi based on one adult female from spring Nimpha on

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

Mt. Propheta. Pesce & Maggi (1983) mentioned this species from several localities of Rhodos, but without any taxonomical data.

Thanks to the deceased Prof. Dr. Jan Stock from Holland, who sent us the samples of genus *Niphargus* collected from various subterranean waters of Rhodos Island, Greece, as well as by some samples sent me by Dr. Hans Malicky from Austria and Dr. Giuseppe Pesce from Italy, we have a possibility to redescribe taxonomically partially known species *Niphargus rhodi* S. Karaman, 1950b, despite the fact that within all numerous samples in hands. not final adult males were found. At the moment, *N. rhodi* is single *Niphargus* taxon known from this island.

MATERIAL AND METHODS

The studied 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; 2012c) regarding the last mandibular palpus article [A= setae on outer face; B= setae on inner face; D= lateral marginal setae; E= distal long setae] and propodus of gnathopods 1 and 2 [S= corner spine; L= lateral slender serrate L-spines; M= facial M-setae; R= subcorner R-spine on inner face]. Terms "setae" and "spines" are used based on its shape, not origin. The study was provided using morphological, ecological and zoogeographical data.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS RHODI S. Karaman, 1950b

Niphargus rhodi, S. Karaman, 1950b, 43, figs. 1-10; G. Karaman, 1972: 7; Pesce & Maggi, 1983: 58; Barnard & Barnard 1983: 694; G. Karaman & Ruffo, 1986: 530.

MATERIAL EXAMINED: **Rhodos Island, Greece**

-688 = "Monte Propheta, spring Nimpha, Rhodos Island, Greece", 2.6.1937, one female 7.0 mm (holotype) (leg. H. Stadler) (incl. 2 slides No. 688/1, 688/2) [? = Profitis Ilias);

S-7385 (No.11). Spring-brook of the river Gaidouras, 1.5 km W of Apollona; slowly running, clear water; pebbles, decomposed leaves; temp.

15.7°C; pH 6.5; Cl 28 mg/l, 12.3; Ca++ 78.5 mg/l; 12.3. 1973, 18 exp. (leg. J. Stock);

No. 4. Stream alongside the road Kallithie-Afantou, 1 km S of Kallithie (16 km S of Rhodos city); slowly running, oligosaprobe to alpha-mesosaprobe; coarse gravel bottom; depth 0-25 cm, width 100-125 cm; pH 6.5-7; Cl 48 mg/l; Ca++ 66.3 mg/l; temp. 16.2°C; 14.3.1973, 3 exp. (leg. J. Stock);

No. 5. Small limnocrene springs on the left bank of the river Loutani, near the mouth of a small affluent named brook of Epta Piges (SW of the village Afantou; slightly thermal (17.4°C); pebbles, loam, decomposed leaves; depth 0-10 cm, width 0-10 cm; pH 6.0; Cl 25 Mg/l; Ca ++68.7 mg/l; 12.3.1973, 5 exp. (leg. J. Stock);

S-5460 (No. 6). The Seven Springs (Epta Piges), in the Loutani river valley, gravel, tree roots, slightly loamy; depth 10-20 cm; width 0-50 cm; temp. 16.8°C; pH 6.0; Cl 24 mg/l; Ca++45.0 mg/l, 10.3. 1973, 5 exp. (leg. J. Stock);

No. 7. Captured well (fountain) on the left bank of the river Loutani, upstream of Epta Piges, dimensions of concrete basin 60x30 cm, depth 10 cm; slowly running, clear; bottom with decomposed leaves and loam; temp. 17.1°C, pH 6.5; Cl 28 mg/l; Ca++53.7 mg., 12.3.1973, one female (leg. J. Stock);

No. 9. Captured well, marked “1951” on the S side of the road Genadion-Vation, 2 km SW of Genadion; dimensions of concrete basin 130x40 cm; depth 0-10 cm; oligosaprobe; bottom muddy with some pebbles; *Chara*; temp. 18.4°C, pH 6.0; Cl 139 mg/l; Ca++43.7 mg/l, 15.3.1973, 9 exp. (leg. J. Stock);

No. 10. Captured well alongside the road Salakos- Embonas, near the side-way to Ag. Issidoros (= 3 km N. of Embonas), dimensions of concrete basin 80x30 cm, depth 30 cm; muddy, stones; alpha- to beta-mesosaprobe; 12.3.1973, 2 exp. (leg. J. Stock);

No. 13. Nameless stream, near small cascade alongside the road Platania-Apollona, just past the side-way to Laerma (= 1 km E of Apollona); slowly running, oligosaprobe, boulders, gravel; *Taxus* leaves; temp. 15.5°C; pH 6.0; Cl 49 mg/l; Ca++133.5 mg/l; 12.3.1973, 25 exp. (leg. J. Stock);

No. 14. Upper course of the river Plati, 0.5 km N of Dimilia (= 36 km SW of Rhodos City); moderately running, oligosaprobe stream; bottom gravel (particles diameter 0.5-5 cm) and some loam; depth 10-40 cm, width 100-150 cm; temp. 15.8°C, pH 6.5-7; Cl 34 mg/l; Ca++77.5 mg/l. 15.3.1973, 8 exp. (leg. J. Stock);

No. 15. Cemented well (fountain) just E of the village Psinthos (alongside the road Psinthos- Archipolis) (= 9.5 km W of the Afantou); slowly running; oligosaprobe; gravel and sand; temp. 18.6°C, pH 6.5; Cl 25 mg/l; Ca++ 77.0 mg/l. 11.3.1973, one female (leg. J. Stock);

No. 16. Cemented well marked “D 1963” N of the road Painthos-Petaloudes (= 24 km SW of Rhodos City); dimensions of basin 800x40 cm; depth 30 cm; slowly to moderately running; oligosaprobe; bottom with one stone, and some filamentous algae; temp. 16.0°C, pH 6.0; Cl 22 mg/l; Ca++ 114 mg/l; 16.3.1973, 25 exp. (leg. J. Stock);

No. 17. Affluent of Plati River system at Petaloudes (= Butterfly Valley, 25 km SW of Rhodos-city; depth 10-40 cm, width 10-150 cm; slowly to moderately running; oligosaprobe; bottom: pebbles and fallen leaves; temp. 15.9°C, pH 6.0; 16.3.1973, one female (leg. J. Stock);

No. 18. Nameless stream SW of Damatria, crossing the road Paradission-Psinthos; depth 0-20 cm (up to 50 cm); width 700 cm; bottom gravel, slightly loamy; temp. 16.5°C; pH 7.0; Cl 50 mg/l; Ca++ 68.5 mg/l. 11.3.1973, 8 exp. (leg. J. Stock);

G-6450= Mandriko, well, June 1981, one exp. (leg. G. Pesce);

S-6451= Tolos, June 1981, one exp. (leg. G. Pesce);

S-6452= airport, Rhodos island, June 1981, one male 5.0 mm, 2 juv. (leg. G. Pesce);

S-6454= Kalavarda, June 1981, 2 females (leg. G. Pesce);

S-6458= Soron, June 1981, one exp. (leg. G. Pesce);

S-6459= "[R-8], Rhodos island", June 1981, one juv. (leg. G. Pesce);

S-6460= "[R-6] Rhodos island", June 1981, one male 5.8 mm (leg. G. Pesce);

R-5= 2 km SW of Laerma, 210 m a.s.l., 6.5.1975, one male 4 mm (leg. H. Malicky);

R-6= 4 km SW of Laerma, 220 m a.s.l., 2 exp. damaged (leg. H. Malicky).

DESCRIPTION

FEMALE 7.5 mm with setose oostegites, from Spring brook of the river Gaidouras (No. 11):

Body moderately slender, metasomal segments 1-3 with 2 stronger and 2 weak dorsoposterior setae (fig. 3F). Urosomal segment 1 on each dorsolateral side with one spine-like seta; urosomal segment 2 on each dorsolateral side with 1-2 spines; urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1-peduncle (fig. 5E).

Epimeral plates 1-3 angular. Epimeral plate 1 along posterior convex margin with 2-3 setae; epimeral plate 2 along posterior straight margin with 4-5 setae (fig. 3F); epimeral plate 3 along posterior straight inclined margin with 4-5 setae. Epimeral plate 2 with 2-3 subventral spines, epimeral plate 3 with 4 subventral spines (fig. 3F).

Head with short rostrum and subrounded lateral cephalic lobes, ventroanterior sinus developed (fig. 1A).

Antenna 1 hardly exceeding half of body (ratio: 45:75); peduncular articles 1-3 progressively shorter (ratio: 53:40:25), scarcely setose (fig. 1B); main flagellum consisting of 17 articles [most of them with one short aesthetasc]. Accessory flagellum 2-articulated, short (fig. 1B). Antenna 2 moderately slender; peduncular article 4 hardly longer than article 5 (ratio: 72:67), each of them with several bunches of short setae; flagellum slender, remarkably longer than last peduncular article and consisting of 8 articles scarcely setose (fig. 1C). Antennal gland cone short (fig. 1C).

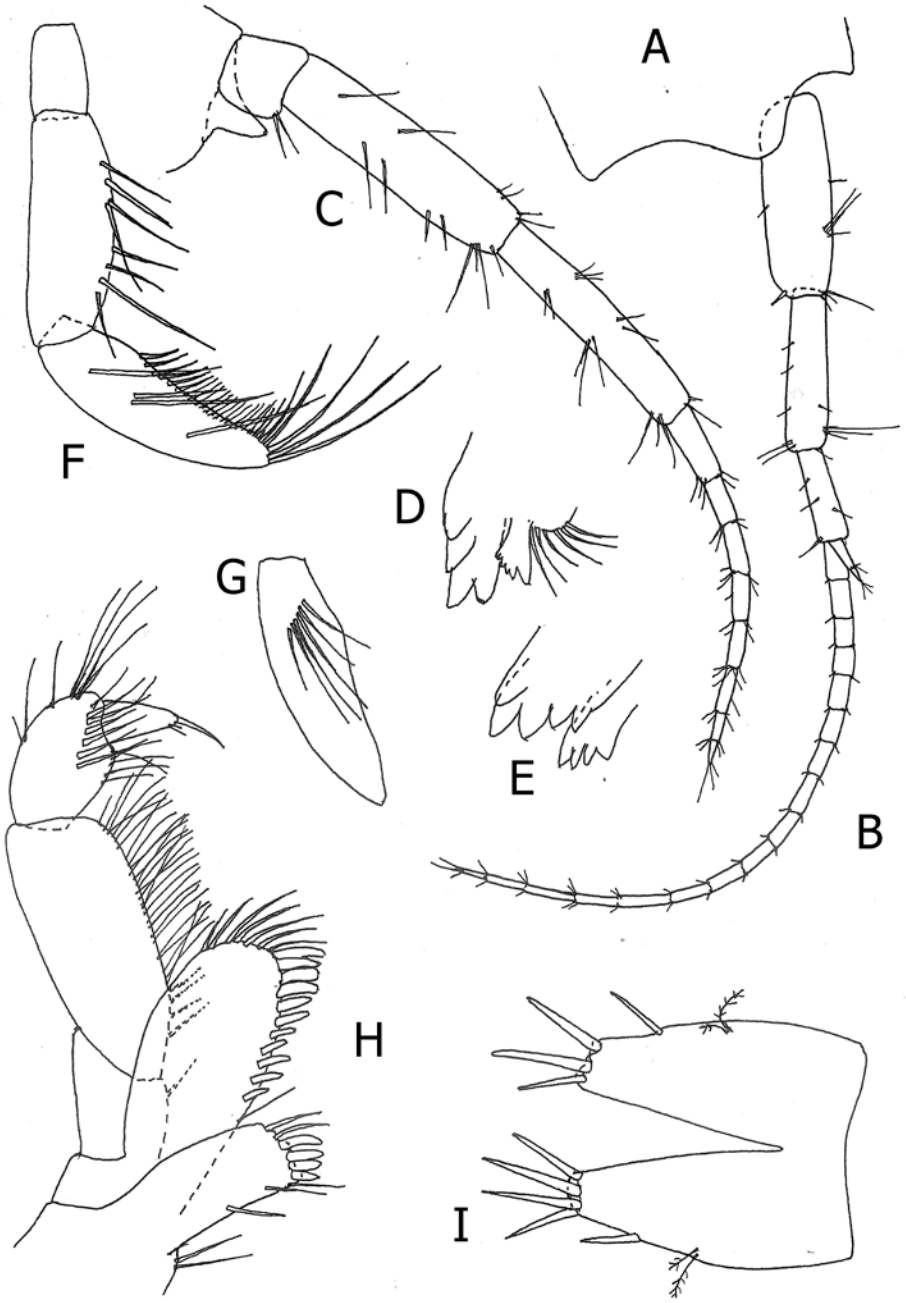

Fig. 1. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, female 7.5 mm: A= head; B= antenna 1; C= antenna 2; D= incisor and lacinia mobilis of left mandible; E= incisor and lacinia mobilis, right mandible; F= mandibular palpus, inner face with facial B-setae; G= mandibular palpus article 3, outer face with facial A-setae; H= maxilliped; I= telson

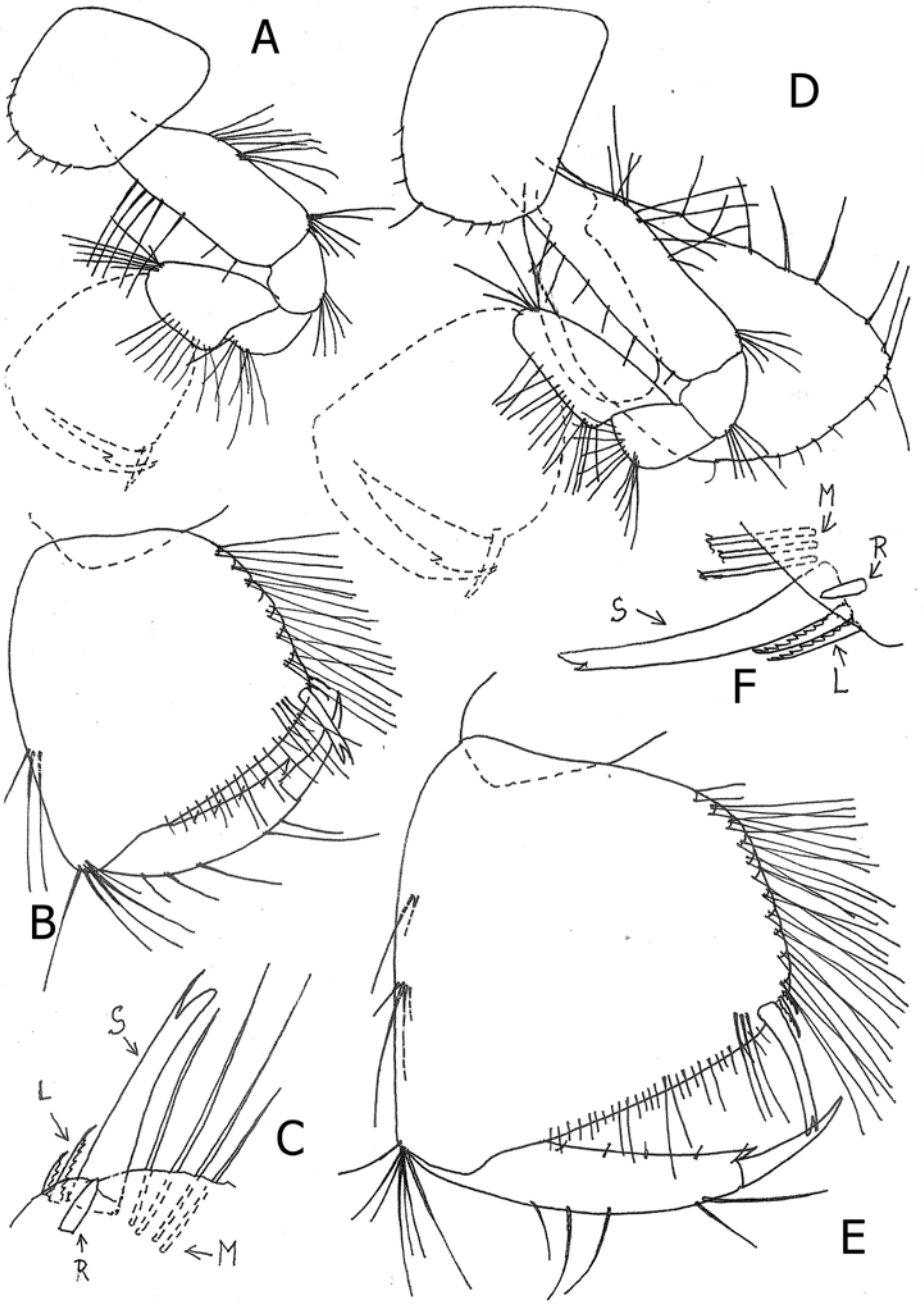

Fig. 2. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, female 7.5 mm: A-B= gnathopod 1, outer face; C= distal tip of gnathopod 1 propodus, inner face [S= corner S-spine; L= slender serrate L-spines; R= subcorner R-spine; M= facial M-setae]; D-E= gnathopod 2, outer face; F= distal tip of gnathopod 2 propodus, inner face [S= corner S-spine; L= slender serrate L-spines; R= subcorner R-spine; M= facial M-setae].

Mouthparts well developed. Labrum much broader than long, with convex anterior margin (fig. 5A). Labium broader than long, with subrounded outer lobes and well developed inner lobes (fig. 5B).

Mandibles with triturative molar. Right mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth and 6 rakers (fig. 1E). Left mandible: incisor with 4 teeth, lacinia mobilis bifurcate, with several teeth, and 6 rakers (fig. 1D). Mandibular palpus article 1 naked, article 2 with 8 strong setae (fig. 1F); article 3 hardly longer than article 2 (ratio: 68:62), subfalciform, provided with nearly 20 marginal D-setae and 5 distal E-setae, on outer face is attached one bunch of 6 A-setae (fig. 1G), on inner face appear 4 B-setae (1-2-1) (fig. 1F).

Maxilla 1: inner plate with 2 unequal setae, outer plate with 7 spines [6 spines with one lateral tooth, one spine with 3 small lateral teeth] palpus 2-articulated, not reaching distal tip of outer plate spines and provided with 6 distal setae (fig. 3A).

Maxilla 2 with subequal both lobes bearing distomarginal setae (fig. 5C).

Maxilliped: inner plate relatively short, with 4 distal spines accompanied by single setae (fig. 1H); outer plate not exceeding half of palpus article 2, with row of nearly 11 strong mesial spines (fig. 1H); palpus article 3 along outer margin with 2 median and one distal bunch of setae; article 4 with 1-2 setae at inner margin near basis of the nail (fig. 1H).

Coxae relatively short. Coxa 1 hardly broader than long (ratio: 45:42), with subrounded ventroanterior corner and bearing nearly 8 short marginal setae (fig. 2A). Coxa 2 longer than broad (ratio: 58:45), along ventral margin appear 9 setae (fig. 2D). Coxa 3 longer than broad (ratio: 66:58), bearing nearly 8 marginal setae (fig. 3B). Coxa 4 nearly as long as broad, with concave posterior margin, along margin appear nearly 9 setae, ventroposterior lobe absent (fig. 3D).

Coxae 5-7 are short. Coxa 5 bilobed, much broader than long (ratio: 63:44), anterior subrounded lobe poorly shorter than coxa 4, provided with 2-3 setae only (fig. 4A); article above coxa with one short seta at ventroposterior margin (fig. 4A). Coxa 6 smaller than 5, bilobed, broader than long (ratio: 52:31) (fig. 4C); article above coxa with one short seta at ventroposterior margin (fig. 4C). Coxa 7 short, entire, much broader than long (ratio: 53:22); article above coxa with one short seta at ventroposterior margin (fig. 4E).

Gnathopods 1 and 2 of moderate size. Gnathopod 1: article 2 along both margins with long setae; article 3 at posterior margin with one bunch of setae (fig. 2A). Article 5 is shorter than propodus (ratio: 36:47), along anterior margin with one distal bunch of setae (fig. 2A). Propodus trapezoid, nearly as large as corresponding coxa, slightly longer than broad (ratio: 87:80), along posterior margin with 7 transverse rows of setae (fig. 2B); palm inclined slightly less than half of propodus-length, convex, defined on outer face by one corner S-spine accompanied laterally by 2 L-spines and 4 long facial M-setae (fig. 2C), 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 5 short setae (fig. 2B).

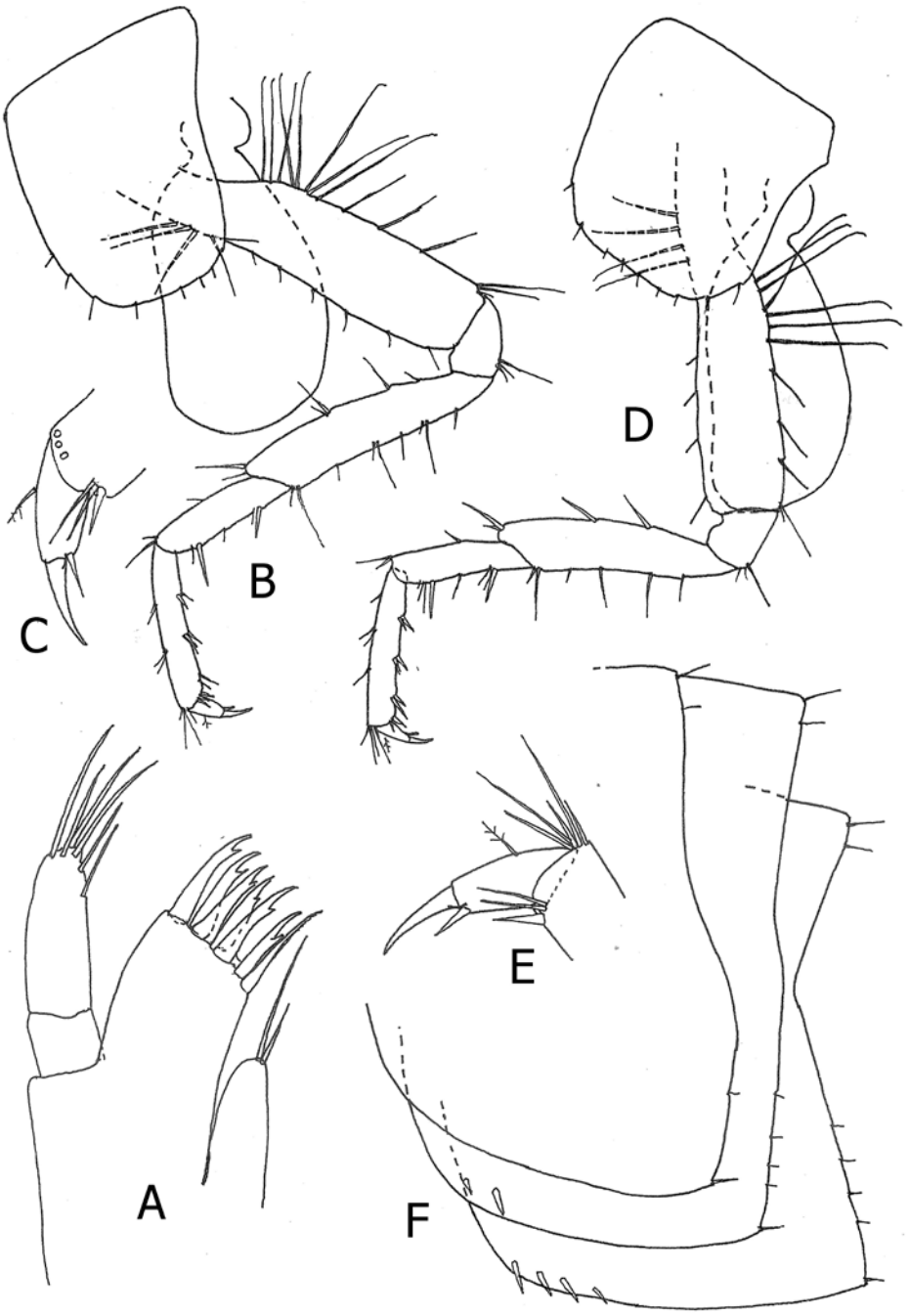

Fig. 3. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, female 7.5 mm: A= maxilla 1; B-C= pereopod 3; D-E= pereopod 4; F= epimeral plates 1-3.

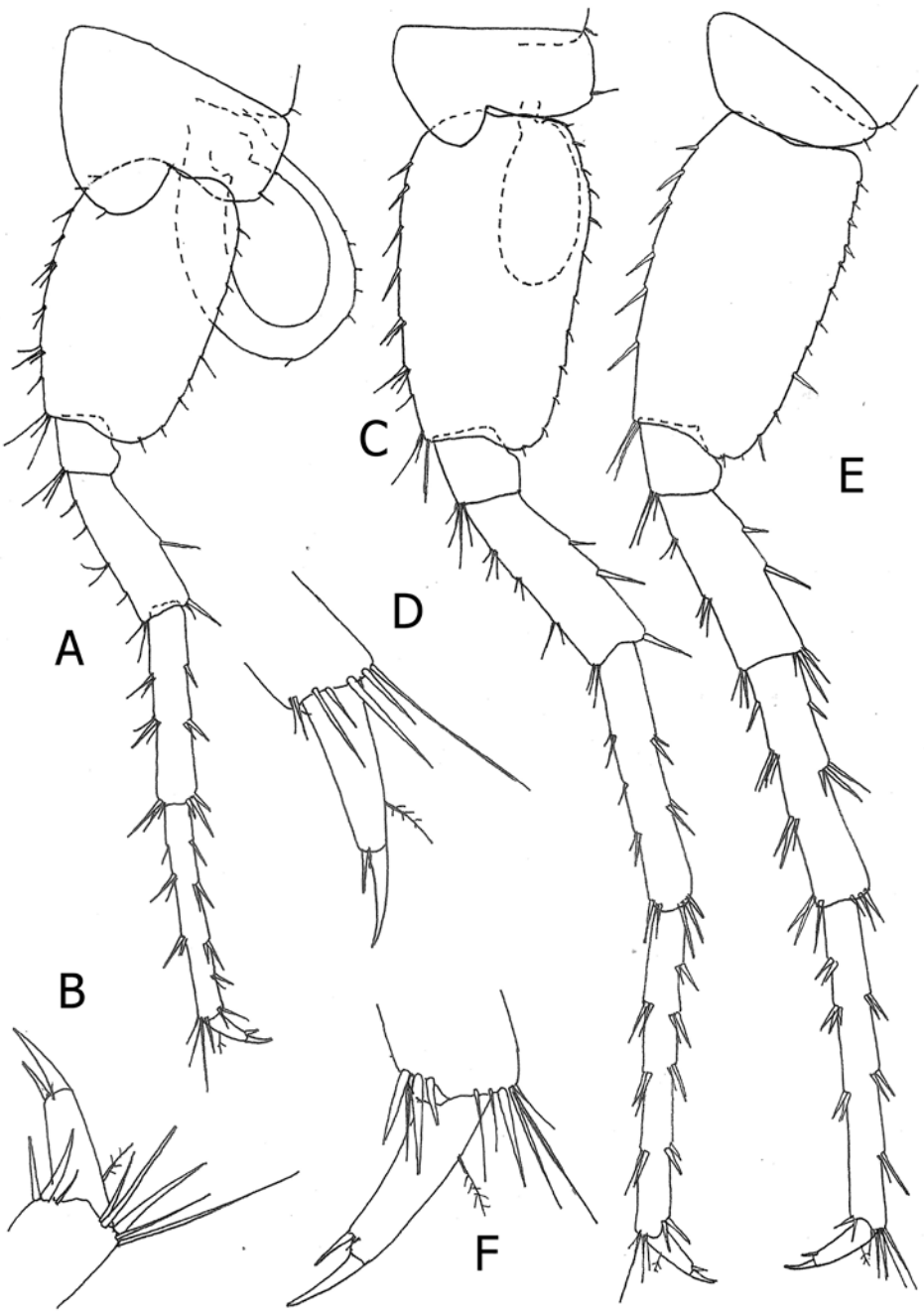

Fig. 4. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, female 7.5 mm: A-B= pereopod 5; C-D= pereopod 6; E-F= pereopod 7.

Gnathopod 2 remarkably larger than gnathopod 1 and corresponding coxa 2 (fig. 2D); article 2 along anterior margin with row of shorter setae, along posterior margin with row of long proximal setae and short distal setae; article 3 at posterior margin with one bunch of setae; article 5 shorter than propodus (ratio: 48:60), along anterior margin with distal bunch of setae. Propodus trapezoid, remarkably larger than that of gnathopod 1, poorly longer than broad (ratio: 112:106), along posterior margin with 11 transverse rows of setae (fig. 2E); palm slightly convex, inclined nearly half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 2 L-spines (close to S-spine) and 3 long facial M-setae, on inner face by one subcorner R-spine (fig. 2F). Dactylus reaching posterior margin of propodus, along outer margin with 5 median setae, along inner margin with 5 short setae (fig. 2E).

Pereopods 3 and 4 moderately slender, scarcely setose. Pereopod 3: article 2 along anterior margin with several long proximal setae and several short distal setae (fig. 3B), along posterior margin setae are longer than these of anterior margin. Articles 4-6 of unequal length (ratio: 61:35:44); article 4 along posterior margin with 5-6 single or paired setae (the longest seta exceeding diameter of article itself); articles 5 and 6 along posterior margin with single or paired short spines. Dactylus short and strong, much shorter than article 6 (ratio: 17:44), with one strong spine at inner margin and one median plumose seta at outer margin (fig. 3C), nail shorter than pedestal (ratio: 24:33).

Pereopod 4: article 2 pilosity is similar to that of pereopod 3; articles 4-6 of unequal length (ratio: 55:34:43); article 4 at anterior margin with 3 single slender spines, along posterior margin with 5 single or paired setae (the longest setae reaching diameter of article itself); article 5 at posterior margin with single spines and setae; article 6 along posterior margin with 4 groups of short spines (fig. 3D). Dactylus short and strong, at inner margin with one spine near basis of the nail, along outer margin with one median plumose seta; nail slightly shorter than pedestal (ratio: 25:35) (fig. 3E).

Pereopods 5-7 are moderately slender. Pereopod 5 is remarkably shorter than pereopods 6 and 7, with article 2 longer than broad (ratio: 72:46), along anterior margin with 9 groups of setae, along posterior convex margin with nearly 10 seta of unequal length (fig. 4A), ventroposterior lobe not fully developed, distoanterior corner is not produced. Articles 4-6 of unequal length (ratio: 45:51:57); article 4 at anterior margin with row of short setae, along posterior margin with one median and one distal spine; articles 5 and 6 along both margins with groups of spines and single short setae. Article 2 is longer than article 6 (ratio:72:57). Dactylus is short and strong, much shorter than article 6 (ratio: 18:57), at inner margin with one strong spine, along outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 22:40) (fig. 4B).

Pereopod 6: article 2 longer than broad (ratio: 86:50), along anterior margin with 8 groups of strong setae, along posterior poorly convex margin with row of nearly 12 setae, ventroposterior lobe shallow (fig. 4C). Articles 4-6 of unequal length (ratio: 55:69:87), article 4 along anterior margin with bunches of

setae, along posterior margin with 3 spines; articles 5-6 along both margins with short spines; article 2 nearly as long as article 6. Dactylus strong and short, much shorter than article 6 (ratio: 23:87), along inner margin with one strong spine near basis of the nail, along outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 26:44).

Pereopod 7: article 2 is much longer than broad (ratio: 88:48), along anterior margin with row of 6-7 spine-like setae, along posterior poorly convex margin with nearly 11 setae and 2 spine-like setae (fig. 4E), ventroposterior lobe shallow. Articles 4-6 of unequal length (ratio: 50:65:86); article 4 along anterior margin with 3 bunches of spines and setae, along posterior margin with 3 groups of spines; articles 5 and 6 along anterior and posterior margin with bunches of spines usually shorter than diameter of articles themselves. Article 6 is almost as long as article 2 (ratio: 86:88), much longer than dactylus (ratio: 86:27). Dactylus strong, along inner margin with one strong spine near basis of the nail, along outer margin with one median plumose seta (fig. 4F); nail is shorter than pedestal (ratio: 55:26).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 along anterior margin with 5 unequal setae; peduncle of pleopod 2 is naked; peduncle of pleopod 3 along posterior margin with 3 strong setae (fig. 7F, G, H).

Uropod 1: peduncle with dorsoexternal row of strong spines and dorsointernal row of spine-like setae (except distal spine) (fig. 5E). Outer ramus as long as inner ramus but shorter than peduncle (fig. 5E), along lateral margins appear 3 pairs of spines accompanied by 2 bunches of simple setae, at the tip are attached 4-5 unequal distal spines. Inner ramus along margins with 3 spines and one bunch of simple setae, at tip appear 4-5 short spines.

Uropod 2: both rami of the same length, bearing several lateral and distal spines, setae are absent (fig. 5D).

Uropod 3 long and slender: peduncle much longer than broad (ratio: 38:24), with several distal short spines (fig. 5F); inner ramus shorter than peduncle, scale-like, with 3 distal spines. Outer ramus 2-articulated, first article along both margins with 7 bunches of short spines; 1-2 plumose setae appears along inner margin (fig. 5F). Second article much shorter than first one (ratio: 40:140), bearing along both lateral margins 3-4 groups of simple setae (fig. 5F).

Telson slightly longer than broad (ratio: 75:66), rather gaping, incised nearly ¾ of telson-length; each lobe with 3-4 distal and one outer marginal spine (fig. 1 I); a pair of short plumose setae appear at outer margin near the middle of each lobe.

Coxal gills on gnathopod 2, pereopods 3 and 4 are ovoid, nearly reaching distal tip of corresponding article 2 (figs. 2D, 3B, D); coxal gills on pereopods 5 and 6 are shorter (fig. 4A, C).

Oostegites very broad, appear on gnathopod 2 and pereopods 3-5, provided with long and short marginal setae (figs. 2D, 4A).

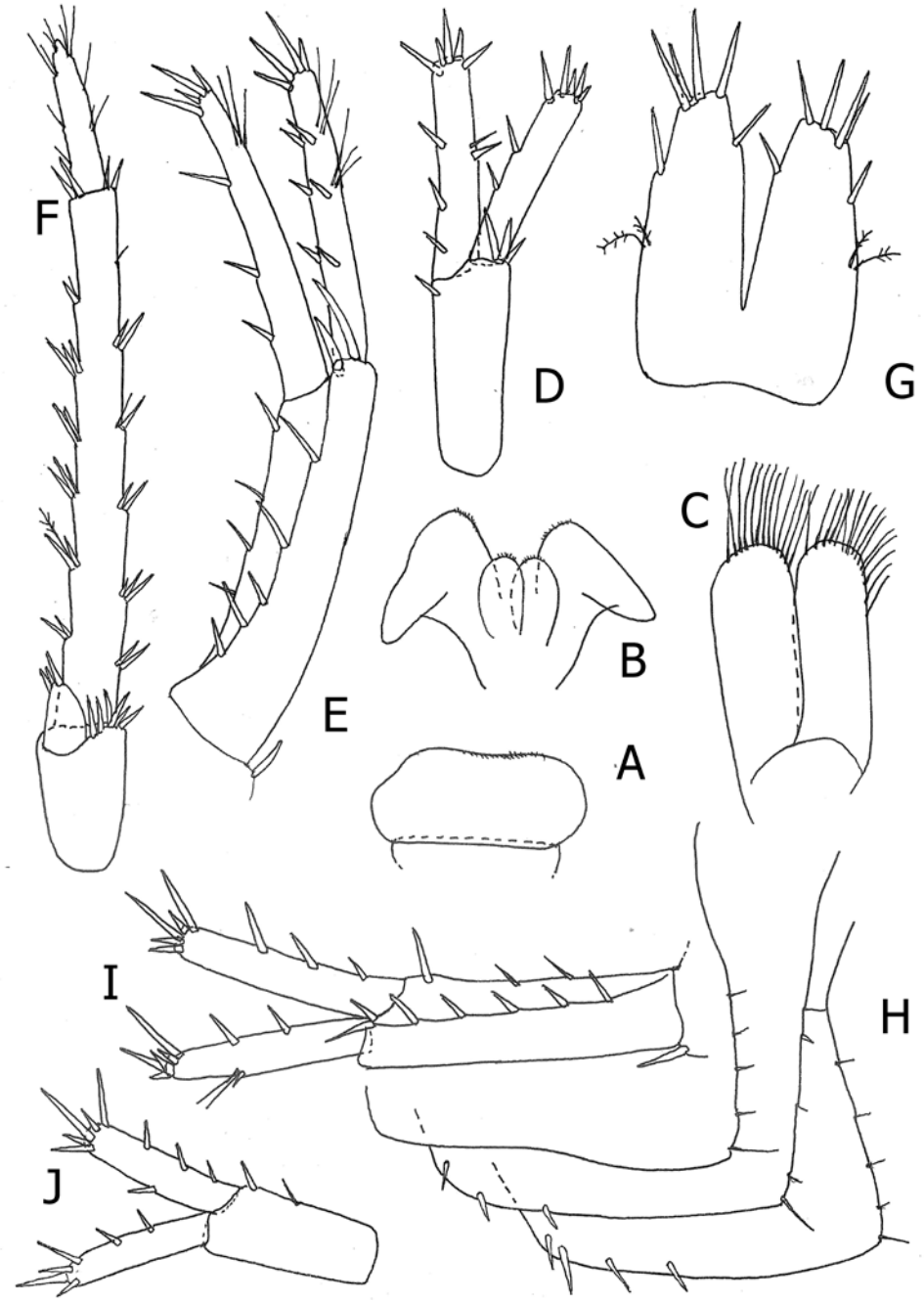

Fig. 5. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, female 7.5 mm: A= labrum; B= labium; C= maxilla 2; D= uropod 2; E= uropod 1; F= uropod 3. G= female 5.8 mm, telson.
Male 5.3 mm: H= epimeral plates 1-3; I= uropod 1; J= uropod 2

MALE 5.3 mm (probably not quite adult): is very similar to adult females.

Metasomal segments 1-3 along dorsoposterior margin with 2-4 short setae; urosomal segment 1 with one seta on each dorsolateral side, urosomal segment 2 on each dorsolateral side with 2 spines, urosomal segment 3 naked.

Epimeral plate 1 angular, with poorly concave ventral margin and poorly convex posterior margin bearing several posterior setae (fig. 5H); epimeral plate 2 angular, with strait posterior margin bearing several setae; epimeral plate 3 angular with inclined posterior margin bearing several setae; epimeral plate 2 is provided with 3 subventral spines, epimeral plate 3 is provided with 4 subventral spines (fig. 5H).

Head and antennae 1-2 like these in female. Antenna 1 hardly exceeding half of the body, consisting of 21 articles (most of them with one aesthetasc); accessory flagellum short, 2-articulated. Flagellum of antenna 2 longer than last peduncular article and consisting of 7 articles.

Mouthparts like these in female. Mandibular palpus article 2 with 6-7 setae; palpus article 3 longer than article 2, falciform, provided with 5 A-setae, 4-5 B-setae, nearly 17 D-setae and 4 E-setae.

Maxilla 1 inner plate with 2 setae, outer plate with 7 spines (6 with one lateral tooth, one spine with 3-4 lateral teeth); palpus not reaching distal tip of outer plate spines and bearing 5-6 setae. Maxilla 2 with subequal both lobes bearing marginal setae only. Maxilliped inner plate reaching outer tip of palpus article 1, bearing 5 pointed teeth, outer plate with 9-10 spines; palpus article 3 along outer margin with 1-2 median and one distal group of setae, article 4 at inner margin with 2 setae near basis of the nail.

Coxa 1 hardly broader than long (ratio: 44:40), with subrounded ventroanterior margin and provided with 5 setae (fig. 6A). Coxa 2 longer than broad (ratio: 56:52) (fig. 6B). Coxa 3 longer than broad (ratio: 62:52) (fig. 6C). Coxa 4 hardly longer than broad (ratio: 56:52) (fig. 6D. Coxae 5-7 short. Coxa 5 is broader than long (ratio: 52:32) (fig. 6E). Coxa 6 is broader than long (ratio: 45:26) (fig. 6F). Coxa 7 shallow, entire, broader than long (ratio: 43:18) (fig. 6G).

Gnathopods 1-2 with articles 2-5 like these in female. Gnathopod 1 propodus trapezoid, longer than broad (ratio: 77:70), along posterior margin with 4 transverse rows of setae (fig. 7A); palm inclined slightly less than half of propodus-length, defined on outer face by 1 S-spine, 2 lateral slender L-spines and 3 facial M-setae (fig. 7A), on inner face by one R-spine; dactylus reaching posterior margin and provided along outer face with 4 median setae.

Gnathopod 2 propodus remarkably larger than that of gnathopod 1, trapezoid, slightly longer than broad (ratio: 92:84), along posterior margin with 7 transverse groups of setae; palm slightly convex, inclined slightly less than half of propodus-length, defined on outer face by 1 corner S-spine, accompanied laterally by 2 L-spines and 3 facial M-setae (fig. 7B), along inner face by one R-spine; dactylus reaching posterior margin of propodus and along outer margin provided with 4 median setae (fig. 7B).

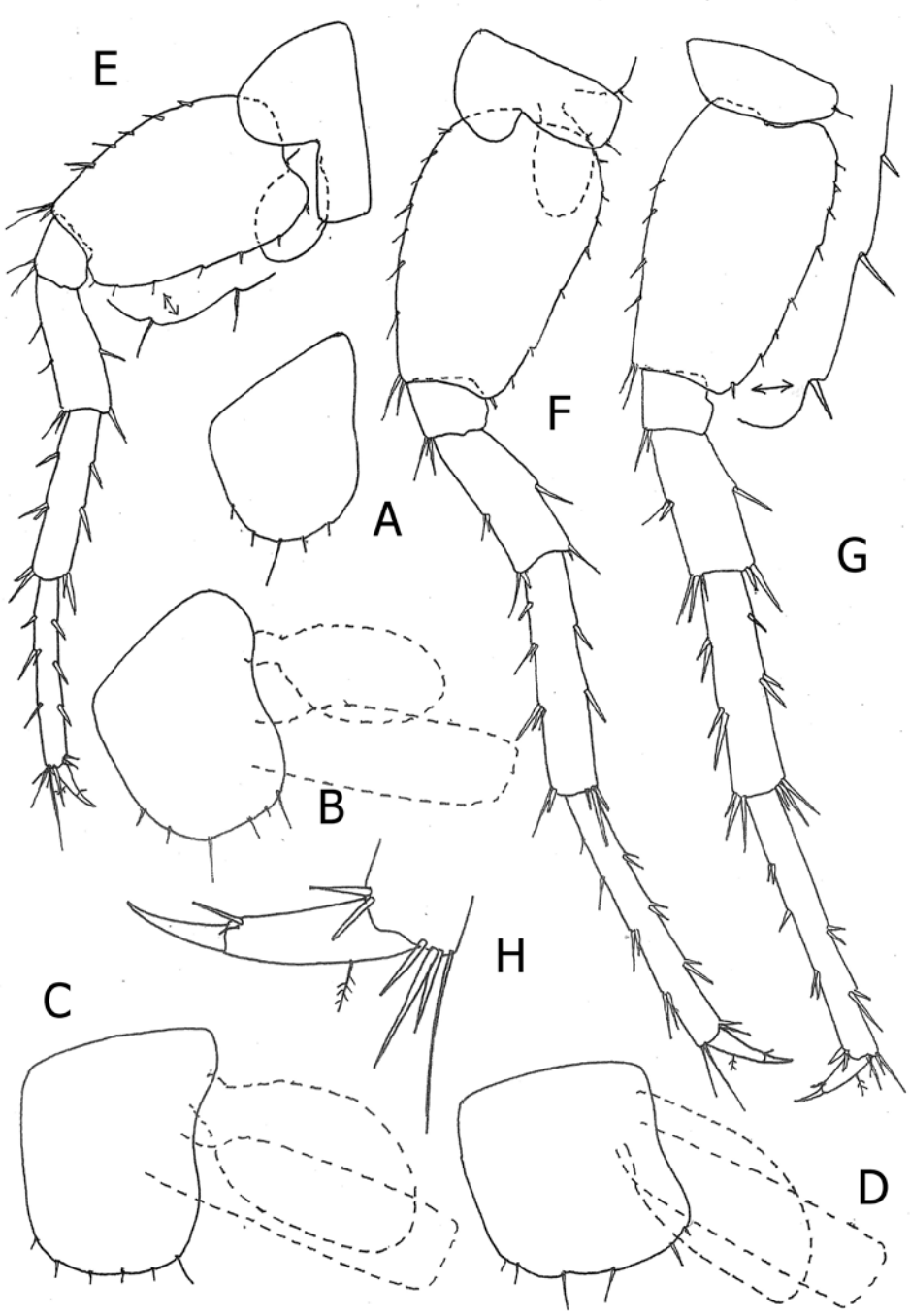

Fig. 6. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, male 5.3 mm: A= coxa 1; B= coxa 2; C= coxa 3; D= coxa 4; E= pereopod 5; F= pereopod 6; G-H= pereopod 7.

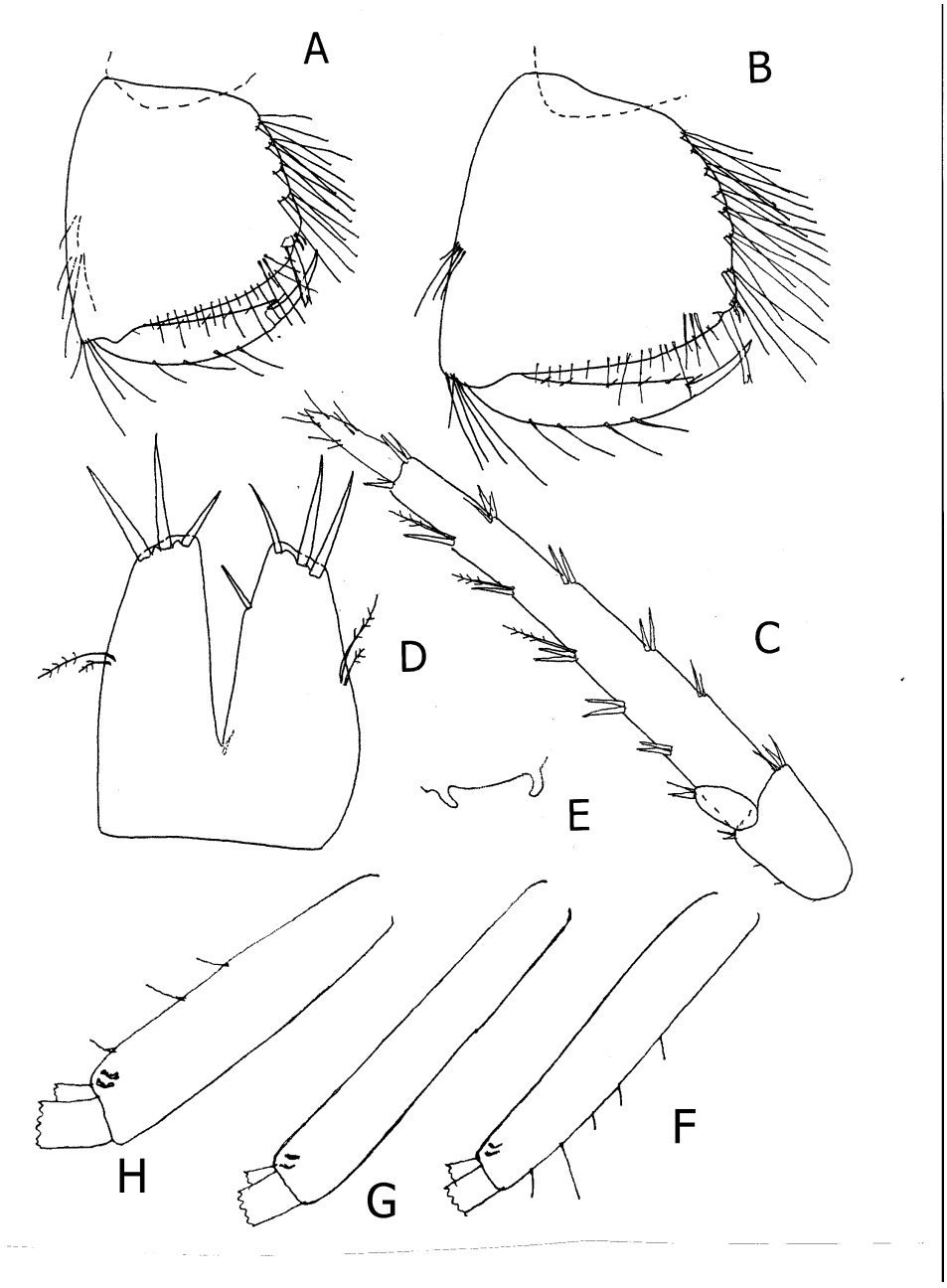

Fig. 7. *Niphargus rhodi* S. Karaman, 1950, Gaidouras, Rhodos island, male 5.3 mm: A= propodus of gnathopod 1, outer face; B= propodus of gnathopod 2, outer face; C= uropod 3; D= telson; E= ventral sexual tubercle on last mesosomal segment. **Female 7.5 mm**.: F= peduncle of pleopod 1; G= peduncle of pleopod 2; H= peduncle of pleopod 3.

Pereopods 3-4 like these in female, with dactylus bearing one spine or spine-like-like seta at inner margin near basis of the nail.

Pereopods 5-7 moderately slender. Pereopod 5 remarkably shorter than pereopods 6 or 7. Article 2 dilated, longer than broad (ratio: 62:43), anterior margin with row of spine-like setae (fig. 6E), posterior poorly convex margin is provided with nearly 7 setae (fig. 6E), ventroposterior lobe not developed; articles 4-6 of unequal length (ratio: 37:46:50, along both margins mainly with short spines. Article 6 is shorter than article 2 (ratio: 50:62); dactylus slender, much shorter than article 6 (ratio: 15:50), at inner margin with one spine-like seta near basis of the nail.

Pereopod 6 with article 2 longer than broad (ratio: 78:49), along anterior margin with 8-9 spine-like setae, along posterior margin with 6 setae and 2 short spines, ventroposterior corner not developed (fig. 6F); articles 4-6 of unequal length (ratio: 43:63:75), articles along both margins with spines (fig. 6F). Article 2 slightly longer than article 6 (ratio: 78:75); dactylus moderately slender, much shorter than article 6 (ratio: 23:75), at inner margin with one spine-like seta near basis of the nail and one median plumose seta at outer margin.

Pereopod 7: article 2 is longer than broad (ratio: 76:47), along anterior margin with several spine-like setae, along posterior convex margin with 8-9 short spine-like setae and setae (fig. 6G), ventroposterior lobe indistinct. Articles 4-6 of unequal length (ratio: 41:60:76), along both margins with bunches of spines (fig. 6G). Article 6 is as long as article 2. Dactylus is much shorter than article 6 (ratio:24:76), at inner margin with one slender spine near basis of the nail, along outer margin with one median plumose seta (fig. 6H), nail shorter than pedestal (ratio: 28:53).

Pleopods 1-3 with 2 retinacula each; pilosity of pleopod`s peduncles like that in female.

Uropod 1: peduncle longer than rami, with dorsoexternal row of spines and dorsointernal row of spine-like setae (except distal spine), distal tubercle absent (fig. 5 I); rami of equal length, bearing several lateral and 5 distal spines; outer ramus with one median group of simple setae only (fig. 5 I).

Uropod 2: rami of equal length, provided with lateral and distal spines, simple setae absent (fig. 5J).

Uropod 3 like that in female: peduncle short, with several distal spines; inner ramus short, scale-like, with one distal spine and seta (fig. 7C); outer ramus 2-articulated: first article long, along outer margin with 5 bunches of short spines, along inner (=mesial) margin with 6 bunches of short spines mixed often with one longer plumose seta (fig. 7C); distal article much shorter than first one (ratio: 34:142), provided with several short simple setae along both margins.

Telson slightly longer than broad (ratio: 88:78), incised slightly over 2/3 of telson-length; each lobe is provided with 3 distal spines and 0-1 spine at mesial margin (fig. 7D); a pair of unequal plumose setae is attached near the external side of each lobe.

Coxal gills on gnathopod 2 and pereopods 3-4 are much longer than these of pereopods 5-6 (figs. 6B-F).

Males of 5.8 mm like these of 5.3 mm.

VARIABILITY.

All our specimens in hands (females up to 7.5 mm and males up to 5.8 mm) agree mainly with description of holotype (female 7.0 mm) of Stanko Karaman (1950b); non quite adult males in hands (up to 5.8 mm long) are similar to the females, including uropods 1-3 and gnathopods.

Lobes of telson are provided with 3-4 distal spines and 0-2 spines at dorsolateral and/or at mesial margin (figs. 1 I, 5G, 7D).

Basipodit (article 2) of pereopod 7 along posterior margin often with short small spines and setae. Inner plate of maxilliped is provided with 4-5 distal spines. Peduncle of uropod 1 is with dorsointernal row of spine-like setae or spines.

The stable characters: short distal article of uropod 3 outer ramus more than twice longer than diameter of first article, equal rami of uropod 1, basipodit of pereopods 5-7 without lobe, dactylus of pereopods with one spine or spine-like seta at inner margin; epimeral plates angular or obtusely angular; telson with 3-4 distal and 0-2 lateral and/or mesial spines, facial spines absent; propodus of gnathopods 1 and 2 with L-spines sitting laterally of corner S-spine; number of facial M-setae is low (3-4 M-setae). Propodus of gnathopod 1 in males with 4-5 transverse rows of setae. Dactylus of gnathopods 1 and 2 is provided with low number (3-5) single median setae along outer margin.

HOLOTYPE: female 7.0 mm, -688 (incl. 2 slides No. 688/1, 688/2); holotype is deposited in Karaman`s Collection in Podgorica, Montenegro.

LOCUS TYPICUS: Spring Nimpha on Propheta Mt. (Eliasberg?) Mt., Rhodos Island, Greece.

DISTRIBUTION. Known from Rhodos Island only (fig. 8).

LOCALITIES CITED ON RHODOS ISLAND:

S. Karaman (1950b): Spring Nimpha on Propheta Mt. (? Elias Mt., Profitis Ilias);

Pesce & Maggi (1983): Road Rodi-Kamiros (near Tolos); road Fane-Kalavarda; road Kamiros-Mandriko; road Petaloudes-Rodi (near Damatria); Kremasti, airport; Harakion (?=Haraki)].

Present work: see sub “Material examined”.

REMARKS AND AFFINITY

Niphargus rhodi is the single known *Niphargus* taxon from Rhodos island, and its closer relation to other taxa of genus *Niphargus* is difficult to establish without the knowledge of adult males. As the ovigerous females are observed already on size of 6.0 mm, and males in hands of 5.8 mm, the adult males are

probably with the similar shortened second article of uropod 3 outer ramus and equal rami of uropod 1.

Fig. 8. Distribution of *Niphargus rhodi* S. Karaman, 1950 in Rhodos Island, Greece (black circles).

Among known *Niphargus* species from Greece, provided with a row of setae along outer margin of gnathopods 1-2 dactylus, there are several taxa with uropod 1 rami of equal length (*N. rhodi*, *N. lindbergi*, *N. lourensis*, *N. impexus*, *N. koukourasi)*.

N. impexus G. Karaman 2016a, described from Creta Island is rather similar to *N. rhodi*, but females of *N. impexus* differ from these of *N. rhodi* by higher number of setae on maxilla 1 inner plate, by low number of spines on maxilliped inner plate as well as by long uropod 3 outer ramus article 2 in all males, etc.

N. koukourasi, Ntakis et al. 1915, described from Springs of Louros River, Vouliasta, Ioannina (continental Greece) differs from *N. rhodi* by highly

spiniferous telson provided with facial spines, by higher number of setae on maxilla 1 inner plate, etc.

Niphargus lourensis Fiser et al. 2006, described from Spring of Louros River, Vouliasta, Ionannina, continental Greece, differs by shorter and more spiniferous outer ramus of uropod 3 having very short distal article, by telson with long distal spines only, etc.

Niphargus lindbergi S. Karaman 1956, described from Cave Draconera (Attique), continental Greece, differs by elevated number of retinacula on pleopods 1-3, etc.

The recently discovery of numerous *Niphargus* taxa by various scientists in Turkey [*Niphargus anatolicus* S. Karaman, 1950a from Emirgan, N of Istanbul; *N. religious* G. Karaman 2012a from Uragavaz Gecidi, Ballidag (Kastamonu), 1350 m. a.s.l., etc.], from Iran [*Niphargus hakani* Esmaeili-Rineh et al. 2017 from Kheder-Goli spring, Razan city, Hamedan Province, etc.], and Iraq [*Niphargus nadarini iraquensis* G. Karaman, 2012b from Haditha (El Hadithah), etc.] show the large distribution of genus *Niphargus* in entire SE Europe, Asia Minor and Near East, and the further investigations of this genus in these regions will help to understand the real taxonomical position of taxa from Greece and adjacent regions.

CONCLUSIONS

Niphargus rhodi S. Karaman, 1950, known from Rhodos island (Greece) only, is redescribed and figured based on numerous localities from Rhodos island (adult females up to 7.5 mm and non quite adult males of up to 5.8 mm.). The males in hands are similar to females, including uropods and gnathopods. Within nearly 15 known *Niphargus* taxa from Greece, *N. rhodi* is well distinguished by various taxonomical characters (uropods 1-3, telson, gnathopods, mouthparts, etc). The exact position of *N. rhodi* within genus *Niphargus* is not possible to determine without knowledge of final adult males.

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I am indebted to Prof. Dr. Giuseppe Pesce from the University of Aquilla (Italy), to Prof. Dr. Hans Malicky from Lunz (Austria) and to the deceased Prof. Dr. Jan Stock from Amsterdam (Holland) for the loan of part of material and data used in this study.

REFERENCES

Barnard, J.L & Barnard, C.M. 1983. Freshwater amphipods of the World. I. Evolutionary patterns. II. Handbook and bibliography.- *Hayfield Associates: Mt. Vernon*, Virginia, 1983, pp. XIX +849 pages, 50 figs., 7 graphs, 98 maps, 12 tables.

Esmaeili-Rineh, S., Mirghaffari, S.A. & Sharifi, M. 2017. The description of a new species of *Niphargus* from Iran based on morphological and molecular data.- *Subterranean Biology* 22: 43–58.

Fišer, C., Trontelj, P. & Sket, B. 2006. Phylogenetic analysis of the *Niphargus orcinus* species-aggregate (Crustacea: Amphipoda: Niphargidae) with description of new taxa.- *Journal of Natural History* 40 (41-43): 2265-2315, 23 figs, 1 pl.

Karaman, G. 1969. XXVII Beitrag zur Kenntnis der Amphipoden. Arten der Genera *Echinogammarus* Stebb. und *Chaetogammarus* Mart. aus der jugoslawischer Adriaküste. *Glasnik Republičkog zavoda za zaštitu prirode i Prirodnjačke zbirke u Titogradu*, 2, 59–84, 51 figs.

Karaman, G. 1972. Le probleme du Genre *Niphargus* en Yougoslavie.- Actes du Ier Colloque International sur le genre *Niphargus*-Verona, 15-19 Aprile 1969, *Museo Civico di Storia Naturale, Verona, Memorie fuori serie*, 5: 1-10.

Karaman, G. & Ruffo, S. 1986. Amphipoda: *Niphargus*-Group (Niphargidae sensu Bousfield, 1982), in: Botosaneanu, L. (edit.): *Stygofauna Mundi, A Faunistic, Distributional, and Ecological Synthesis of the World Fauna inhabiting Subterranean Warers (including the Marine Interstitial*), Leiden, E. J. Brill/ Dr. W. Backhuys, pp. 514-534.

Karaman, G. 2012a. New species *Niphargus religiosus*, sp. n. (Fam. Niphargidae), with remarks to *Amathillina cristata* G.O. Sars, 1894 (Fam. Gammaridae) in Turkey - Contribution to the Knowledge of the Amphipoda 257 - *Agriculture & Forestry*, Podgorica. 53 (07) (1-4): 49-76, 11 figs.

Karaman, G. 2012b. Further studies on genus *Niphargus* Schiödte, 1849 (Fam. Niphargidae) from the Near East (Contribution to the Knowledge of the Amphipoda 260).- *Agriculture & Forestry*, Podgorica, 55 (09) (1-4): 49-74, 7 figs.

Karaman, G. 2012c. Further investigations of the subterranean genus *Niphargus* Schiödte, 1849 (fam. Niphargidae) in Serbia. (Contribution to the Knowledge of the Amphipoda 264). - *Agriculture and Forestry*, Podgorica, 58 (2): 45–64, 7 figs.

Karaman, G. 2016a. On two new or interesting species of the family Niphargidae from Greece and Croatia. (Contribution to the Knowledge of the Amphipoda 286).- *Ecologica Montenegrina*, Podgorica, 5: 1-17, 9 figs.

Karaman, G. 2016b. Two new genera of the family Niphargidae from Greece (Contribution to the Knowledge of the Amphipoda 287). - *Agriculture & Forestry*, Podgorica, 62 (1): 7-27, 8 figs.

Karaman, S. 1934. Weitere Beiträge zur Kenntnis griechischer Süsswasser-Amphipoden.- Zoologischer Anzeiger, Leipzig, 105 (7/8): 215-219, figs. 1-2.

Karaman, S. 1950a. Amfipoda Male Azije I. (= Die Amphipoden Kleinasiens I.).- *Srpska Akademija Nauka, Posebna Izdanja knj. 158, Odelenje Prirodno-matematičkih nauka*, Beograd, 2: 33-46, figs. 1-18.

Karaman, S. 1950b. Novi amfipodi podzemne faune Grčke.[Neue Amphipoden der unterirdischen Fauna Griechenlands]- *Rad, Jugoslavenska Akademija znanosti i umjetnosti, 280 (Odjel za prirodne i medicinske nauke)*, Zagreb, 3: 106-114, figs. 1-20 (pp. 43-50, figs. 1-20).

Karaman, S. 1956. III Beitrag zur Kenntnis griechischer Niphargiden.- *Folia Balcanica, Zavod za Ribarstvo na N. R. Makedonija*, Skopje, 1 (1): 1-8, figs. 1-9.

Ntakis, A., Anastasiadou, A., Zakšek, V. & Fišer, C. 2015. Phylogeny and biogeography of three new species of NIphargus (Crustacea: Amphipoda) from Greece.- Zoologische Anzeiger, 255: 32-46, 16 figs.

Pesce, G.L. & Maggi, D. 1983. Ricerche faunistiche in acque sotterranee freatiche della Grecia Meridionale ed insulare e stato attuale delle conoscenze sulla stygofauna di Grecia.- *Natura*, Milano, 74 (1-2): 15-73.

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THE LEGACY OF COLLECTING MISSIONS TO THE VALORISATION OF AGRO-BIODIVERSITY

SUMMARY

The history of the civilizations is deeply linked to the history of agriculture, and this has direct links to germplasm collecting and management.

Plant collecting activities date back to the beginning of agriculture, with the first steps of plant domestication.

Since the most remote times, mankind has depended on plant species collecting to address its basic needs. For the same reasons, or for cultural and economic reasons, for millennia, people collected and carried seeds, cuttings, seedlings and plants from the places they visited or settled in, and whenever they inhabited new places, they carried the species they knew and which cultivation they mastered.

The germplasm collecting is an historic activity used in the conservation of genetic resources, especially species for food and agriculture and represents an activity of primary importance within the genetic resources conservation strategies.

Germplasm collecting missions have the following main goals: to prevent genetic erosion; to expand or complete the genetic base available in the existent collections; and to meet specific needs (breeding programmes, research or development).

Between 1977 and 2014, Banco Português de Germoplasma Vegetal (BPGV), carried out 126 collecting missions in Portugal, which resulted in 12,540 accessions of several species.

The history of collecting plant genetic resourcesin the world, its connection to plant dissemination among the continents, and the plant germplasm collecting missions carried out by the BPGV in the last 40 years is the study object of this communication, in order to demonstrate the contribution of collecting missions to the valorisation of agro-biodiversity.

Keywords: collecting missions, Genebank, Portugal

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INTRODUCTION

Plant exploration is one of the oldest activities of humanity. Since the beginning of human civilization, people collect and carry around with them new and useful plants from faraway locations. In addition, whenever people explored new territories and settled in new lands, they took with them seeds and seedlings.

Plant exploration is a fascinating study field that has been attracting many adventurists, naturalists, travelers and plant hunters. However, the golden age of plant exploration and collecting was the later part of eighteenth century when a wider concern on plants of economic utility arose, with emphasis on their introduction (Arora R.K. 1991).

From the moment when it was discovered that the plant genetic diversity is the key component of global food security, the collecting missions began to provide genetic variation useful to crop improvement and to conservation of plant biodiversity. Plant collecting has in fact been one of the most powerful stimuli to exploration through the ages. (IPGRI *et al.*, 1995).

History of plant germplasm collecting

The search for exotic plants and new crops and varieties is linked to human history. With the beginning of agriculture, which replaced the collected species of wild plants by crop cultivation, naturally arose research, discovery and introduction of new crops and new farming techniques, through travel, trade and conquest.

The first recorded plant exploration expeditions took place in 2500 BC, when the Sumerians made excursions in Asia Minor in search for grapes, figs and roses (Walter and Cavalcanti, 2005).

Records also show earlier exploits of Sankhkara Mentuhotep III (2010-1998 BC), the Egyptian pharaoh, who sent ships to the Gulf of Aden (Yemen) to collect cinnamon (*Cinnamomum verum* J. Presl) and cassia (*Cinnamomum aromaticum* Nees), which were used in the embalming of the dead (Damania, 2008; Janick, 2007).In 1495 BC, the Egyptian Queen Hatshepsut sent a team under Prince Nehasito the Horn of Africa, Northeastern Coast of Africa(i.e., the area that now includes Somalia, Djibouti, Eritrea, and Ethiopia), with the main objective of collecting plants whose fragrant resins produced frankincense (*Boswellia serrata* Roxb. ex. Colebr.) and myrrh [*Commiphora myrrha* (Nees) Engl.] (Damania, 2008; Janick, 2007; Walter and Cavalcanti, 2005; IPGRI *et al*., 1995). The history of Egyptian civilization is marked by persistent collection and domestication of plants from the region and their subsequent introduction in the Nile valley (Damania, 2008; Janick, 2007).

Streams of immigrants and captives, as well as invasions by others such as the Persians (492-490 BCand 480-479 BC), the Greeks (335-323BC) and the Romans (27 BC-476 AD) contribute to new introductions of germplasm and technology.

Alexander the Great (356- 323BC), son of Philip of Macedonia and student of Aristotle, had a profound influence on the introduction of west Asian

plants in Europe. Alexander conquered Persia, Turkistan, Afghanistan, Pakistan, and north west India, including the Indus Valley. Greek settlements and commercial posts were founded between the Mediterranean and India along the western section of trade routes, which became known as the Silk Road. Alexander's campaign led to increased botanical knowledge concerning herbs and spices, and the cities that he established became the western routes for many Asian fruits. With the conquest of Egypt, the new city of Alexandria became the most important trading center between the Mediterranean and Indian Ocean, and was known as the Gateway to the East. (Janick, 2007).

The Roman Empire (27 BC-476 AD) is responsible for the introduction of a large amount of agricultural crops (cereals, forage, vegetables, vines, medicinal and aromatic plants, fruit trees) in the conquered territories (Ferrão *et al*., 2008). The Roman Empire was one of the largest in history and ruled a continuous territorial extension throughout Europe, North Africa and the Middle East, occupying an area of about five million square kilometers, which is currently apportioned among forty countries.

Although there are few detailed records and despite the fact that germplasm was not necessarily the objective of the incursions, many collecting missions and the introduction of germplasm have always existed in all ancient civilizations.

In 1011-1012, Emperor Chen-Tsung ordered the shipment of 30,000 Champa varieties of rice from Vietnam to China's Yangtze Delta, to improve agriculture. This was perhaps the first large-scale germplasm introduction (IPGRI *et al*., 1995; Twitchett and Fairbank, 2009).

Agricultural technology and new crops via the Arabs reached the rest of Europe after the incursions of the West into Jerusalem (the crusades) and from the re-conquest of the Iberian Peninsula by Christian warriors (Janick, 2007).

At the same time, the aggressive mounted tribes of Central Asia, consisting of Turks, Mongols and Tibetans, became a dominant power, under the leadership of Genghis Khan, who was proclaimed ruler of all Mongols in 1206. The Mongol Empire (1206–1368) eventually stretched from Eastern Europe to the Sea of Japan, extending northwards into Siberia, eastwards and southwards into the Indian subcontinent, Indochina, and the Iranian plateau, and westwards as far as the Levant and Arabia. During the Mongol Empire new crops and varieties were introduced in the conquered territories. Mongol invasion into India was a decisive turning point in the subcontinent. The Mughal Empire opened the way for a host of European visitors, missionaries and traders to travel throughout Eurasia. The Mughal gardens still remains in India, indicating the influence of this culture on horticulture (Janick, 2007).

During the 15th and 16th centuries, with the Age of Discovery of the New World, the Iberian navigators and conquerors unwittingly started a major programme of germplasm exchange with the Old World, generating a true food revolution (Ferrão, 2013; Janick, 2007; IPGRI *et al*., 1995; Gusmão *et al*., 1996).

Names like Marco Polo, Henrique the Navigator, Cristóvão Colombo, Bartolomeu Dias, Pedro Álvares Cabral and Vasco da Gama are remembered in various fields of knowledge. These men had as great motivator the search for desired and valuable products of plant origin, known by the generic name of spices. Such products had great value in important families of the Renaissance, who spared no efforts to have seeds, fruits, vegetables, bark, pulp, roots, rhizomes, bulbs, tubers, stems, grains, resins, leaves, herbs and berries at their table (Bracht and Dos Santos, 2011).

The Portuguese navigators and conquerors took with them seeds and seedlings that were known to ensure their own survival andto test how they adapt to new environments. The chroniclers of the Age of discovery mention their introduction on the islands of St. Helena, Cape Verde, São Tomé and Príncipe (which functioned as intermediate stations), African Coast and Brazil's,such as: almond (*Prunus dulcis* (Mill.) D. A. Webb), barley (*Hordeum vulgare* L.), black mustard (*Brassica nigra* L.), borage (*Borago officinalis* L.), cabbage (*Brassica oleracea* L.), carob (*Ceratonia silique* L.), carrots (*Daucus carota* L.), cinnamon (*Cinnamomum* spp.), coriander (*Coriadrum sativum* L.), cucumber (*Cucumis sativus* L.), fennel (*Foeniculum vulgare* Mill.), fig (*Ficus* spp.), garlic (*Alium sativum* L.), lemon (*Citrus limon* (L) Burm.), *lemon balm (Melissa officinalis* L.), lettuces (*Lactuca sativa* L.), melon (*Cucumis melo* L.) mint (*Mentha* spp.), onion (*Allium cepa* L.), orange (*Citrus sinensis* (L.) Osbeck), olive (*Olea europea* L.), pea (*Pisum sativum* L.), pomegranate (*Punica granatum* L.), radish (*Raphanus sativus* L.), rye (*Secale cereale* L.), sugarcane (*Saccharum* spp.), sesame (*Sesamum indicum* L.) spinach (*Spinacia oleracea* L.), turnip (*Brassica rapa* L.), vines (*Vitis* spp.), and wheat (*Triticum* spp.), among other species that were part of long and detailed lists which greatly influenced the agriculture and food of the people where they were introduced (Ferrão, 2004; Ferrão and Loureiro, 2013; Madeira et al, 2008).

In the New World, the Portuguese navigators came into contact with new and numerous plants, which somehow had some immediate use, particularly those who were grown and/or operated by indigenous peoples. Many of these species have earned the seas and eventually succeed to be transported and transplanted by the colonizers to regions as different as the Indian subcontinent, the southern Iberian Peninsula, the northern Europe and the American continent (Bracht and Dos Santos, 2011). From the American continent, the Portuguese collected and brought a huge number of plants. Some of these plants have quickly spread throughout the world and have deeply changed the agricultural scene and alimentary, such as: achiote (*Bixaorelana* L.), avocado (*Persea americana* Mill.), bean (*Phaseolus vulgaris* L.), cacao (*Theobroma cacao* L.), cashew tree (*Anacardium occidentale* L.), custard apple (*Annona* spp.), guava (*Psidium gujava* L.), manioc (*Manihot esculenta*Krantze), maize (*Zea mays* L.), papaya (*Carica papaya* L.), passion fruit (*Passiflora* spp.), peanut (*Arachis hypogaea* L.), peppers and chillies (*Capsicum* spp.), pineapple (*Ananas comosus* L.), potato (*Solanum tuberosum* L.), pumpkins (*Cucurbita* spp.), quinine

(*Cinchona* spp.), runner bean (*Phaseolus coccineus* L.), sunflower (*Helianthus annuus* L.), sweet potato (*Ipomea batatas* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Solanum lycopersicon* L.) and vanilla (*Vanilla planifolia* Jacks. ex Andrews) (Ferrão and Loureiro, 2013).

In this period, there was a great exchange of plants between West Africa and America. The WestCoast of Africaintroduced in America plants consumed by slaves, such as: palm oil (*Elaeis guineensis*a cq.), pearl millet (*Pennisetum glaucum* (L.) R.Br.), sorghum (*Sorghum bicolor* (L.) Moench) and yam (*Dioscorea* spp.). From America to African West Coast, several plants were introduced, for example: cacao (*Theobroma cacao* L.), manioc (*Manihot esculenta* Krantze), maize (*Zea mays* L.), papaya (*Carica papaya* L.), peanut (*Arachis hypogaea* L.), peppers and chillies (*Capsicum* spp.), pineapple (*Ananas comosus* L.), potato (*Solanum tuberosum* L.), sweet potato (*Ipomea batatas* L.), quinine (*Cinchona* spp.), tobacco (*Nicotiana tabacum* L.), tomato (*Solanum lycopersicon* L.) yam (*Dioscorea* spp.) and many fruits (Ferrão, 2013).

The introduction of various species and vegetables, and the diversity of the supply of the first colonizers, served as the basic material for plant breeding, in search for a better adaptation of these species to different soil and climatic conditions.

During the Age of Discovery, the collected plants were sent mainly to European Botanic Gardens in order to adapt to the new weather and soil conditions (Walter and Cavalcanti, 2005). In Portugal, the Botanical Garden of the National Palace of Ajuda (1768) and the Botanical Garden of the University of Coimbra (1772) had a very important role in the philosophic trips carried out in the Portuguese colonies between 1777 and 1808. This vast project, led initially by Domingos Vandelli, had the mission of collecting, preparing, sending and transporting the natural products of the Portuguese colonies established in America, Africa and Asia, in order to discover new plant species to develop agriculture (Pataca, 2011). As the exploration of the tropics by the emerging colonial powers continued, the first Botanic Gardens outside Europe were created, such as those at the Cape of Good Hope (South Africa), Pamplemousses (Mauritius), Buitenzorg (now Bogor, Indonesia), Calcutta (lndia) and Bath (Jamaica). These served as introduction and acclimatization centers for a wide range of crops, fruits, spices and ornamental (IPGRI *et al.*, 1995).

According to Walter and Cavalcanti (2005), the collectors that stood out in the 18th century were: Francis Masson (1741-1806), who collected plants in South Africa, Madeira and the Canary Islands, Azores and West Indies; David Nelson (? -1789) who explored the Middle East and Oceania; George Caley (1770-1829) which collected for over 10 years in Australia; and Joseph Banks (1743-1820) who explored the South Pacific. With Banks's death, the germplasm collecting expeditions were reduced, until in 1841, when William Jackson Hooker (1785-1865) took over part of the activities being succeeded by his son Joseph Dalton Hooker (1817-1911).

Under direction of Sir Joseph Banks (1743-1820), the British carried out many collecting expeditions in the world in the search for species that were potentially useful as food, ornamental, medicinal and timber, and brought back plants to botanic gardens, especially to the Royal Botanic Gardens at Kew, near London. They also established the Botanic Garden at St. Vincent in the Windward Islands in the Caribbean. (Damania, 2008; Walter and Cavalcanti, 2005; IPGRI *et al*., 1995).

In the 19th century and early 20th century, many germplasm collectors also stood out, including Robert Fortune (1812-1880), who collected in China and Japan; George Forrest (1873-1932), who made several expeditions to the Himalayas and to China; Ernest Henry Wilson (1876-1930) who also collected in China; Peter Barr (1825-1909) who explored areas of Spain, Portugal and the Alps; Richard Spruce (1817-1893) who collected for over 20 years in South America, covering major routes in Amazonas and Pará; Frank N. Meyer (1875-1818) who collected in Europe, China, Russia, Korea and Tibet, introducing many species from other continents in North America; David G. Fairchild (1869-1954) who made explorations in different parts of the world, particularly in Asia and South America; and Wilson Popenoe (1892-1975) who explored Central America in the early XX century (Walter and Cavalcanti, 2005; IPGRI *et al* 1995; Ford-Lloyd & Jackson, 1986).

Until the early 20th century, Portuguese, Spanish, British, Dutch and French were particularly active in the dissemination of plants between continents (Walter and Cavalcanti, 2005).

From 1922 to 1940, Nikolai I. Vavilov and his team conducted numerous expeditions in the USSR and in different parts of the world, covering more than 50 countries in Asia, Africa, Central and South America, collecting around 50,000 germplasm accessions. Vavilov organized and took part in over 100 plant collecting missions. His co-workers, including S.M. Bukasov and M.G. Popov, conducted several other missions. His major expeditions outside the Soviet Union included those to Iran (1916), America (1921), Afghanistan (1924), Mongolia (1926), Central and South America (1930), the United States (1932), the Mediterranean region, including Portugal (Bettencourt & Gusmão, 1995), Lebanon, Syria, Ethiopia (1926–1927) and Central Asia (1929). Vavilov's works on the origin and geography of cultivated plant species, has formed the basis of much of the study of plant genetic resources that is performed today. Nowadays, Vavilovis considered to be the father of crop plant exploration and collection (Damania, 2008; IPGRI *et al*., 1995; Loskutov, 1999; Walter and Cavalcanti, 2005).

Germplasm collection and conservation worldwide

After World War II, the Food and Agriculture Organization for the United Nations (FAO) became the leading organization to promote the conservation of plant genetic resources. In 1971, the Consultative Group on International Agricultural Research (CGIAR) was formed with co-sponsorship from FAO, the United Nations Development Program (UNDP) and the World Bank, with the

main objective to contribute to the agrobiodiversity's preservation by establishing *ex situ* collection of plant genetic resources. The International Board for Plant Genetic Resources (IBPGR nowadays Bioversity International) was created in 1974 by the CGIAR with the basic function to advance the conservation and use of plant genetic resources for the benefit of present and future generations (IBPGR, 1983).

From 1974 onwards, Bioversity International supported a series of collecting expeditions worldwide, with the objective to systematically collect and conserve landraces cultivated by farmers and their crop wild relatives which were being lost from fields and natural habitats (Bioversity International, 2015).

Collectors from national and international institutions collected over 225,000 plant samples during more than 500 collecting expeditions to most countries of the world. Samples of approximately 4300 different species were collected, with a focus on landraces and crop wild relatives of major crops. The most collected genera were *Oryza*, *Zea*, *Phaseolus* and *Sorghum* with more than 10,000 samples each, followed by *Triticum, Vigna, Solanum, Ipomoea* and *Pennisetum* with more than 5000 samples. This wealth of landraces and CWR was distributed to over 500 genebanks for conservation (Bioversity International, 2015).

During expeditions, for each one of the samples collected, the collectors make notes in field books and fill the forms with passport data about the plant and its environment. They also record valuable knowledge shared by farmers about traits they value in the plants, and the ways they cultivate, harvest and process them. This information recorded by the collectors is a treasure trove of fascinating data. It also contains information that can help us understand better the consequences of climate change and agricultural practices changes (Bioversity International, 2015).

RESULTS AND DISCUSSION

Collecting missions in Portugal
Period from 1930 to 1950

From 1930 to 1950, in Portugal, agronomists and plants breeders were aware that plant genetic resources were the starting point for their breeding programmes, so they carried out the collection and the establishment of several active and breeders' collections in various points of the country. Some examples are cited:

- In 1933, with the creation of the *Estação Agrária de Ensaio de Sementes e Melhoramento de Plantas* in Belém, Lisbon, João de Carvalho eVasconcellos established the first collection of Portuguese wheat. An extensive collection of traditional Portuguese varieties of hexaploid and tetraploid wheat, including 48 varieties of soft wheat and 51 varieties of durum wheat, were collected throughout the territory of Portugal

mainland (Vasconcellos, 1933). After the extinction of the *EstaçãoAgrária* in 1936, that collection was transferred to the *Estação de Melhoramento de Plantas*, in Elvas, and nowadays is preserved in the National Genebank (BPGV), in Braga.

- In 1941 at the *EstaçãoAgronómica Nacional* (EAN), located in Oeiras, António Marques de Almeida started the organization, maintenance and enrichment of the collection of the main forage species, traditional and exotic, cultivars and ecotypes, of the most diverse geographical origins in Portugal and from international breeding centres. In 1948, this collection had more than 12,000 species and strains (Campos Andrada, 2011).
- In 1949 at the *Estação de Melhoramento de Plantas*, work of characterization of the genus *Trifolium* was carried out, describing about 40 species of the genus (annual and perennial forms) and indicating the locations of dispersion and forage interest.
- From 1940 to 1950, researchers and maize breeders Luiz Távora, Antonio Lacerda and Luis Freire de Andrade conducted some collection missions of the traditional varieties of maize (*Zea mays* L.) in Minho and Algarve Regions, to support the maize breeding programme, in Braga and Tavira (Farias and Marcelino, 1993; Farias, 1999).

Much of this germplasm, maintained during that period on several active and breeder's collections, such as the improvement programme of forage species conducted in Elvas and Oeiras, maize in Braga and cereals in Elvas, have contributed to the obtainment of numerous varieties, listed in the National Catalogue of Varieties (CNV) and commercialized by different seed companies (Ministério da Agricultura e do Mar, 2015). However, some of the material collected and maintained in various parts of the country was lost due to the lack of appropriate conditions for medium and long term conservation (Farias and Marcelino, 1993; Farias, 1999).

Period from 1977 to 2014

In the early 1970s, a new momentum started in Portugal in order to collect and conserve the national plant genetic resources, through actions carried out by genetic resources program for the Mediterranean region(Bettencourt, 1999; Ministério da Agricultura e do Mar, 2015).In 1977, still under the genetic resources programme for the Mediterranean region, supported by FAO and Bioversity International, the Banco Mediterrânico de Milho, in Braga,was created, with the responsibility of maize collecting and conservation, that subsequently gave birth to the Banco Português de Germoplasma Vegetal (BPGV), the National Genebank (Barata *et al*., 2011b; Bettencourt, 1999; Farias and Marcelino, 1993; Farias, 1999; Ministério da Agricultura e do Mar, 2015).

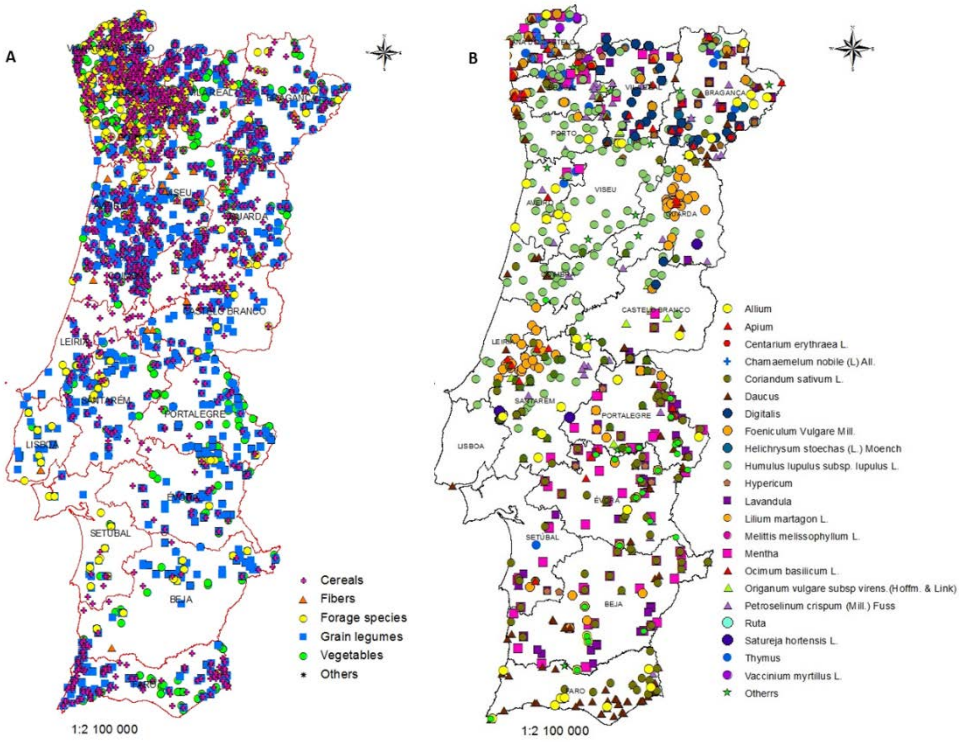

Figure 1. Collecting sites in Portugal, by species group: (A) Cereals, Fibers, Forage species, Grain legumes and Vegetables; (B) Medicinal and aromatic plants.

From 1977 to 2014, numerous germplasm collecting missions for cereals, fibers, forage species, grain legumes, medicinal and aromatic plants, vegetables and crop wild relatives of major crops were carried out in Portugal (Barata *et al*., 2009; Barata, *et al*., 2011a; Barata *et al.*, 2011b; Barata *et al*., 2014; Bettencourt and Gusmão, 1981; Bettencourt and Gusmão, 1982; Bettencourt, 1999; Farias, 1989; Farias *et al.*, 1992; Farias and Marcelino, 1993; Farias, 1999; Farias, 2002; Lopes *et al*., 2015; Marcelino, 1994; Marcelino, 2002; Mota *et al*., 1981; Mota *et al*., 1982a; Mota *et al*, 1982b; Mota *et al*, 2005; Rocha, 2000; Rocha, 2005; Rocha *et al*., 2008; Rocha *et al*., 2010a; Rocha *et al*., 2010b; Rocha *et al*., 2010c; Rocha *et al*., 2011; Rocha *et al*., 2013; Rocha *et al*., 2014;Rocha *et al*., 2017).

The Key point dates of collecting missions are:

- 1977 - First germplasm landraces collecting mission of cereals;
- 1978 - Systematic landraces collecting missions of grain legumes;
- 1979 - Collecting mission for maize landraces in Azores;
- 1980 to 1983 - Collecting missions for cereals and grain legumes and in Portugal and Spain;
- 1985 - Systematic collecting missions of forage species;

- 1987 - Systematic collecting missions for flax;
- 1990 to 1994 - International collecting missions for vegetables;
- 1991 - International collecting missions for cereals, grain legumes, fibers and vegetables in Madeira; International collecting for broad beans in Portugal mainland;
- 1994 - Systematic prospection and collecting missions for genus *Allium*;
- 1997 to 1999 - Systematic prospection and collecting for wild hop;
- 2000 - Systematic collecting for medicinal and aromatic plants;
- 2001 - International germplasm collecting mission for genus *Daucus*;
- 2006 - International collecting mission for genus *Lupinus*;
- 2009 to 2010 - International germplasm collecting missions for forage species, medicinal and aromatic plants and grain legumes, in Portugal and Spain;
- 2014 - Systematic collecting of crop wild relatives of major crops (*Avena*, *Daucus*, *Hordeum*, *Lathyrus*, *Lens*, *Malus*, *Medicago*, *Pisum*, and *Vicia*) in Portugal.

During this period, BPGV carried out 126 collecting missions in Portugal, which resulted in 12,540 accessions of several species.

Figure 1 are represented the collecting sites in Portugal by species group: (A) Cereals, Fibers, Forage species, Grain legumes and Vegetables; (B) Medicinal and aromatic plants, as a result of collecting missions from 1977 to 2014.

The following table – Table 1 – shows the number of collecting missions and accessions by decades.

Table 1. Number of collecting missions and accessions by decades.

Decade	Number of collecting missions	Number of accessions
1977 to 1987	14	1690
1988 to 1997	39	5712
1998 to 2007	43	2506
2008 to 2014	30	2632
Total	126	12540

According to the results, the decade in which more collecting missions were carried out is 1998-2007 (43), followed by the decade from 1988 to 1997 (39). However, the decade in which more genetic material was collected was the period of 1988 to 1997 (5712).

The original passport data of each accession (which includes collecting number, scientific name, common names, date of collecting, collectors name, geographical location, topography, habitat characteristics, characterization of the collecting site, sample characteristics, ethnobotanical information) weredocumented and now are available in the Grin-Global database platform (http://bpgv.iniav.pt).

During this period, there were some colleagues who have stood out in germplasm collecting missions in Portugal and abroad, namely Rena Martins

Farias, Eliseu Bettencourt, Miguel Mota, Manuel Tavares Sousa and Luís Gusmão. Along almost 40 years of plant genetic resources conservation in Portugal, many others, from many different institutions in the country and around the World, have been involved in germplasm collecting missions of different crops and crop wild relatives.

CONCLUSION

The history of the civilizations is deeply linked to the history of agriculture, and this has direct links to germplasm collecting and management.

Plant collecting activities are one of the oldest activities of mankind. Since the dawn of civilization, individuals have gathered new and useful plants from faraway places. Seeds and seedlings were routinely included as part of the household as people explored new territories and settled in new lands. From centuries ago until the middle of the 20th century, many collecting expeditions were carried out worldwide in the search for species that were potentially useful as food, ornamental, medicinal and timber.

Today, because genetic diversity is the key to maintaining and improving agriculture, plants are collected and valorised in order to preserve genetic variability.Over the last four decades Bioversity International supported a series of collecting expeditions worldwide, with the objective to systematically collect and conserve representative samples of genetic diversity for many crops and their crop wild relatives.

Nowadays, many collections of crop diversity are conserved in the world gene banks and can be found in online accessible databases, which include collecting mission information.

With the same objective, between 1977 and 2014, numerous systematic germplasm collection missions have been held in Portugal, for cereals, fibers, forage species, grain legumes, medicinal and aromatic plants, vegetables and crop wild relatives of major crops.

The germplasm collection in Portugal resulted in the preservation of a large legacy, representative of the genetic variability in the country. These collections are an important source of germplasm to address future needs of organic farming production and other sustainable agriculture programmes, as well as a response to global climate change conditions and to ensure local and global food security.

The spread of crops throughout the world, which is occurring since the first plant exploration expeditions, as well as the amount of plant genetic resources, which are currently conserved and are available in Genebanks, as a result of the collecting expeditions carried out in the last years worldwide, demonstrates the contribution of this international activity for the valorisation of agro-biodiversity and agricultural systems.

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REFERENCES

Arora R.K. 1991. Plant exploration and germplasm collection. In Paroda RS and Arora RK, editors. Plant Genetic Resources Conservation and Management. IBPGR, Regional Office, New Delhi, India, 55–88.

Barata, A.M.; Xavier, D.; Bettencourt, E.; Rocha, F.; Farias, R.; Lopes, V.R. 2009. Genetic variability in field collections of *Origanum*,*Thymus* and *Mentha* spp. from NW Portugal. BioversityInternacional. In Report of a Working Group on Medicinal and Aromatic Plants. Second Meeting, 16-18 December 2004, Strumica, Macedonia FYR / Third Meeting- E. Lipman, editor, Rome, Italy.117-182.

Barata, A.M.; Rocha, F.A.; Lopes, V.M.; Morgado, J.; Maia, J.; Bettencourt, E.; Dias, S.; Delgado, F.; Costa, M.; Farinha, N.; Póvoa, O.; Salgueiro, L.; Figueiredo, A.C. 2011a. Networking on conservation and use of medicinal, aromatic and culinary plants genetic resources in Portugal. ActaHort 925:21–35.

Barata A.M.; Rocha, F.; Reis, A.; Lopes V.R. 2011b. O Banco Português de Germoplasma Vegetal e a conservação dos recursos genéticos em Portugal. AGRORRURAL: CONTRIBUTOS CIENTÍFICOS, Instituto Nacional de Recursos Biológicos, I. P. e Imprensa Nacional-Casa da Moeda, S. A. editedby Paula S. Coelho, Pedro Reis. Chapter V - Ecofisiologia, recursos genéticos e melhoramento de plantas. Imprensa Nacional-Casa da Moeda, S. A. 964-974.

Barata, A.M.; Rocha, F.; Gaspar, C.; Vaz, M.; Reis, A. 2014. Variedades de Feijão do Concelho de São Pedro do Sul: Valorização, Colheita e Conservação deste Património Genético. In Agricultura Familiar e Desenvolvimento Rural: Biodiversidade e promoção da saúde. EditedbyVitor Barros. Associação Portuguesa para o Desenvolvimento Local. 13-22.

Bracht, F.; Dos Santos, C.F.M.2011. A disseminação e uso de plantas do novo mundo no século XVI: A difusão de elementos da flora americana a partir da expansão marítima européia. Anais do XXVI Simpósio Nacional de História. ANPUH, São Paulo.

Bettencourt, E;Gusmão, L. 1981. Maize collecting in the Azores. FAO/IBPGR Plant Genetic Resources Newsletter 46: 15–17.

Bettencourt, E.; Gusmão, L. 1982. Colheita de germoplasma de milho, nos Açores. I. S. Miguel e Terceira. Agronomia Lusitana 41(3-4): 241–258.

Bettencourt, E.; Gusmão, L. 1995. Vavilov Mission Confirmed Pivotal Importance of Portugal's Plant Wealth. Diversity, Vol. 11(1-2):84-85.

Bettencourt, E. 1999. A coleção do Banco de Germoplasma da secção de Genética da EAN. In Actas da I Conferência Técnica sobre Recursos Genéticos Vegetais, 3-5 junho de 1996, Direção Regional de Agricultura de Entre Douro e Minho, Braga, 31-34.

Bioversity International. 2015. The history of Bioversity International collecting missions. Available at: http://www.bioversityinternational.org/e-library/databases/collecting-missions/history/ (accessed 16 September 2015).

Campos Andrada, M.P. 2011. Recursos genéticos e melhoramento de plantas forrageiras - 75 anos de investigação na EAN. AGRORRURAL: CONTRIBUTOS CIENTÍFICOS, Instituto Nacional de Recursos Biológicos, I. P. e Imprensa Nacional-Casa da Moeda, S. A. editedby Paula S. Coelho, Pedro Reis. Chapter V - Ecofisiologia, recursos genéticos e melhoramento de plantas. Imprensa Nacional-Casa da Moeda, S. A. 927-938.

Damania, A.B. 2008. History, Achievements, and Current Status of Genetic Resources Conservation. AgronomyJournal 100:9–21.

Farias R.M. 1989. Recursos genéticos. Banco de Germoplasma de milho. Sua Majestade... o milho rei. Revista da Feira do Milho, Gualtar, Braga, 3-6 de outubro. 17–18.

Farias R.M; Varandas E; Marcelino FR. 1992. O tesouro de Basto e a agricultura biológica. Vida Rural Nº 8/92. 2ª Quinzena de Abril. 46–47.

Farias, R.M.; Marcelino, F.R. 1993. O Banco Português de Germoplasma Vegetal Guardião do Tesouro Nacional. Revista Vida Rural, Nº 17/93. 1ª Quinzena de Setembro, Lisboa. 7-10.

Farias, R.M. 1999. Banco Português de Germoplasma Vegetal – Testemunho Vivo da Biodiversidade em Portugal. In Actas da I Conferência Técnica sobre Recursos Genéticos Vegetais, 3-5 junho de 1996, Direção Regional de Agricultura de Entre Douro e Minho, Braga, 23-29.

Farias, R. 2002. Status of vegetatively propagated *Allium* collection in Portugal. In Maggioni. L., Keller, J. and Astley, D. compilers. European collection of vegetatively propagated *Allium*. Report of a Workshop, 21-22 May 2001, Gatersleben, Germany. Internacional Plant Genetic Resources Institute, Rome. 62-63.

Ferrão, J.E.M. 2004. A Aventura das Plantas e os Descobrimentos Portugueses. Instituto de Investigação Científica Tropical, Lisboa. 241 p.

Ferrão, J.E.M.; Caixinhas, M.L.; Liberato, M.C. 2008. A ecologia, as plantas e a interculturalidade. In Portugal: Percursos de interculturalidade. Volume I - Raízes e Estruturas. Alto Comissariado para a Imigração e Minorias Étnicas (ACIME). 132-223.

Ferrão, J.E.M. and Loureiro, R.M. 2013. Plantas Viajantes. O legado do Novo Mundo. Available at: http://www.palato.org/plantas-viajantes-o-legado-do-novo-mundo/ (accessed 18 September 2015).

Ferrão, J.E.M. 2013. Na linha dos descobrimentos dos séculos XV e XVI. Intercâmbio de plantas entre a África Ocidental e a América. Exchange of plants between West Africa and America in the 15th and 16th centuries discoveries. Revista de CiênciasAgrárias. 36 (2) 250-259.

Ford-Lloyd, B.; Jackson, M. 1986. Plant Genetic Resources: an introduction to their conservation and use. Edward Arnold Publishers, London, UK. 152 p.

Gusmão, L.; Bettencourt, E.; Alves, J.; Godinho, C.; Varela, C. & Pereira, L. 1996. Early Portuguese Multidirectional Promotion of New Geographical Diversification Areas.International Technical Conference on Plant Genetic Resources, 23-27 June. Leipzig Germany.

IBPGR. 1993. Crop Genetic Resources. A slidepack with text. International Board for Plant Genetic Resources, Funny Press, Rome, Italy.

IPGRI, FAO, UNEP, IUCN. 1995. A brief history of plant germplasm collecting. In: Collecting plant genetic diversity: Technical guidelines. Edited by L. Guarino, V. Ramanatha Rao and R. Reid. Chapter 1. pp- 1-11. CABI

Janick, J. 2007. Plant Exploration: From Queen Hatshepsut to Sir Joseph Banks. Hortscience Vol. 42(2): 191-196.

Lopes, V.R.; Rocha, F.; Gaspar, C.; Barata, A.M. 2015. Contribution to *ex situ* conservation of wild *Lavandula* populations in Portugal. MESMAP-2. Abstract Book. Antalya, Turquia. 311-521.

Loskutov, I.G. 1999. Vavilov and his institute. A history of the world collection of plant genetic resources in Russia. IPGRI, Rome, Italy: 188 p.

Madeira, N.R.; Reifschneider, F.J.B.; Giordano L.B. 2008. Contribuição portuguesa à produção e ao consumo de hortaliças no Brasil: uma revisão histórica. *Horticultura Brasileira* 26: 428-432.

Marcelino, F.R; Farias, R.M. 1994. Colheita de germoplasma vegetal na Madeira. Vida Rural Nº 1589, Ano 41º, abril. 29–33.

Marcelino, F.R. 2002. Conservação e utilização sustentada de variedades tradicionais. Revista O Minho, a Terra e o Homem, DRAEDM, Braga, 46: 13–18.

Ministério da Agricultura e do Mar, 2015 Plano Nacional para os Recursos Genéticos Vegetais. Lisboa. 30 p.

Mota, M., Gusmão, L., Bettencourt, E. 1981. *Lupinus* and *Secale* collecting in Portugal. FAO/IBPGR Plant Genetic Resources Newsletter 47: 26–27.

Mota, M., Farias, RM, Teixeira, M., Bettencourt, E. 1982a. *Zea* and *Phaseolus*collecting in Portugal. I. FAO/IBPGR Plant Genetic Resources Newsletter 50: 21-22.

Mota, M., Gusmão, L., Bettencourt, E. 1982b. *Lupinus* and *Secale* collecting in Portugal. II. FAO/IBPGR Plant Genetic Resources Newsletter 50: 22–23.

Mota, M.; Nunes, E.; Farias, R. 2005. Colheita e caracterização morfológica de populações regionais de cenoura silvestre. In Actas Portuguesas de Horticultura, V Congresso Ibérico de Ciências Hortícolas/IV Congresso Iberoamericano de Ciências Hortícolas, Associação Portuguesa de Horticultura, Lisboa, 1, 8: 141-146.

Pataca, E.M. 2011. Coletar, preparar, remeter, transportar – práticas de história natural nas viagens filosóficas portuguesas (1777-1808). Revista da Sociedade Brasileira de História da Ciência, Rio de Janeiro, 4(2):125-138.

Rocha, F. 2000. Colheita de Germoplasma espontâneo de*Humuluslupulus*L.Revista Melhoramento, Estação Nacional de Melhoramento de Plantas, Elvas, Volume 36. 163-175.

Rocha, F.; Farias, R.; Marreiros, A.; Sousa, J.B.; Farinhó, M. 2004. Colheita de germoplasma de Cucurbitáceas – Abóboras e Melancias no Algarve. Encontro sobre Cucurbitáceas, Escola Superior Agrária de Santarém, Santarém. 6 p.

Rocha, F.A.S. 2005. Distribuição e ecologia do lúpulo *(Humuluslupulus*L.subsp. *lupulus*) em Portugal. Tese de mestrado, Universidade do Minho, Braga, 173 p.

Rocha, F.; Bettencourt, E.; Gaspar, C. 2008. Genetic erosion assessment through the re-collecting of crop germplasm. Countiesof Arcos de Valdevez, Melgaço, Montalegre, Ponte da Barca and, Terras de Bouro (Portugal).Plant Genetic Resources Newsletter, 154: 6-13.

Rocha, F.; Reis, A.; Gaspar, C. 2010a. Portuguese *Allium* collection maintained at the BPGV. EURISCO catalogue. E-bulletin, April 2010.

Rocha, F.; Lopes, V.; Gaspar, C. 2010b. Portuguese *Brassica* collection maintained at the BPGV. EURISCO catalogue. E-bulletin, April 2010.

Rocha, F. Barata, A.M.; Gaspar, C. 2010c. Portuguese Maize collection maintained at the BPGV. EURISCO catalogue. E-bulletin, April 2010.

Rocha, F.A.; Barata, A.M.; Quedas, F.; Lopes, F.; Marreiros, A.; Leitão, J. 2011. *Portuguese Cucurbita spp. and Citrulluslanatus: Conservation, Evaluation and Breeding*. ActaHorticulturae ISHS. 545-549.

Rocha, F.A.; Pereira, G.; Farinha, N.; Carneiro, J.P., Campos-Andrada, M.P.; Gaspar, C.; Póvoa, O.; Duarte, I.; Carita, T.; Sousa, M.T.2013. Prospecção e colheita de populações autóctones de espécies forrageiras, aromáticas e medicinais, ornamentais e leguminosas para grão na Região do Alentejo. Revista da Sociedade Portuguesa de Pastagens e Forragens, vol. 32/33. 45-59.

Rocha, F.; Rodrigues,C.; Gaspar,C.; Barata,A.M. Miranda, J. 2014. Traditional cuisine and cultural heritage: the case of Sistelo, in the Minho region, Northwestern Portugal. ICEB 2014 VI Congreso Internacional de Etnobotánica/ VI thInternationalCongressofEthnobotany, Córdoba, Espanha, S6 - O13. 361-362.

Rocha, F.; Gaspar, C.; Lopes V. R.; Barata A. M. 2017. Medicinal and Aromatic Plants collecting missions in Portugal. Arabian Journal of Medicinal & Aromatic Plants, v.3(1):19-32

Twitchett, D.; Fairbank, J.K. 2009. The Cambridge history of China. Volume 5. Part One: The Sung Dynasty and Its Precursors, Cambridge University Press.907–1279.

Vasconcellos, J.C. 1933. Trigos portugueses. Subsídios para o seu estudo botânico. Bol. Agron. Lisboa 1: 1-150.

Walter, B.M.T.; Cavalcanti, T.B. 2005. Fundamentos para coleta de germoplasma vegetal. Embrapa Recursos genéticos e Biotecnologia. Brasília. 27-56.

DOI: 10.17707/AgricultForest.63.2.03

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THE CORRELATION BETWEEN DBH AND THE HEIGHT OF SOME FLOOD PLAIN STANDS FROM NATURAL PARK „LUNCA MURESULUI"

SUMMARY

On the background of climate changes, in the last period concerns regarding the forest vegetation have increased due to its role in improving the effects of these climatic changes. The stands from meadow have a great importance because they represent habitat and food source for a large variety of living beings. In the last decade, the occupied surface with these types of ecosystem decreased. Research on the influence towards diminishing of these areas is the main purpose of the paper. The research objectives refer to capturing the relationship between main variables of the trees: diameters and heights.

Following research was highlighted a strong relation between those two variables, both through graphical representation and through the values of correlation coefficients (0.69-0.93). The mathematical relationships used in the description of these correlative links caught very well the stands structure, fact indicated by the standard error values (0.05-3.18).

In the case of oak stand, regarding the distribution on variation coefficient of heights in relation with diameters, it has been noticed a greater stability around the d_{50}, reference diameter for h_{50}height, from uneven aged stands. This aspect supports the theory from literature, according to which the stands, subject to selection cuttings, the reference diameter of height indicator is 50 cm.

Keywords: meadow forests, uneven-aged forests, oak from meadow, DBH-height relationship, Mures Natural Park.

INTRODUCTION

On the background of climatic changes, the forest vegetation is a fundamental component for a sustainable management of these ecosystems and those who depend on them (Trettin et al., 2016). In Romania, some of the most sensitive forest ecosystems with a commendable reduction are the flood plain oak stands (Calinovschișı Palaghianu, 2007). This representative diminution is due, in

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general, to the anthropic impact (Stanescu, 1979, Calinovschișși Palaghianu, 2007). In this way, the current researches are of great interest towards protection and conservation of them through a sustainable management.

All the more, the current researches goes on the flood plain of the most important rivers from Romania- Mureș river, these ecosystems are able to fulfil the protection functions of river beds against erosion and the protection against floods of towns or farmland neighbouring river. As well as their importance for the Terra climate and biodiversity, the forest are a major component of rural development which offers protection functions of soil, water and infrastructure, contributing through goods and services in economic and social field(Ojea et al., 2012, Briner et al., 2013, García-Nieto et al., 2013).

At the same time, these researches are part of some multidisciplinary studies included in a project for enlargement of LTER Romania network in forest ecosystems from flood plain (Silaghi, Chisăliță, 2014).

Research purpose was to understand, as detailed, the structure and functional characteristics of flood plain stands, which have in composition, beside the characteristics species of this ecosystem (poplar, common ash etc.) other species, which due the climate changes start slowly to disappear from this area (oak).

Research objectives, are correlated with their purpose, it refers to tying the correlation between the most important trees characteristics, respectively diameter and height.

MATERIALS AND METHODS

2.1. Location of permanent plots

The researches were conducted in Natural Park "Lunca Muresului", situated in the west part of Romania, across Arad andTimiș county (Fig. 1). The occupied surface is 6466,6 ha (37,1% from all the natural park surface) and contain forest ecosystems with semi natural character, but with small anthropic influences.

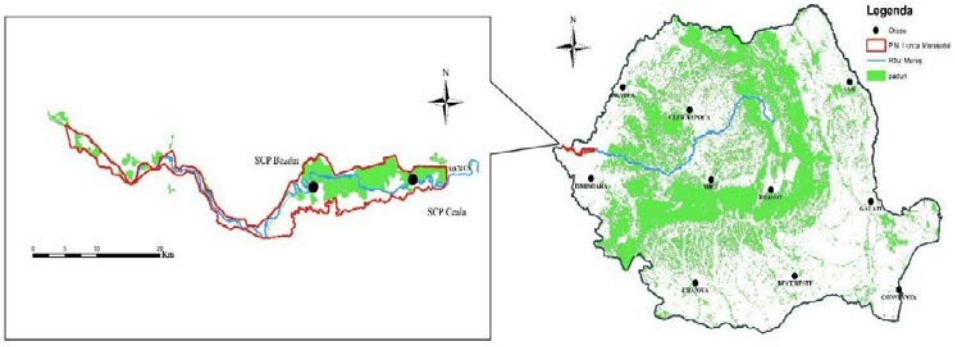

Figure 1: The location of permanent plot in Natural Park Lunca Muresului and Romania territory (EEA, 2000, MMP, 2014).

These studies were conducted in the project „The action of climate changes and other stress factors on the forest ecosystems situation from Natural Park" Lunca Mureșului", achieved in the collaboration between INCDS" Marin Drăcea" and National Forest Administration "Romsilva" from Romania. Within the research were placed two experimental plots, with square form (100x100m) with an area of 1ha, located across the yield management unit I Bezdin and V Ceala of Iuliu Moldovan forest district (FD) from County forest administration (CFA) (Table 1). To permanent research plots location (PRP) has been taken into account the degree of representation for flood plain ecosystems and the field configuration.

Table 1: The principal indicators of sample plots characterization

Permanent research plod (PRP)		PRP Bezdin	PRP Ceala
County (CFA)		Arad	Arad
Forest District (FD)		Iuliu Moldovan	Iuliu Moldovan
Yield management unit (UP)		UP. I Bezdin	UP. V Ceala
Compartment (u.a.)		33C	17L
Forest formation		Mixed foliage forest	Mixed foliage forest
Principal species		Oak	Poplar
Age* (years)		105	30
Structure		Uneven-aged	Uneven-aged
Alt. (m)		96	105
Coordinates	Lat	46° 08' 07" N	46° 09' 03" N
	Long	21° 00' 32" E	21° 12' 37" E

* Extracted from Management Plans

2.2 Research method

In the permanent research plots PRP Bezdin and PRP Ceala were taken into the inventory all the trees with diameter at breast height (d) greater than 80 mm, have been achieved in this way the main characteristics of trees (diameter, height, quality class, cenotic position etc.). The diameter (d) was measured with measuring-tape, materializing the place on tree with paint towards any reviews, and height of trees with the help of ultrasonic hypsometer Vertex IVfor all the trees in inventory (Badea, 2008). Furthermore, was determined the position class considering the stand floor and the quality class considering work wood proportion from total height of tree (Giurgiu, 1979);

The information obtained following inventory were registered in a typified sheet, after, this sheet was used for developing a database and for calculation.

In the case of structure analysis of stands and the relationships between dendrometric characteristics were taken into account the main species from this permanent research plots. Thus,the main species which form the PRP composition are: oak (*Quercusrobur)*, common ash (*Fraxinus excelsior)*, common maple (*Acer campestre)*, white poplar *(Populus alba)* and elm tree *(Ulmus minor)*. The relationship between the diameter at breast height and height have a great importance in stand characterization (Zhang, 1996). For

highlighting the correlation between the diameter and height (Giurgiu,1999, Giurgiu et al., 2004) was calculated the variation coefficient of height and after was represented graphically in relation with the diameter (d). To draw the height curve was tested a series of mathematical relations, which was used in the past for describing the connection between those two characteristics of the stands from the permanent research plots (d and h). The most suitable equations to describe the diameter - height relation were in accordance with the specific methodology (Giurgiu et al., 2004, Mahmut, 2004, Zhang, 1997) and the standard error have small values with a normal curve form. In the wake of testing more biometric equations, the most suitable for stands characterization, from permanent research plots, turn out to be the following:

$$h = a_0 + a_1 \log d \quad (1)$$

$$h = a_0 + a_1 d + a_2 d^2 \quad (2)$$

$$\log h = a_0 + a_1 \log d + a_2 \log^2 d \quad (3)$$

where: d – diameter at breast height (cm);

h – tree height (m);

a_0 -a_2–coefficients for regression equations, corresponding each species(Giurgiu *et al.*, 2004).

In the process of applying the mathematical models, were used the packets S*tats* (Bates and Chambers, 1992), *Base* and *graphics* (Chambers and Hastie, 1992) of the program R.

RESULTS AND DISCUSSION

For permanent research plots characterization was determined a series of statistical indicators with a dependency towards stands structure. In general, was calculated limit values, arithmetical mean diameter, mean height and standard deviation (Table 2).

Table 2: Main characteristics of stands from permanent research plots

Permanent research plots	Characteristic	Number of tree (N)	Min (Min)	Max (Max)	Average ($\bar{X}$)	Standard deviation (s)	Variance (s^2)
PRP Bezdin	Diameter (cm)	562	3	88	22,13	19,43	377,74
	Height (m)	562	2	45	17,14	11,77	138,65
PRP Ceala	Diameter (cm)	602	3	48	17,09	9,48	90,02
	Height (m)	602	2	34	17,27	6,98	48,85
Total	Diameter (cm)	1164	3	88	19,55	-	-
	Height (m)	1164	2	45	17,20	-	-

These two areas have close values in what concern the mean height of tree with a value of 17,20 m, and standard deviation, it fit in the interval 9,48 (PRP Ceala) - 19.43 (PRP Bezdin) in the case of diameters and for height, standard deviation have a specific range 6,98 (PRP Ceala) and 11,77 m(PRP Bezdin). The studied stands have a similar structure, with small exceptions, PRP Ceala shows a major homogeneity as regard the diameters and also the height compared to PRP

Bezdin. In what concern the statistical parameters of central tendency, was determined the arithmetical mean diameter which reaches the value of 22,13 cm for PRP Bezdin and 17,09 for PRP Ceala. The minimum and maximum, characteristics of this areas have defined limits by the intervals 2-88 cm for diameters, and 2-45 m for heights, highlighting a great diversity, due to uneven-aged structure of stands which are in the permanent research plots.

The relationships between diameter and height of tree is highlighted by the connection between the most fundamental characteristics of stand, diameter and height, used for growth describing and forest good development (Meng et all, 2008). In the past, this connection was studied by various foreign researchers (Huang et al., 1992, Peng et al., 2001, Calama and Montero, 2004, Temesgensi Gadow, 2004, Sharma and Parton, 2007, Temesgen et al., 2008), but also by Romanian researchers (Prodan, 1965, Giurgiu, 1969, 1974, 1999, 2004). The graphical representation of heights curves has, in most of the cases, an ascendant bearing, which linearize gradually, following the "normal, biological motivated" form from literature (Giurgiu, 1999, 2004) (Fig. 2).

In many instances, the most suitable relationship for describing the connection between diameters and heights was relation (3) used forPRP Ceala – Common ash, PRP Ceala – White poplar and PRP Ceala – Elm tree.In the past, this equation was used with success for describing the flood plain stands (Trettinand Stringer, 2016).

Relation (2), likewise, has registered good results, curve bearing being characteristic for relationship diameter-height. The connection between those two variables was represented through the value of correlation coefficient, and to check if the used equation was the most suitable it was calculated the value of standard deviation (Table 3).

Table 3: Main statistical parameters from permanent research plots

Statistical parameters	PRP Bezdin			PRP Ceala		
	Common Ash	Common Maple	Oak	Common ash	White Poplar	Elm
Standard deviation	2,35	3,18	0,09	0,07	0,05	0,08
Correlation coefficient (r)	0,71	0,68	0,82	0,89	0,81	0,93

Thus, can be observed that the equations used for describing the relationship diameter-heights follows the criteria from literature (Giurgiu et al., 2004) with regard to standard error, having values between 0,05 and 3,18.In what concern the correlation between this two variables, can be appreciated that in many instances have a strong connection (PRP Bezdin – Oak, PRP Ceala – Common ash, PRP Ceala White poplar and PRP Ceala – Elm). PRP Bezdin – Common ash and PRP Bezdin Common Maple, don't follows this trend, them have a connection with medium intensity between variables. This aspect is due to

lower participating proportion of these species in forest stand composition. Also, these values can be found in the determined limits in other studies, respectively 0,6 and 0,9 (Giurgiu, 1979).

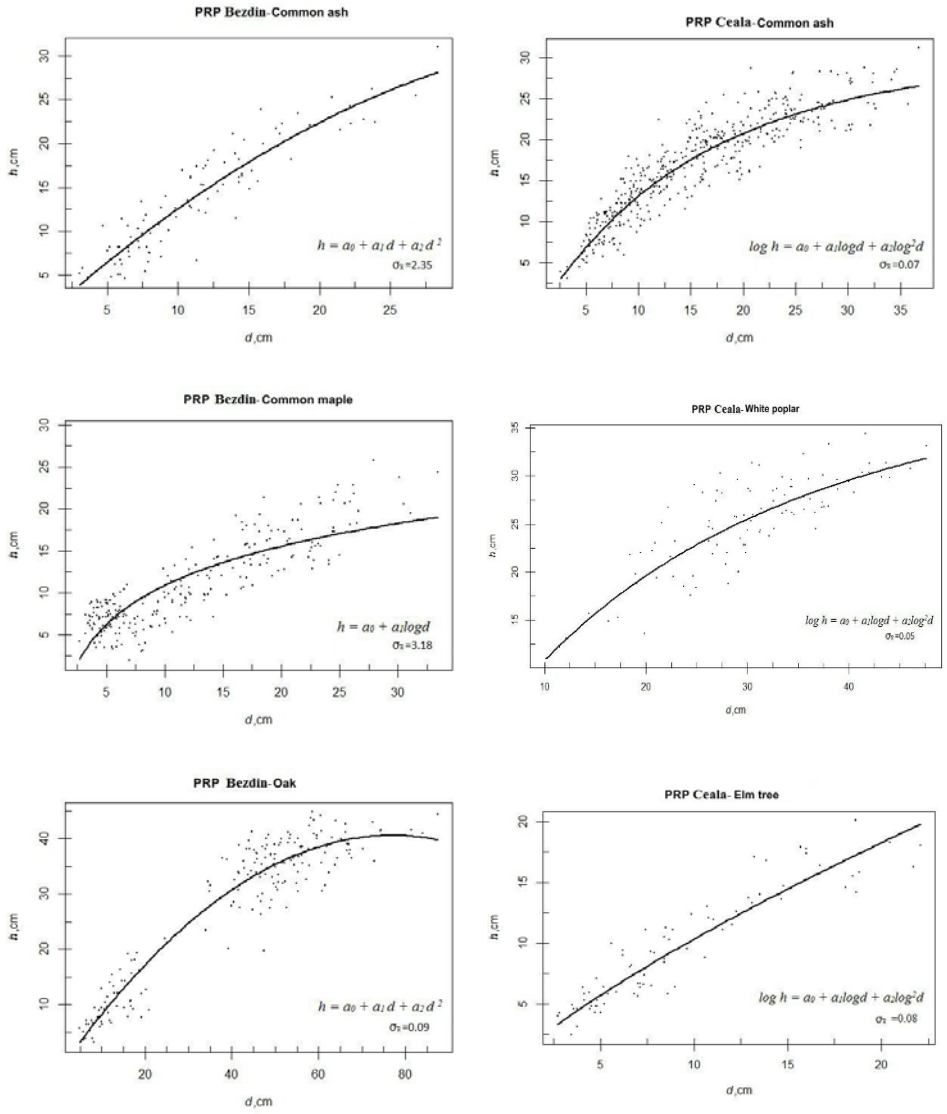

Figure 2: The correlation between diameter and height for permanent research plot

Further to highlight clearer the intensity of diameter-height connection, it was passed to graphical representation of height variability, expressed through variation coefficient ($S\%_h$) in relation with the diameter (Fig. 3).

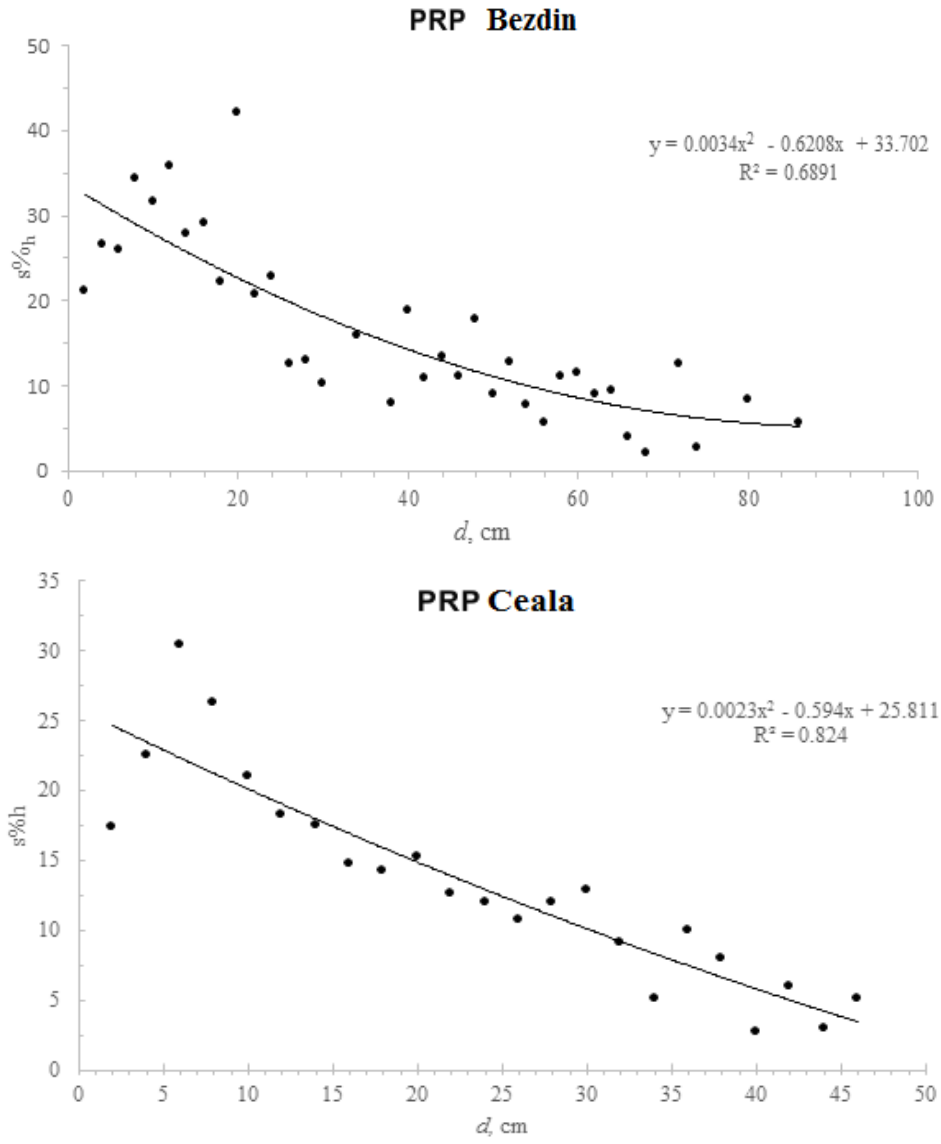

Figure 3: Distribution of variation coefficient for height in relation with diameter from permanent research plots

It can be observed that the studied stands follow the lawfulness according to which the stands with heliophilous species (oak, sessile oak, poplar etc.) have small values for variation coefficient of heights (Giurgiu, 1999). For the stand from PRP Bezdin, where can be found trees with large diameters can be observed a small stability near the 50' cm diameter(d_{50}), reference diameter for indicator height h_{50}, characteristic for uneven-aged stands, coming to support

the hypothesis from literature according to which the stand shows a maximum stability at the level of d_{50} (Chivulescu et al, 2014). In accordance with the same hypothesis, after this reference diameter, the variability should grow, but in the case of stand from PRP Bezdin, it does not happen. This aspect is due to the old age of stand (Giurgiu, 1999), respectively105 years (I.C.A.S., 2012), number of trees from the higher categories considerable reducing, make it this way a instability in the structure of stands.

In the case of stand PRP Ceala, it can be observed that the distribution curve form of variation coefficient for height in relation with the diameters is exponentially negative, where many value can be found in the small categories of diameters, this fact is explained by the inhomogeneity of stands due the presence of more stands elements (Ichim, 1968; Assman, 1970; Roibu, 2010).

Also through all mentioned above, can be noticed the gradual deletion of oak from pond stand composition, making possible in the future a complete deletion of this kind of ecosystems. Most likely, the cause of these strikes from natural cycle of these ecosystem is due to climate change in the last period. Some of these aspects it would be clarified in the following studies regarding stands auxology and the influence of disruptors factors, like climate effects on those.

CONCLUSION

In the last period, a considerable drop has developed of the forest stands from flood plain rivers, and a detailed research regarding this depreciation is absolutely necessary to understand the cause of it. The actual studies are a part from a series of multidisciplinary researches, conducted in an institutionalized background, highlighting in this way the enhancement of researchers and decisional forums preoccupation regarding this subject.

The researches were conducted in two uneven-aged stands of oak and white poplar from Mureș flood plain, situated across Natural Park "Lunca Mureșului". The research methods were based on enshrined methodologies in forestry domain, but also modern ways like automatically processing of data. In this way, in shorter time were tested a series of regression equations for the relationships diameters-heights, finally choosing the most suitable solution, taking into account all validation criteria.

Thus, were determined the main statistical parameters, highlighting, with small exceptions, the similarity of stands structure. The form of the most heights curves is "a normal one, biological motivated" taking into account in this way the lawfulness regarding their form from literature. Also at these stands was remarketed a strong link between diameters and height, fact highlighted through the values of the correlation coefficient, with a range between 0,68 and 0,93. Small strikes from this strong link were possible due to the lower participating proportion of these species in the stand total composition.

The used equations for describing the relation between those two variables (diameters and heights) were most suitable, fact proven by the values of standard error, ranging 0,05 and 3,18.

In the case of the stand from PRP Bezdin, were can be found also trees with bigger diameters, was identified a small stability around diameter d_{50}, reference diameter for height h_{50}, demonstrating in this way the theory according to the fact that in stands with selection system, the reference diameter for indicator height is that one previously mentioned. According to the same theory the variability should grow after this diameter, aspect not proven in the case of the studied stands in this paper, due the lack of superior diameter. This aspect is due to the old age of stand and climate change from the last period, which have a great influence on the trees vitality located in the permanent research plots.

For materialization of these researches in the future will be set more ample studies regarding the trees auxology and the influence of climatic factors on these ecosystems.

REFERENCES

Assman, E., 1970. The principles of forest yield study. Trad. Inglês: SH Gardiner.

Badea, O.,2008: Manual privindmetodologia de supravegherepetermen lung a stăriiecosistemelorforestiereaflate sub acţiuneapoluăriiatmosfericeşimodificărilorclimatice , EdituraSilvică, Voluntari, 98p.

Bates, D. M. &Chambers, J. M. (1992) Nonlinear models. Chapter 10 of Statistical Models in S, Editors J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole

Briner, S., et al., A., 2012. Assessing the impacts of economic and climate changes on land-use in mountain regions: A spatial dynamic modeling approach. Agriculture, Ecosystems & Environment, 149, pp.50-63.

Calama, R. &Montero, G., 2004. Interregional nonlinear height diameter model with random coefficients for stone pine in Spain. Canadian Journal of Forest Research, 34(1), pp.150-163.

Chambers, J.M. & Hastie, T.J., 1991. Statistical models in S. CRC Press.

Chivulescuet et al., 2016. Structural Features of virgin beech forests in Semenic mountains. The dynamic structure of virgin beech forest P20 Semenic between 2005–2013.

Clinovschi, F.&Palaghianu, C., 2007. Studiuasuprastructuriivegetatieilemnoase din RezervatiaZamostea-Lunca. AnaleleUniversitatii Stefan cel Mare Suceava-SectiuneaSilvicultura, 9(1), pp.19-28. ,

García-Nieto et al., 2013. Mapping forest ecosystem services: from providing units to beneficiaries. Ecosystem Services, 4, pp.126-138.

EEA ,2000. Corine Land Cover, European Environment. Agency (EEA). Available at: http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-2

Giurgiu, V., 1969. Dendrometrie. Ed. Agro-Silvică. 1974,

Giurgiu, V., 1979: Dendrometrics and forestry auxology, Editura Ceres, Bucureşti, 692p.

Giurgiu, V., 1999: Corelaţiadintreînălţimileşidiametrelearborilorînarboreteleechieneşipluriene din România, Giurgiu 1999, Silvologie, vol. II, EdituraAcademieiRomâne, Bucureşti,1999, 9-64p.

Giurgiu, V.,2004: Metodeşitabeledendrometrice, Editura Ceres, Bucureşti, 575p.

Huang, S., Titus, S.J. and Wiens, D.P., 1992. Comparison of nonlinear height–diameter functions for major Alberta tree species. Canadian Journal of Forest Research, 22(9), p.1297-1304.

I.C.A.S., 2012. Amenajamentul silvic U.P. V Bezdin

Meng, S.X., et al.,2008. Wind speed and crown class influence the height–diameter relationship of lodgepole pine: nonlinear mixed effects modeling. Forest Ecology and Management, 256(4), pp.570-577.

MinisterulMediuluișiPădurilor (MMP), 2014, Limitele GIS ale ariilornaturaleprotejate de interes national /international, data available through next link: http://www.mmediu.ro/articol/arii-naturale-protejate/33

Ojea, E., Martin-Ortega, J. &Chiabai, A., 2012. Defining and classifying ecosystem services for economic valuation: the case of forest water services. Environmental Science & Policy, 19, pp.1-15.

Peng, C.et al., 2001. Developing and validating nonlinear height–diameter models for major Tree species of Ontario's boreal forests. Northern Journal of Applied Forestry, 18(3), pp.87-94.

Prodan, M., 1965. Holzmesslehre (p. 644). JD Sauerländer.

Radu, I., 1968. Variabilitateacaracteristicilordendrometrice ale arborilor din arboretele de molidexploatabileși de tip regulat.Revistapădurilor

Roibu, C.C., 2010. Biometrics and dendrochronological researches in beech forests from Suceava Plateau at Eastern limit of the European areal (Doctoral dissertation, PhD thesis," Ștefancel Mare" University, Suceava 274 p.

Temesgen, H. &Gadow, K.V., 2004. Generalized height–diameter models—an application for major tree species in complex stands of interior British Columbia. European Journal of Forest Research, 123(1), pp.45-51.

Temesgen, H., et al., 2008. Analysis and comparison of nonlinear tree height prediction strategies for Douglas-fir forests. Canadian Journal of Forest Research, 38(3), pp.553-565

Trettin, C.C., et al., 2016. Composition, biomass and structure of mangroves within the Zambezi River Delta. Wetlands Ecology and Management, 24(2), pp.173-186.

Sharma, M. & Parton, J., 2007. Height–diameter equations for boreal tree species in Ontario using a mixed-effects modeling approach. Forest Ecology and Management, 249(3), pp.187-198.

Silaghi, D.,&Chisaliță,I. 2014. Acțiuneaschimbărilorclimaticeși a diferiților factori de stress asuprastăriiecosistemelorforestiere din Parcul Natural LuncaMureșului – 14.17. Raportștiințificanual

Stănescu, V., 1979. Dendrologie. EdituraDidacticășiPedagogică.

Zhang, L.,1996: Cross-validation of Non-linear Growth Function for Modelling Tree Height-Diameter Relationships, Annals of Botany 79, 251-257p., 1997.

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PHYTOSOCIOLOGIAL STUDY OF RABOR REGION, KERMAN, IRAN

SUMMARY

The patterns of plant associations are correlated with environmental factors. Therefore, information on association between vegetation and ecological factors is important for understanding the stability and dynamics of plant communities. This work studied phytosociology of Rober (Kerman, Iran) by analyzing the associations between vegetation and environmental factors based on Braun Blanquet method using 25 randomly sampled relevés (plots or quadrats) and determining growth form based on Raunkiaer. The relevé size was determined using Minimal Area method. For vegetation data, species richness and evenness, and percentage plant surface covers were recorded within the relevés. For environmental data, different edaphic and topographic information e.g. soil moisture, pH, electrical conductivity, slope degree and direction and altitude were measured for the relevés. Similarity among relevés was estimated using cluster analyzing based on Ward' coefficient. Canonical Corresponding Analysis (CCA) was used to study the relationship between relevés and environmental factors and to determine the most important environmental factors affecting the relevés distribution. A total of 34 species was identified. The species number per relevé varied from 23 to 29 with average of 25.2. Analyzing of species diversity among therelevés using diversity indices of Simpson, Shannon-H, Menhinick and Margalef showed that three relevés with 23 species and only one out of 8 relevés with 24 species had smallest species diversity, while relevé with 29 species had highest diversity level. Cluster analysis showed the study site composed of three sub-associations. CCA indicated that different edaphic and topographic variables affected differently on the distribution of the relevés.

Keywords: canonical correspondence analysis, phytosociology, species richness and evenness, vegetation analysis, relevés

INTRODUCTION

The development of human civilization in the last centuries has caused different environmental problems including the habitat destruction and

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fragmentation that consequently have dramatically changed the structure, distribution and functioning of natural ecosystems (Saunders etal., 1991; Vitouseket al., 1997). The fragmentation and destruction of the habitats has consequently caused loss of biodiversity (Sala et al., 2000;Balmfordet al., 2005;Piessens et al., 2005) and also produced main problems in the conservation biology (Fahrig, 2003;Hanski, 2005;Strantfordand Robinson, 2005).

The first changes in the environments can be observed in the vegetation. Therefore, the knowledge on vegetation pattern and its relationship with environment shave vital importance in surveying and/or monitoring of the environment and conservation biology.

Phytosociological studies mostly focused on the interaction between plant communities and the environmental factors e.g.topography, soil properties and human inferences such as grazing. Therefore, these studies are necessary for designing of the conservation programs in order for protecting the natural plant communities and biodiversity as well as understanding the vegetation changes (occurred in the past) and vegetation dynamics in the future.

Because of long phytosociological tradition, Europe has many more vegetation relevés than any other part of the world. In the last decade many electronic databases e.g. TURBOVEG (Hennekens and Schaminee, 2001)designated for vegetation plots especially phytosociological relevés were established in many European countries, which included over 4,300,000 vegetation-plots (Schaminee et al., 2009).However, the vegetation studies conducted so far in Iran are not sufficient for constructing a complete vegetation map of Iran, although many studies were recently carried out in different regions i.e. Arasbaran Forestland (Ebrahimigajoti et al., 2013) Alborze Mountanins (Naqinezhad et al. 2012; Noroozi et al. 2014; Ravanbakhsh et al. 2016).

Iran has over 1.6 million Km^2 with two Mountains Chainsof Alborz and Zagrosstretching in Northwest-Northeast and northwest- southeast directions, respectively. They determine the precipitation distribution in the country so that a very large part of the country located between these Mountains Chains in the middle of the country is very dry and has two large deserts.This Central part of the country is a high plateau. The major part of the country has arid or semi-arid climate, except the coastal area of Caspian Sea in the North and Western parts of the country. The precipitation in the country is mainly affected by Mediterranean synoptic systems. Therefore, the amount of the rainfall gradually decreases from west to east of the country. There are four vegetation zones in Iran based on Zohary (1973): 1) Hyrcanian zone, which covers the coastal area of Caspian Sea, 2) Zagross zone, which extends along Zagross Chain Mountains in the West of the country, 3) Khalij-o-Ommanian zone covers the coastal areas of Persian Gulf and 4) Iran-o-Turanian zone, which is the largest vegetation zones and covers the rest of the country including the Central Plateau. The last vegetation zone includes two large deserts i.e. a vast saline desert of Dasht-Kavir in the north and a sandy very hot desert of Dasht-Lut in the south(Zohary, 1973).

The impact of environmental variations on species richness has been less investigated using field data, especially at local scale (Statzner and Moss, 2004). The study site is located in Rabor Town, Kerman Province in Southeast part of the Central plateau between Baft Town and desert of Dasht-Lut with dry climate.

Due to a worrying situation on loss of biodiversity and environmental problems e.g. global warming and ongoing changes in land use, there is an urgent need for wide-scale scientific and applied vegetation research (Schaminee et al., 2009) especially in developing countries. This work aimed to carry out a phytosociological survey in South of Iran.

MATERIALS AND METHODS

Study site

The study site is a Protected area and situated in Kerman Province between Kerman and Jiroft cities (latitude: 29°, 15' N; longitude: 57°, 7' to latitude: 29°, 20' N; longitude: 57°, 6'). The area covers 2600hc and its altitude ranges from 2269m to 3652m.

Vegetation data

The size of relevé in the study site was determined using the Minimal Area method. Consequently, a number of 25 quadrats were randomly sampled in the site. The vegetation data for each relevé were recorded on the basis of species number/relevé (N= the presence and absent of species), individual numbers of a given species/relevé, percentage plant surface cover (%COV), total plant surface cover (COV), surface cover of plant base (P) and surface cover of plant canopy (A).

Environmental data

For environmental data several edaphic and topographic factors including soil moisture (SM), soil acidity(pH), soil electrical conductivity (EC), slope angle degree(Slope A),slope direction (Slope D) and altitude from the sea level (Alt) were studied. Soil moisture, EC and pH were measured following Seshagiri (2013) and Piper (1947).

The level of species diversity was estimated for the relevés using different diversity coefficient indices i.e. Simpson, Shannon-H, Menhinick and Margalef. Consequently, species evenness was calculated for each relevé based on Shannon's diversity index. The species evenness calculated the similarity among species within a given relevé in terms of individual numbers.

The levels of similarity among the relevés were estimated using clustering analysis on the basis of the Ward's similarity index using all vegetation data: the presence and absent of species, individual numbers of a given species per relevé, percentage plant surface cover, total plant surface cover, surface cover of plant base and surface cover of plant canopy.

To determine the relationship among the environmental and vegetation data, the ordination analysis of Canonical Correspondence Analysis (CCA) was conducted in order to find out the most effective environmental factors on the distribution of relevés.

RESULTS AND DISCUSSION

Using Minimal Cover Area method, the size of relevé was determined to be 16×16m. A total of 34 species was identified in 25 relevés sampled in the study site with average richness value (species/relevé) of 25.2 (Table 1). The systematic identification of the plant species was carried out using Flora Iranica (Rechinger 1963 -2005) and double checked with herbarium type specimens. The smallest number of species/relevé (23 species) was found in relevés number 17, 21 and 25, while the highest value of species/relevé (29 species)was found in relevé number 1.The dominant species among all plots was *Juniperus excelsa*, which occurred in all 25 relevés with a total number of 117 individual plants, averaging 4.7 plant per plot. Therefore, the site was herein called Juniperetum association. The individual plant number of the species within the relevés ranged from 2 to 9 plants with overage of 4.7.

Table 1: The alphabetic order of the species identified within 25randomly sampled relevés in Juniperetum association

1	*Acer monspessulanum* L.	18	*Ephedra intermedia* Schranket C. A. Mey.
2	*Aegopordon berardioides* Boiss.	19	*Euphorbia hebecarpa* Boiss.
3	*Allium scabriscapum* Boiss. & Ky.	20	*Geranium tuberosum*L.
4	*Alyssum inflatum* Nyarady.	21	*Hieracium hoppeanum* Schultes.
5	*Artemisia Aucheri* Boiss.	22	*Hordeum bulbosum* L.
6	*Astragalus cephalanthus* DC.	23	*Ixiolirion tataricum*(Pall.) Herb.
7	*Astragalus microcephalus* Willd.	24	*Juniperus excels* M. B.
8	*Astragalus rhodosemius* Boiss.	25	*Muscari longipes*Boiss.
9	*Astragalus spachianus* Boiss. & Buhse.	26	*Oryzopsis barbellata* (Mez.) Bor.
10	*Bromus rubens* L.	27	*Paronychia Bungeii* Boiss.
11	*Campanula kermanica* Rech. f.	28	*Pistacia atlantica*Desf.
12	*Cirsium* sp.	29	*Sonchus asper* (L.) Hill.
13	*Colchicum Schimperi* Janka.	30	*Stipa parviflora* Desf.
14	*Cotonea sterintegerrima* Medicus.	31	*Thalictru misopyroides*C.A.Mey.
15	*Cousinia calcitrapa* Boiss.	32	*Tragopogon crocifolius* L.
16	*Daphne mucronata* Royle.	33	*Vulpia myuros* (L.) C.C. Gmel.
17	*Ebenus stellate* Boiss.	34	*Ziziphora tenuior* L.

Analyzing of species diversity among 25 relevés in the study site using different diversity indices showed that the relevés number 4 (with 24 species), 17, 21 and 25 (all with 23 species) had the smallest diversity level, while plot number 1 with 29 species had the highest diversity coefficient (Table 2). For example, based on Simpson diversity coefficient, relevés number 4, 17, 21 and 23 had smallest diversity level of 0.9565 while relevé number 1 had highest level of 0.9655. Similarly, based on Shannon, relevés No. 4, 17, 21 and 23 had minimal species diversity of 3.135 while relevé No. 1 had largest value of 3.367.

These values on the basis of Menhinick diversity coefficient were 4.796 against 5.385, and based on Margalef diversity coefficient were 7.016 versus 8.315 (Table 2). The species diversity coefficient among the relevés is similar. This indicates that the distribution of the species is constant in the study area. Moreover, the values of Simpson diversity coefficient are close to 1 in all of the relevés , indicating that the species richness within the relevés is high.

The levels of species richness in 23 out of 25 relevés were larger than 0.9998 with a range varying from 0.9865 to 1.0000, (Table 2).

Table 2: Species richness and evenness in 25 relevés in Juniperetum vegetation

Relevé No.	No. of Species (Richness)	Diversity indices				Evenness (Shannon)
		Simpson-1-D	Shannon H	Menhinick	Margalef	
1	29	**0.9655**	**3.367**	**5.385**	**8.315**	0.99991
2	28	0.9630	3.296	5.196	7.889	0.98913
3	26	0.9615	3.258	5.099	7.673	0.99997
4	24	**0.9565**	**3.135**	**4.796**	**7.016**	0.98645
5	26	0.9615	3.258	5.099	7.673	0.99997
6	24	0.9583	3.178	4.899	7.237	0.99998
7	24	0.9583	3.178	4.899	7.237	0.99998
8	26	0.9615	3.258	5.099	7.673	0.99997
9	24	0.9583	3.178	4.899	7.237	0.99998
10	26	0.9615	3.258	5.099	7.673	0.99997
11	27	0.9630	3.296	5.196	7.889	1.00005
12	24	0.9583	3.178	4.899	7.237	0.99998
13	23	0.9583	3.178	4.899	7.237	1.01356
14	26	0.9615	3.258	5.099	7.673	0.99997
15	27	0.9630	3.296	5.196	7.889	1.00005
16	26	0.9615	3.258	5.099	7.673	0.99997
17	23	**0.9565**	**3.135**	**4.796**	**7.016**	0.99984
18	24	0.9583	3.178	4.899	7.237	0.99998
19	25	0.9600	3.219	5.000	7.456	1.00004
20	24	0.9583	3.178	4.899	7.237	0.99998
21	23	**0.9565**	**3.135**	**4.796**	**7.016**	0.99984
22	26	0.9615	3.258	5.099	7.673	0.99997
23	26	0.9615	3.258	5.099	7.673	0.99997
24	24	0.9583	3.178	4.899	7.237	0.99998
25	23	**0.9565**	**3.135**	**4.796**	**7.016**	0.99984

The variations of environmental factors among 25 relevés in the study site are shown in Table 3. The soil EC among relevés ranged from 146 to 357, and the SM varied from 28 to 46. The range of variation of soil pH was 7.8 - 8.7. Slope A varied from 21 to 44 degree while Slope D ranged from 45 to 360 (Table 3).

Correlation analysis showed that species diversity coefficient werevery significantly correlated with the number of species in the relevés (Figure1; N=25, V_{alue}< 0.00001, at P <0.05 level, Pearson rank correlation test).

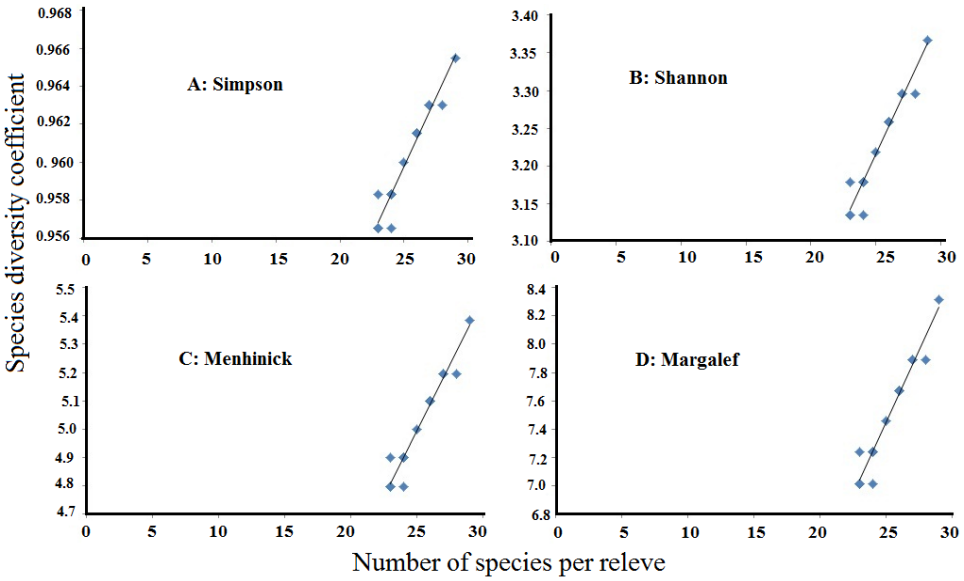

Figure 1. The positive significant correlation between species number and diversity coefficient among 25 relevés.

The clustering analysis of the relevés based on the similarity showed that the study site composed of three sub-associations (A, B and C in Figure 2). The sub-association A comprising of the relevés numbers 1, 2, 3, 4, 5, 7, 12, 13, 16, 17 was mostly affected by soil EC and pH (Figure 3), while the environmental factors of slop-A had strong impact on the sub-association C, which is composed of plots numbers 9, 14, 22, 23. SM had higher impact on plots 19 and 24 (Figure 3).

The ordination analysis of CCA between environmental and vegetation data revealed that the highest value of total vegetation variations (48.83%) was reflected in axis 1 while small amount of the total variation (21.73 %) was shown in the axis 2 (Figure3). CCA also revealed the impact of environmental factors on percentage plant surface covers (% COV), individual numbers of species (N) and cover surface of plant base (P) in the study site.

Furthermore, the impact of environmental factors on the distribution of the relevés was diverse. In other words, the relevés were differently affected by the

environmental factors. For instance, the relevés numbers 14, 15 and 22 were strongly affected by COV and %COV, while the relevés numbers 25 and 23 were respectively influenced by N and P, whereas soil pH had strong impact on relevés 3 and 11, while relevé 19 was affected by soil moisture (SM).

Table 3: Variation of environmental factors among 25 relevés in Juniperetum vegetation

Variable	Minimum	Maximum	Mean	S.D.[1]
EC	146.0	357.0	222.7	64.4
pH	7.8	8.70	8.30	0.25
Soil Moisture	28.0	46.0	38.60	5.25
Altitude from sea level	2269.0	3652.0	2585.3	285.75
slop A	21.0	44.0	30.5	5.4
slop D	45.0	360.0	183.6	105.5

[1]: S.D. = Standard Deviation

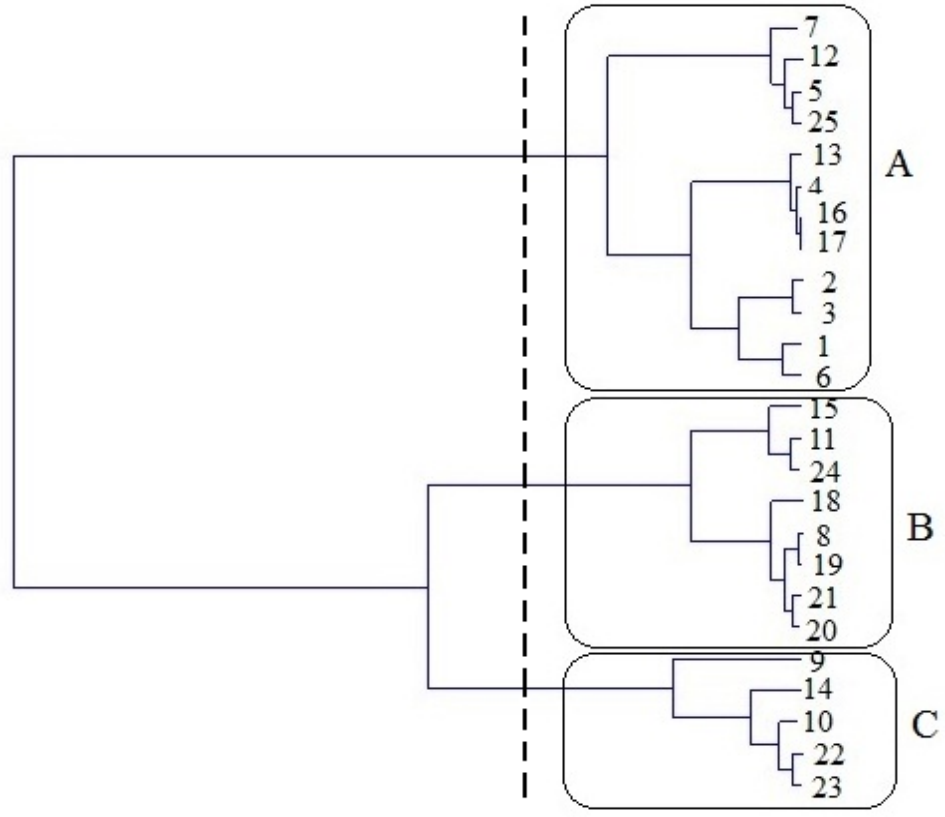

Figure 2: Clustering analysis of the relevés in the study site based on Ward's similarity showed that the vegetation of the site is composed of three sub-association of A, B and C (the broken line indicates the cutoff point, determined by clustering software analysis).

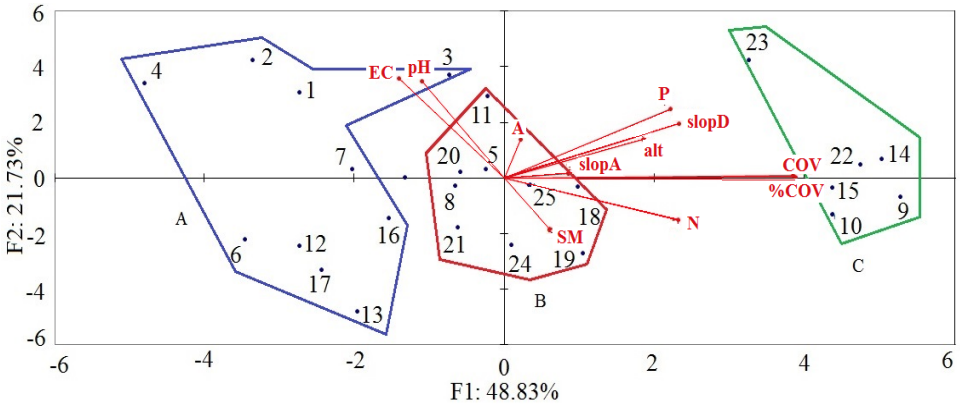

Figure3. Ordination analysis showing the relationship between environmental (vectors) and vegetation (numbered dots representingrelevés)componentsin thestudy sitebased on the CCA. The classification of the relevés into three sub-association was based on clustering analysis (% COV= Percentage of plant covers surface; COV = total plant covers surface, P= covers surface of plant base, A= cover surface of plant canopy, N= species number/relevé, SM = soil moisture, pH= soil acidity, EC = soil electrical conductivity, Slope A = slope angle degree, Slope D slope direction, Alt = altitude from the sea level).

Based on the vegetation classification form of Raunkiaer, the majority (30%) of the species growing in the study site were the rophytes followed by cryptophytes (28%), of which 26% was geophytes.

Grouping the species growing in the study site in three different clusters indicates that there are three vegetation types, which might be corresponds with three sub-associations. However, the high similarity of indices in the species evenness among all 25 relevés sampled in the study site (close to 1) indicates that the variation among relevés is very low. This shows that the study site is a consistent plant vegetation. In the current study the results obtained from ordination and clustering analyses were corresponding with each other, both showing the three sub-associations in the study site. Similar results were reported by a phytosociological study in North of the country, in which both clustering and ordination analyses identified two corresponding vegetation types (Naqinezhad et al. 2012). While a phytosociological investigation in Arasbaran Forestland recognized six vegetation types while ordination analysis identified only three groups (Ebrahimigajoti et al. 2010).

Despite locating in semi-arid region, the vegetation types of the study site were not strongly affected by soil moisture. This fact can be also implied from the fact that in the species compositions of the relevés there were not xerophyte plants (see Table 1).The species composition of the vegetation types recognized in the present study were different from those of other vegetation types identified in other semi-arid neighboring regions in the country reported by previous studies (Akhani and Ghorbanli, 1993).

CONCLUSION

This study showed that different ecological factors (i.e. edaphic and topographic) had differently impact on the species composition of the study site.

The current study showed that different environmental factors have diverse impact on distribution of different species compositions in any plant vegetation. Moreover, detecting the most affecting environmental factors on the species biodiversity and composition can be used in management of plant vegetations and renovating of the destruction sites of plant vegetations.

REFERENCES

Akhani H., Ghorbanli M.(1993). A contribution to the halophytic vegetation and flora of Iran, in H. Leith and A. Al- Masoom (eds.). Towards the Rational use of High Salinity Tolerant Plants, vol. 1, p. 35-44, Kluwer Academic publishers, Netherlands.

Balmford A., Bennun L., Brink BT., Cooper D., Cote LM., Crane P. (2005). The convention on biological diversity, 2010 target. Science, 307: 212-213.

Braun BlanquetJ. (1983). *Plant Sociology*. Translated by G. D. Fuller and H. S. Conard. McGraw.Hill Book Company, Inc., New York, 439 p.

Ebrahimigajoti T., Nosrati H., Razban-Haghighi A., Khanbabai M. (2013). Studying biodiversity of plant associations in sutan-Chay Basin in Arasbaran, Northwest of Iran. Agriculture & Forestry, 59(1): 85-98.

Fahrig L. (2003) Effects of habitat fragmentation on biodiversity.Annual Review of Ecology, Evolution and Systematics.34: 487-515.

Hanski I. (2005). The Shrinking World: Ecological Consequences of Habitat Loss. International Ecology Institute, Oldendorf/Luhe, Germany.

Hennekens SM., Schaminee JHJ. (2001). TURBOVEG, a comprehensive data base management system forvegetation data. Journal of Vegetation Science. 12: 589–591.

Naqinezhad A., Bahari SH., Gholizadeh H.,Esmaeili R., Hamzeh'ee B., Djamali M., Moradi H. (2012). A phytosociological survey of two lowland Caspian (Hyrcanian) remnant forests, Northern Iran, for validation of some forest syntax. PhytologiaBalcanica 18(2): 173-186.

Noroozi J., Willner W., Pauli H.,Grabherr G. (2014).Phytosociology and ecology of the high-alpine to subnival scree vegetation of N and NW Iran (Alborz and Azerbaijan Mts.).Appl Veg Sci, 17(1): 142–161. DOI:10.1111/avsc.12031

Piessens K., Honnay O., Hermy M. (2005). The role of fragment area and isolation in the conservation of heathland species.Biological Conservation. 122: 61-69.

Piper CS. (1947). Soil and Plant Analysis. 1st Edn.,University of Adelaide, Australia, pp: 368.

Ravanbakhsh H., Hamzeh'ee B., Etemad V., Mohadjer MR.,Assadi M. (2016).Phytosociology of *Juniperusexcelsa*M.Bieb. forests in Alborz mountain range in the north of Iran. Plant Biosystems 150 (5)

Rechinger KH. ed., *Flora Iranica: Flora des iranischenHochlandes und der umrahmendenGebirge*, Graz, Austria, 1963- (in progress).

Sala OE., Chapin FS., Armesto JJ. (2000). Global biodiversity scenarios for the year 2100. Science 287: 1770-1774.

Saunders DA., Hobbs RJ.,Margules CR. (1991). Biological Consequences of Ecosystem Fragmentation: A Review.Conservation Biology, 5(1) 18-32.

SchaminnJHJ.,Hennekens SM., Chytry M., Rodwell JS. (2009). Vegetation-plot data and databases in Europe: an overview*Preslia 81: 173–185,*

Seshagiri-Rao P, Sujatha B, Lakshminarayana K, Ratnam SV. 2013. A study on phytosociology, soil conservation and socio-economic aspects in red sand dunes near Bhimili of Visakhapatnam.Archives of Applied Science Research, 5 (1):45-56.

Statzner B., Moss B.(2004). Linking ecological function, biodiversity and habitat: a mini-review focusing on older ecological literature. *Basic Applied Ecology*. 5:97-106.

Strantford JA., Robinson WD. (2005). Gulliver travels to the fragmented tropics: geographic variation in mechanisms of avian extinction. Front Ecology and Environment, 3: 85-92.

Vitousek PM., Aber JD., Howarth RW. (1997). Human alteration of the global nitrogen cycle: sources and consequences. Ecological Application. 7, (3) 737–750.

Zohary M. (1973). Geobotanical foundations of the Middle East, volume1,2. Gustav Fischer Verlag, Stuttgart,Germany.

DOI: 10.17707/AgricultForest.63.2.05

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DIFFERENCES IN WATER DEFICIT ADAPTATION DURING EARLY GROWTH OF BRAZILIAN DRY FOREST CAATINGA TREES

SUMMARY

Previous studies have shown that adaptation to drought in Brazilian Caatinga trees may involve different physiological and morphological strategies. Particular climatic and soil characteristics occur along this dry ecosystem leading to non-homogeneous water availability. Therefore, in certain niches, some species predominate over others, indicating that best performance of a species is not widespread. We studied changes in initial growth, which is the most critical factor during the life of a plant, of two common species of Caatinga (*Erythrina velutina* Willd. and *Enterolobium contortisiliquum* (Vell.) Morong) with variable occurrence through the ecosystem. Young plants were subjected to three water regimes: 450 (control), 225 (moderate stress) and 112.5 mm (severe stress) of water spread over 40 days, which represent years with heavy, moderate and scarce rainfall, respectively. Analyses were performed at 20 and 40 days after the initiation of treatments. When compared to the control group, treatments with water restriction reduced the growth of shoots and roots, the number of leaves and leaflets, the total biomass and the leaf area more in *E. velutina* than in *E. contortisiliquum*. Taking into account the adaptation of both species in Caatinga, we present evidence of different drought tolerance strategies. The ecological importance of early changes in the growth of species is discussed in the paper.

Keywords: Arid environment, *Enterolobium contortisiliquum* (Vell.) Morong, *Erythrina velutina* Willd., initial growth, water stress.

INTRODUCTION

Caatinga is the predominant biome in the semiarid region of north-eastern Brazil. Most of its area presents average annual rainfall lower than 1,000 mm (Menezes *et al*. 2012). Nonetheless, some very dry years often occur, with the last example being 2012 (Santos *et al*. 2014). Due to variable soil depth, which is most often low (Menezes *et al*. 2013), and local climate characteristics, the water availability is concentrated in some months but non-homogeneous over Caatinga. Environmental degradation in many areas has been observed particularly due to slash-and-burn systems of agriculture, overgrazing and intensive fuel wood extraction and also due to soil erosion processes (Menezes *et al*. 2012).

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

Water stress is one of the major limiting factors for plant growth and development (Shane *et al*. 2010).In Caatinga, the availability of water affects the abundance of species in a population (Andrade *et al.* 2009). In such conditions the plants must balance strategies to maintain growth and to survive (Claeys and Inzé 2013). On the other hand, moderate drought can increase root growth (Baluska *et al*. 2010) and the net accumulation of solutes per cell (Krasensky and Jonak 2012), which ensures the exploration of soil layers that still have available water. Studies of the ecosystem have led us to suggest that these mechanisms are present but with variable intensities over Caatinga species.

The initial growth is generally critical for most plants species (Rathcke and Lacey 1985). *Erythrina velutina* Willd., popularly known as mulungu, and *Enterolobium contortisiliquum* (Vell.) Morong, popularly known as tamboril or orelha de nego, both belong to the Fabaceae family and occur naturally in Caatinga. In certain niches, one species is abundant over the other, but this is not reported in other niches, suggesting different adaptation strategies to drought between species. In a greenhouse experiment, we aimed to study changes in the initial growth of the two species under different water regimes, which could simulate differences in water supply over growth seasons and/or local niches. We also studied the morphological characteristics of leaves, which may be related to drought adaptation strategies. This paper contributes to the understanding of water adaptation strategies of Caatinga trees.

MATERIALS AND METHODS

The study was carried out in a greenhouse in the Unidade Acadêmica Especializada em Ciências Agrárias (UAECIA) of Universida de Federal do Rio Grande do Norte located in the district of Jundiaí, Macaíba-RN, Brazil. Duringthe experiment the average temperature in the greenhouse was25.9°C.

For both species, these eds were removed from fruits immediately after collection and stored in glass containers. The sowing was conducted in polyethylene pots of 10 x 15 cm (diameter x height) with soil from UAECIA. Analysis of soil fertility was performed by Empresa de Pesquisa Agropecuária do Rio Grande do Norte, Brazil (EMPARN) (Table 1). Each pot received three seeds sownata depth of 0.5 cm. The pots were watered daily in the evening with distilled water to the field capacity. Twenty-one days after sowing, thinning was carried out, leaving one plant per pot.

The pots containing one plant each were divided into three groups, each subjected to a water regime as follows: 450 (control), 225 (moderate stress) and112.5 mm (severe stress) distributed over 40 days. The water regimes tested simulate years with good rain, as well as dry and very dry, in East Seridó of Rio Grande do Norte State, according to EMPARN (2016) historical data. The pots were randomly distributed in the greenhouse. At this stage, irrigation occurred at intervals of two days. To calculate the daily amount of water to be applied to each pot, the surface area and total amount of water to be applied divided by the number of days of the experiment were taken into account. The water volumes applied were measured using a graduated provete.

Table 1 – Analysis of fertility of the soil used in the experiment

pH in water (1:25)	5.53
Calcium ($cmol_c/dm^3$)	1.46
Magnesium ($cmol_c/dm^3$)	0.64
Aluminum ($cmol_c/dm^3$)	0.00
Hydrogen+Aluminum ($cmol_c/dm^3$)	1.33
Phosphorus (mg/dm^3)	14
Potassium (mg/dm^3)	83
Sodium (mg/dm^3)	11

Five plants per treatment were collected 20 and 40 days after the beginning of the treatments (DAT). The plants were divided into leaves, roots and shoots (stems + petioles). Plant parts, along with a graduated scale for reference, were photographed with a digital camera. The images were analysed with Image J software, version 1.47v, developed by the National Institutes of Health, Bethesda, MD (public domain: http://rsb.info.nih.gov/ij/). The area, perimeter, length and width of leaflets were then determined. These data were then used for the determination of leaf and leaflet area, specific leaf area (SLA; leaf area divided by leaf dry mass), leaflet index (LI; length divided by width) and, as an index of the degree of margin dissection independent of leaf size, the $perimeter^2$/area (Sack *et al*. 2003) was calculated. As the analyses were destructive, we obtained the number of required plants plus 40% to ensure uniformity during collections.

To determine the dry mass of plant parts, they were dried in an oven at 70°Cuntil a constant weight was achieved. Partition analyses were performed: leaf area fraction (LAF; leaf area divided by the total plant dry mass) and biomass fractions (dry mass of plant parts divided by the total dry mass) of root (RMF), stem (SMF) and leaf (LMF).

The experiment was carried out with a 2×3 factorial design (two species ×three water regimes), completely randomised with five replications. After assessing the homogeneity of variances and normality, according to Fligner–Killeen and Shapiro–Wilk tests, respectively, the data were subjected to analysis of variance and the treatments were compared by the least significant difference obtained by the Student's *t*-test at 5% (Crawley 2012). To analyse the data and obtain figures, the software R(R Development Core Team2016) for Linux, version 3.2.2 was used.

RESULTS AND DISCUSSION

The water restriction caused greater effects in *E. velutina* (Figure 1). In *E. contortisiliquum,* the shoot length was not significantly affected by treatments, but at the end of the experiment, there was a slight trend of inhibition due to water restriction (Figure 1A). In *E.velutina*, however, the growth of shoots was affected in moderate and severe water stress conditions at 20 and 40 DAT (Figure 1B). In *E. contortisiliquum,* there was no effect of water stress on the

number of leaves and leafletsuntil20DAT (Figure 1C and E), but a trend of reduced leaf are under severe water stress (Figure 1G) was observed, suggesting smaller leaves and leaflets (see Figure 3). At 40 DAT, the severe water stress had induced a reduction in the number of leaves (significant; $p< 0.05$) and a trend to reduce the number leaflets and leaf area in this species, but moderate water stress caused only small changes (Figure 1C, E and G). The reduction in the number of leaves and leaflets from 20 to 40 DAT under severe water deficit indicates senescence, which was verified. In *E. velutina*, until 20 DAT, only under severe water stress, there was inhibition of the number leaves, leaflets and leaf area (Figure 1D, F and H). However, at 40 DAT, under moderate stress, there was a reduction in these parameters (Figure 1D, F and H), indicating leaf senescence, as described above in *E.contortisiliquum*. Under severe stress, acclimation must have occurred earlier in this species because there was a tendency towards an increase in the number of leaves, leaflet sand consequently leaf area throughout the experiment, but at a much lower rate than in the control plants (Figure 1D,F and H). At 20 DAT,no significant effect of water restriction on the accumulation of total biomass was seen in either species. However, at 40 DAT, there was inhibition under severe stress in *E. contortisiliquum* and under the two water regimes tested in *E. velutina* (Figure 1I an dJ).

The LAF was not significantly affected by treatments on *E.contortisiliquum* throughout the experiment; however, a slight trend to increase under water restriction was shown (Figure 2A). In *E.velutina* there were also no significant changes (Figure 2B). In relation to biomass partitioning, there was a trend towards increasing LMF in *E.contortisiliquum* under water stress; this increase was significant at 40 DAT (Figure 2C). In *E. velutina*, however, no changes were found under water restriction (Figure 2D). In *E.contortisiliquum*, minor but not significant variations in SMF and RMF were observed on the two collection dates (Figure 2E and G). In *E.velutina*, however, effects that indicate an interaction between the stress restrictions tested and the investment of plants on stems or roots were observed at 20 DAT. Under moderate stress, there was greater investment in roots rather than stems, while under severe water stress, the plants invested more in the stems instead of roots (Figure 2F and H). Interestingly, this effect did not persist at 40 DAT (Figure 2F and H).

The drought stresses tested had little or no effect on the size of leaves and leaflets and SLA in *E.contortisiliquum* (Figure 3A, C and E). In *E.velutina*, however, we verified significant reductions in the size of both leaves and leaflets in the treatments under water restriction at 40DAT (Figure 3B and D), but no effect occurred in SLA for the same time period (Figure 3F). Despite this effect in *E.velutina*, there was no effect onleaf format parameters, indicated by the absence of change in LI and dissection index (DI) (Figure 3 H and J). In *E. contortisiliquum* also, no significant effects of water stress on leaf format were observed. The only significant effect was the reduction in DI at 40DAT in stressed plants (Figure 3G and I).

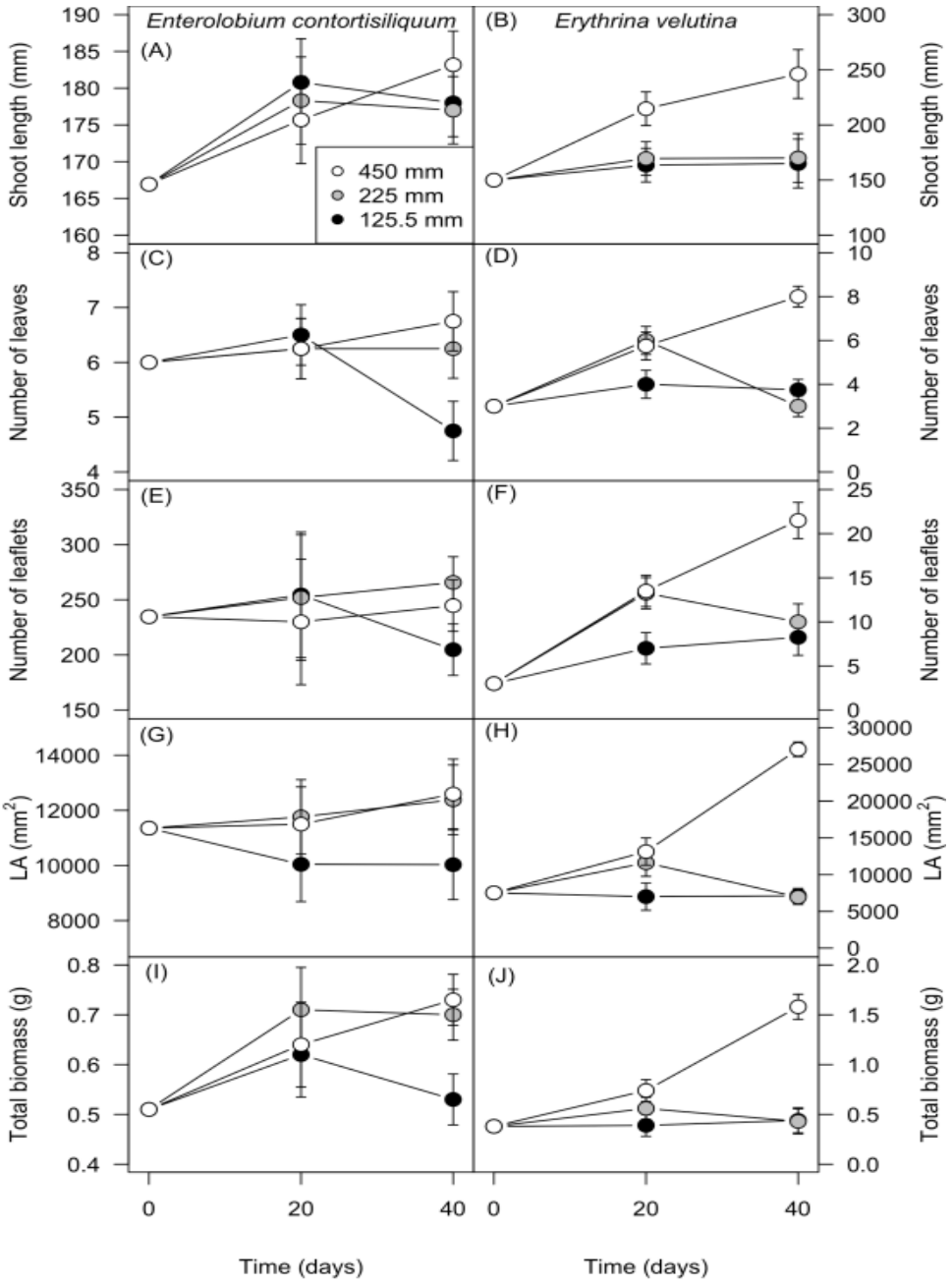

Figure 1 – Shoot length (A, B), number of leaves (C , D) number of leaflets (E , F), leaf area (G, H) and total biomass (I, J) in young plants *E. contortisiliquum* (Vell.) Morong. and *E. velutina* Willd., at three water regimes: 450 mm (control group), 225 mm (moderate stress) and 112.5 mm (severe stress) distributed over 40 days. The error bars represent the least significant difference by Student's *t*-test at 5 %.

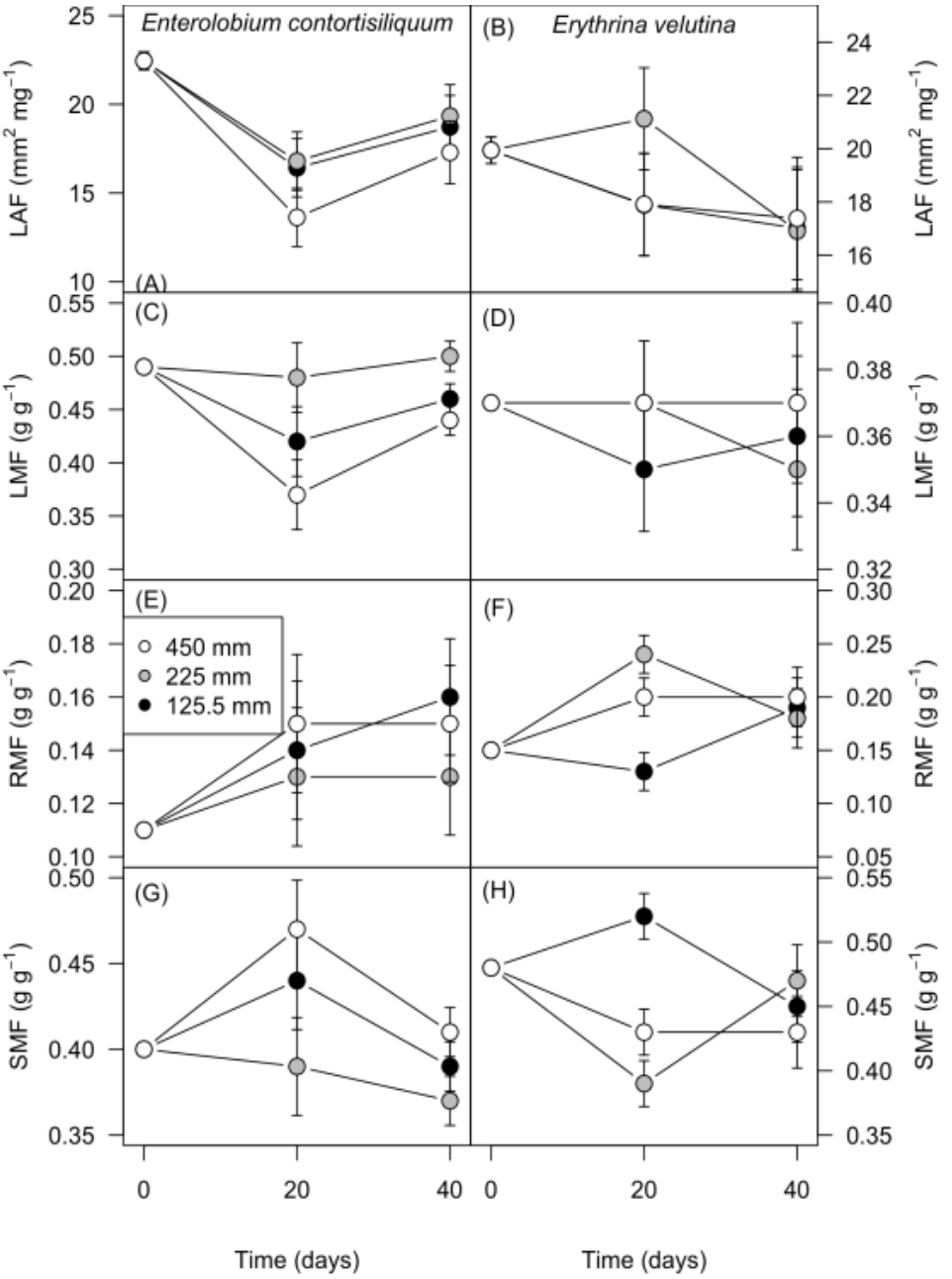

Figure 2 – LAF (A, B), LMF (C, D) RMF (E , F) and SMF (G, H) in young plants *E. contortisiliquum* (Vell.) Morong and *E.* Willd., at three water regimes: 450 mm (control group), 225 mm (moderate stress) and 112.5 mm (severe stress) distributed over 40 days. The error bars represent the least significant difference by Student's *t*-test at 5 %.

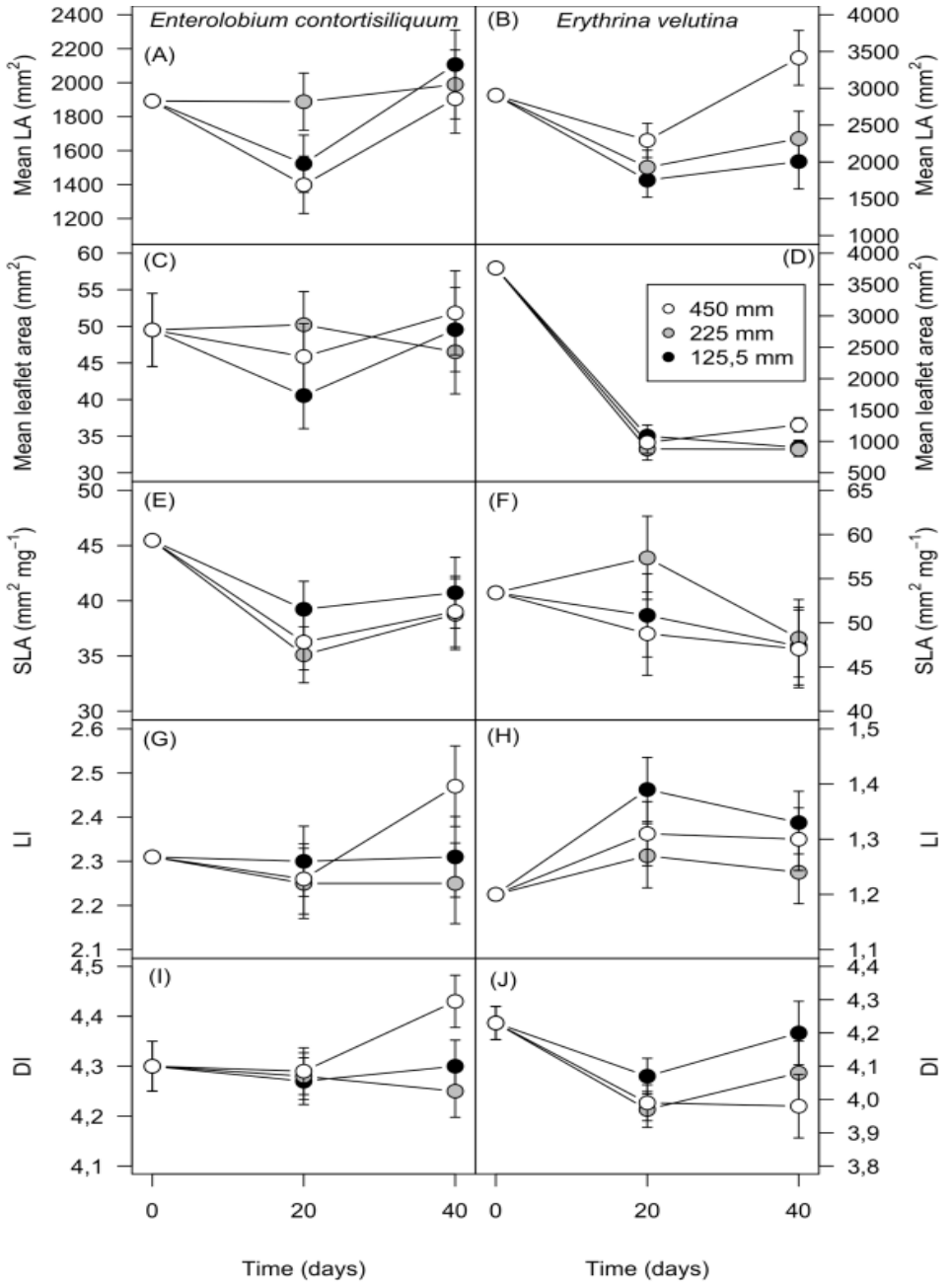

Figure 3 – Mean leaf area (A, B), mean leaflets area (C , D) specific leaf area (E, F), leaflet index (G, H) and dissection index (I, J) in young plants *E. contortisiliquum* (Vell.) Morong and *E. velutina* Willd., at 20 and 40 DAT with three water slides: 450 mm (control group), 225 mm (moderate stress) and 112.5 mm (severe stress). The error bars represent the least significant difference by Student's *t*-test at 5 %.

Studies of several species have demonstrated the inhibitory effect of water stress on plant growth (Claeys and Inzé 2013). Generally, a reduction in biomass accumulation due to water restriction is observed (Magalhães Filho *et al*.2008, Suassuna *et al*.2012). Morphological and physiological changes occur to avoid the negative effects of water stress (Machado *et al*.2009), leading us to hypothesise that photosynthesis should have been less inhibited in *E.contortisiliquum* in our study. Ina field study with *Auxem maoncocalyx*, *Caesalpinia ferrea*, *Caesalpinia pyramidalis*, *Calliandra spinosa*, *Tabebuia aurea* and *Mimosa caesalpiniifolia* (Dombroski*et al*.2011), species which occur in Caatinga, it was found that even in the dry season, only the latter species showed significant inhibition of stomatal conductance and thus photosynthesis. *M. caesalpiniifolia* is a specie that produces leaves very early in the rainy season and which loses them faster than other species in the dry season (Maia, 2004), indicating a differential survival strategy. Our results lead us to consider that *E.contortisiliquum* should present strategies for dealing with drought that are similar to the majority of species studied by Dombroski *et al*. (2011); that is, this species must invest in mechanisms to maintain high water status in tissues for the maintenance of gas exchange. *E. velutina,*on the other hand, should have a strategy similar to *M. caesalpiniifolia*: faster growth or inhibition of growth, depending on the availability of water.

In *M.caesalpiniifolia*, *Prosopis juliflora* and *T.aurea* plants under water stress, reduction but not complete inhibition of the production of new leaves were observed (Silva andNogueira2003). In the same study, the authors verified leaf abscission in *E. contortisiliquum*, as reported in both species in our experiment. The availability of water in the environment determines the leaf area of a plant (Baerenfaller *et al*. 2012, Massonnet *et al*. 2015), inducing larger areas in humid and smaller in arid environments. This feature is an important defence mechanism against excessive water loss; reduced leaf area is xeromorphic characteristic among the many that are identified in plants under water shortage conditions (Villagra and Cavagnaro 2006). A large leaf area can be advantageous to plants for the production of biomass, but in conditions of water stress, this maybe disadvantageous because it increases the surface transpiration, leading to the rapid depletion of ground water. Taking into account that no major changes in leaf form characteristics were found in either species, the inhibition of leaf and leaflet size in *E. velutina* supports our hypothesis that water restriction tolerance in this species should be related to the inhibition of growth.

In *E. contortisiliquum*, we may parallel the small effect of water restriction on biomass partitioning with the small growth inhibition. Thus, this should be a species that maintains higher water status under restriction but not investing more in roots or stems. In *E.velutina,* variation in investment preference in roots or stems, under moderate and severe water restrictions, respectively, may indicate that different parts of the plants suffered the effects of water restriction differently, or that the partition of biomass is a tolerance strategy in this species. Greater biomass investment in roots under water restriction is well documented

in the literature (Baluska *et al*. 2010,Praxedes *et al*.2010, Xu *et al*. 2013), which must be a strategy to explore a larger volume of soil. However, based on our study, we cannot yet indicate a controlled change in growth in response to water deficit.

In most of the studies with water restriction, the authors state that less inhibition of growth is correlated with stress tolerance. Therefore, it should be said that *E.velutina* suffered most as a result of our experimental conditions. However, both species studied here are well adapted to the dry climate of Caatinga. Thus, the restriction in growth should be a strategy for living with drought as important as the strategies aimed at maintaining growth.

CONCLUSION

Generally the growth of *E.velutina* was more inhibited by water stress than in *E.contortisiliquum*. Inhibition of growth in adverse situations must be one important strategy for dealing with drought in Caatinga.

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REFERENCES

Andrade, W.M., Lima, E.A., Rodal, M.J.N., Encarnação, C.R.F. & Pimentel, R.M.M. 2009. Influência da precipitação na abundância de populações de plantas da Caatinga. *Revista de Geografia*, 26:161-184.

Baluska, F., Mancuso, S., Volkmann, D. & Barlow P.W. 2010.Root apex transition zone: a signaling-response nexus in the root. *Trends in Plant Science*, 15:402-408.

Baerenfaller, K., Massonnet, C., Walsh, S., Baginsky, S., Buhlmann, P., Hennig, L., Hirsch-Hoffmann, M., Howell, K.A., Kahlau, S., Radziejwoski, A., Russenberger, D., Rutishauser, D., Small, I., Stekhoven, D., Sulpice, R., Svozil, J., Wuyts, N., Stitt, M., Hilson, P., Granier, C. &Gruissem, W. 2012. Systems-based analysis of Arabidopsis leaf growth reveals adaptation to water deficit. *Molecular Systems Biology*, 8:1-18.

Claeys, H. &Inzé, D. 2013. The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiology*,162:1768-1779.

Crawley, M.J. 2012.*The R Book*.John Willey & Sons Ltd, West Sussex, UK.1076 pp.

Dombroski, J.L.D., Praxedes, S.C., Freitas, R.M.O. & Pontes, F.M. 2011. Water relations of Caatinga trees in the dry season.South African Journal of Botany, 77:430-434.

EMPARN, Empresa de Pesquisa Agropecuária do Rio Grande do Norte. 2016. *Monitoramento Pluviométrico*. http://186.250.20.84/monitoramento/ (accessed November 13, 2016).

Krasensky, J. &Jonak, C. 2012.Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks.*Journal of Experimental Botany*, 63: 1593-1608.

Machado, R.S., Ribeiro, R.V., Marchiori, P.E.R., Machado, D.F.S.P., Machado, E.C. & Landell, M.G.A. 2009. Respostas biométricas e fisiológicas ao déficit hídrico em cana-de-açúcar, em diferentes fases fenológicas. *Pesquisa Agropecuária Brasileira*, 44: 1575-1582.

Magalhães Filho, J.R., Amaral, L.R., Machado, D.F.S.P., Medina, C.L. & Machado, E.C. 2008. Deficiência hídrica, trocas gasosas e crescimento de raízes em laranjeira 'Valência' sobre dois tipos de porta-enxerto. *Bragantia*, 67: 75-72.

Maia, G.N. 2004. *Caatinga: árvores e arbustos e suas utilidades*. Leitura e Arte, São Paulo, Brazil.413 pp.

Menezes, J.A.L., Santos, T.E.M., Montenegro, A.A.A. & Silva, J.R.L. 2013. Comportamento temporal da umidade do solo sob Caatinga e solo descoberto na Bacia Experimental do Jatobá, Pernambuco. *Water Resources and Irrigation Manegement*,2: 45-51.

Menezes, R.S.C., Sampaio, E.V.S.B., Giongo, V. & Pérez-Marin, A.M. 2012.Biogeochemical cycling in terrestrial ecosystems of the Caatinga Biome.*Brazilian Journal of Biology*, 72: 643-653.

Massonnet, C., Dauzat, M., Bédiée, A., Vile, D. &Granier, C. 2015. Individual leaf area of early flowering Arabidopsis genotypes Is more affected by drought than late flowering ones: A multi-Scale analysis in 35 genetically modified lines. *American Journal of Plant Sciences*, 6: 955-971.

Praxedes, S.C., Lacerda, C.F., DaMatta, F.M., Prisco, J.T. & Gomes-Filho, E. 2010. Salt tolerance is associated with differences in ion accumulation, biomass allocation and photosynthesis in cowpea cultivars. *Journal of Agronomy and Crop Science*, 196: 193-204.

R Core Development Team. 2016. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-project.org/(accessedNovember 13, 2016).

Rathcke, B. & Lacey, E. 1985.Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, 16: 179-214.

Sack, L., Cowan, P.D., Aikumar, N.J. & Olbrook, N.M.H. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment*, 26: 1343-1356.

Santos, M.G., Oliveira, M.T., Figueiredo, K.V., Falcão, H.M., Arruda, E.C.P., Almeida-Cortez, J., Sampaio, E.V.S.B., Ometto, J.P.H.B., Menezes, R.S.C.,Oliveira, A.F.M., Pompelli, M.F. & Antonino, A.C.D. 2014Caatinga, the Brazilian dry tropical forest: can it tolerate climate changes?*Theoretical and Experimental Plant Physiology*, 26: 83-99.

Shane, M.W., McCully, M.E., Canny, M.J., Pate, J.S., Huang, C., Ngo, H. &Lambers, H. 2010. Seasonal water relations of *Lyginiabarbata* (Southern rush) in relation to root xylem development and summer dormancy of root apices. *New Phytologist*, 185: 1025–1037.

Silva, E.C. & Nogueira, R.J.M. 2003. Crescimento de quatro espécies lenhosas cultivadas sob estresse hídrico em casa-de-vegetação. *Revista Ceres*, 50: 203-217.

Suassuna, J.F., Fernandes, P.D., Nascimento, R., Oliveira, A.C.M., Brito, K.S.A. &Melo, A.S. 2012. Produção de fitomassa em genótipos de citros submetidos a estresse hídrico na formação do porta-enxerto. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 16: 1305-1313.

Villagra, P.E. &Cavagnaro, J.B. 2006. Water stress effects on the seedling growth of *Prosopis argentina* and *Prosopis alpataco*. *Journal of Arid Environments*, 64: 390-400.

Xu, W., Jia, L., Shi, W., Liang, J., Zhou, F., Li, Q. & Zhang, J. 2013. Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytologist*, 197: 139–150.

DOI: 10.17707/AgricultForest.63.2.06

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ANALYSIS OF ATTITUDES OF HONEY CONSUMERS IN THE MUNICIPALITY OF NOVI GRAD (BOSNIA AND HERZEGOVINA)

SUMMARY

Beekeeping is one of the important branches of animal husbandry, and it is also important in the municipality of Novi Grad in Bosnia and Herzegovina (BaH). Honey producers should be interested in what consumers think of honey and which factors are crucial for their commitment to purchase honey. Therefore, there has been a study of honey consumer attitudes conducted by a survey of 100 randomly selected consumers. The subject of the survey was to determine the consumers' attitudes regarding the amount of honey they consume, honey types, prices, quality, purchasing and other characteristics. Systematization of data from surveys and their statistical processing led to the result that more than a half of consumers consume honey every week, with a high average consumption of 1.33 kg per month. Women consume honey more often than men, older people more frequently than younger ones. The largest number of consumers purchase honey directly from beekeepers. The commitment of consumer's choice to buy honey is greatly influenced by the quality of honey, and it is followed by the price. These and other attitudes of consumers of honey are compared with the attitudes of consumers of similar studies in other countries and it was found that there is sometimes a greater or lesser coincidence among them, and sometimes there are significant differences. Honey producers, taking into account the views and preferences of consumers, should focus their future marketing activities for better promotion of their products and look ahead trying to establish direct contacts with consumers.

***Keywords**:* honey consumption, the price of honey, honey types, quality, honey, consumer attitudes, Novi Grad

INTRODUCTION

Honey is one of the oldest sweets, and beekeeping is one of the activities which were dealt with before the new era. Honey is still present in the human diet, and beekeeping is an important source of income for rural households.

Novi Grad municipality is located in north-western Bosnia and Herzegovina. Climate and geographical characteristics of the municipality Novi Grad are favorable for the development of beekeeping. Honey production is the main activity of the majority of beekeepers, but only a small number of experienced beekeepers produce pollen, royal jelly and propolis. For the purpose

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

of institutional organization of beekeepers of Novi Grad municipality and in order to facilitate implementation of the rights towards the institutions of the Republic of Srpska and other organizations that are ready to help to improve beekeeping the Association of Beekeepers of "Novi Grad" was founded in 1997.

On the territory of the municipality of Novi Grad, according to the Department of Economy and Agriculture of the Novi Grad, there are 56 beekeepers that have 4 000 hives. Honey production in 2013 amounted to 35 tons, while the average production per hive was 8.8 kg, which is less than the national level (Republic of Srpska).

In the municipality of Novi Grad, every year, there is an event called "Days of beekeeping, fruit growing and our village" in the organization of Beekeepers Association promoting bee products and beekeeping as production with great opportunities.

For the purposes of this study, the data of statistical organizations that deal with the movement and development of beekeeping, as well as the survey with the use of a questionnaire by which the data on the habits and attitudes of consumers regarding the consumption of honey in the area of the municipality of Novi Grad were collected.

Some previous researchers have dealt with research of chemical characteristics of honey as Dimitrevska-Stojković at al. (2016) and Milošević at al. (2013), or bee influence on pollination as Jacimovic et al. (2012). Methods of market survey and consumer perception of honey can be found in published survey results of domestic and foreign authors. Similar surveys as this on honey consumer behavior were made by the following scholars: Arvan-Vanyi et al. (2011) in Hungary, Saner et al. (2007) in Turkey, Ismaiel (2014) in Saudi Arabia, Gyau et al. (2014) in Congo, Pacol et. al. (2007) and Pacol (2011) in Romania, Pidek (2001) in Poland, Ćirić et al. (2015) and Otović and Miličić (2014, 2015) in Serbia, Špoljarić (2010) in Croatia, Ostojić et al. (2010) in Bosnia and Herzegovina. Certain results from the aforementioned surveys are associated and compared with the results of this study in the discussion section.

MATERIALS AND METHODS

The survey topic is the analysis of key trends and market conditions, the purchase and consumption of honey, and the comparison and evaluation of the results obtained in market survey in the area of the municipality of Novi Grad. The aim is to establish the basic parameters that affect the supply, purchase, perception and behavior in the consumption of honey in the municipality of Novi Grad.

For the purpose of this survey, the questionnaire was used for testing of the poll of honey consumers in the area of municipality of Novi Grad, and the probing was used as method by personal surveys. The survey included 100 respondents. The questionnaire had 27 questions. The questions in the questionnaire were open or closed. There were four open type questions in the survey and their aim was to find out what we associate with honey, the reasons for consumption of honey, honey price and size of monthly consumption, while there were 23 closed type questions. Through the survey, there were the following data collected: age and number of family members, the reasons for the

consumption of honey, mode of consumption, types of honey consumed, characteristics that are important to them when buying honey and much more. The use of questionnaires has enabled the collection of primary data on the facts, motives and attitudes of consumers, as well as the further processing and linking of interesting factors affecting honey market.

Honey consumer evaluated characteristics were analyzed through general linear mixed models ($p < 0.05$). Analysis and graphical presentation of data was done and R 3.1 (RDCT-crane, 2016) and SPSS Statistics 22 (IBM 2013). Dependent variables were related to the respondents' age, gender, education, family size and number of children.

RESULTS AND DISCUSSION

Honey production in Bosnia and Herzegovina

While the number of hives in recent years in Bosnia and Herzegovina (BaH) moderately and continuously increased, the amount of honey produced has varied significantly from year to year, largely depending on the availability of "pastures" or favorable agro-ecological conditions. The most significant example is sawn in two Novi Grads in a row 2014 (a bad year) and 2015 (best year).

Table 1: Number of hives and honey production in Bosnia and Herzegovina

Year	2008	2009	2010	2011	2012	2013	2014	2015
Hives (000)	334	347	367	382	384	393	392	393
Honey (tons)	2 571	3 261	3 340	3 059	3 107	3 644	2 678	4 926

Source: Agency of Statistics of BaH.

Socio-demographic characteristics of respondents

The data on the following socio-demographic characteristics of the sample were collected through the survey: respondents' gender, age structure, level of education, status in the household, employment status and the amount of monthly income (Table 2).

Table 2: Socio-demographic characteristics of the sample

Characteristic	Variable	Percentage
Sex	Male	56%
	Female	44%
Age	≤ 20 year	4%
	21-35	31%
	26-50	29%
	>50	36%
Education	Elementary school	1%
	Secondary school	52%
	Higher or University	47%
Monthly household income (€)	Up to 250 €	36%
	251 - 500 €	39%
	> 500 €	25%

Source: Own research.

Honey consumption

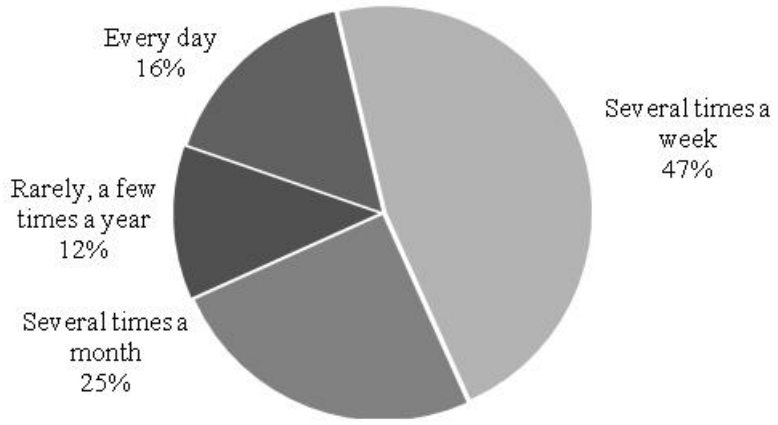
Consuming honey in the municipality of Novi Grad is very frequent. The largest number of respondents, i.e. 47% gave the answer that they consume honey several times a week. Daily consumption of honey was declared by 16% of respondents, and it can be concluded that this consumption represent the habit for these consumers, while 12% of respondents said that they eat honey very rarely, a few times a year, while 25% of them stated that they consume honey several times a month (Graph 1).

Respondents in Novi Grad municipality usually prefer two types of honey (Meadow grass 42%, and Acacia 44%). Other choices were mainly chestnut honey, which was preferred by 12% of respondents, Tilia honey and Salvia honey 1% of respondents. Majority of respondents said that they would like to consume Salvia honey, because they believe that it is very good, but that a lack of honey on the market and the very high cost, prevent them to buy it in most cases.

Consumers generally consume less amounts of honey, from 1 kg to 2 kg per month (78% of respondents), followed by 18% of the respondents who declared to use up to 0.5 kg per month, while 4% of the surveyed consume more than 2 kg of honey per month (Graph 2).

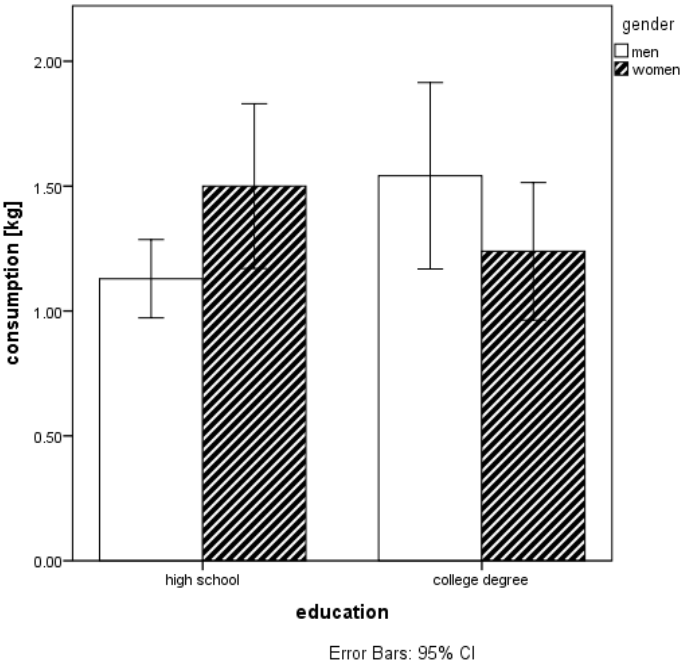
One of the best measures of honey consumption is how many kilograms were consumed in average. In general, the respondents consumed 1.33 kilograms of honey per month.

There was a significant interaction between the education and gender ($p=0.028$) in honey consumption. Namely, with higher education honey consumption increases in men population, but decreases in women. There was no statistically significant influence of respondents' age ($p=0.651$) on average monthly consumption of honey.



Graph 1: Honey consumption frequency

Graph 2: Monthly consumption of honey (kilograms)

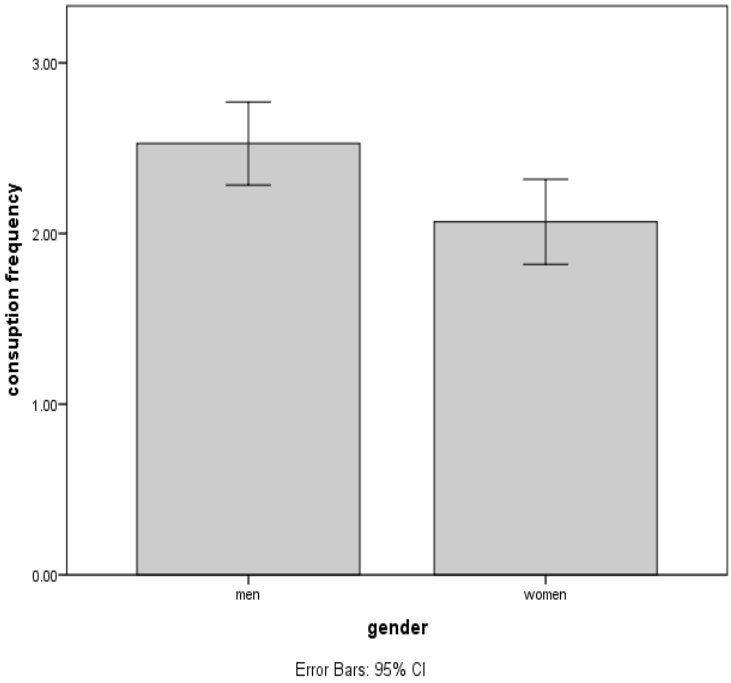


Graph 3. Consumption of honey in kilograms per month based on education and gender categories

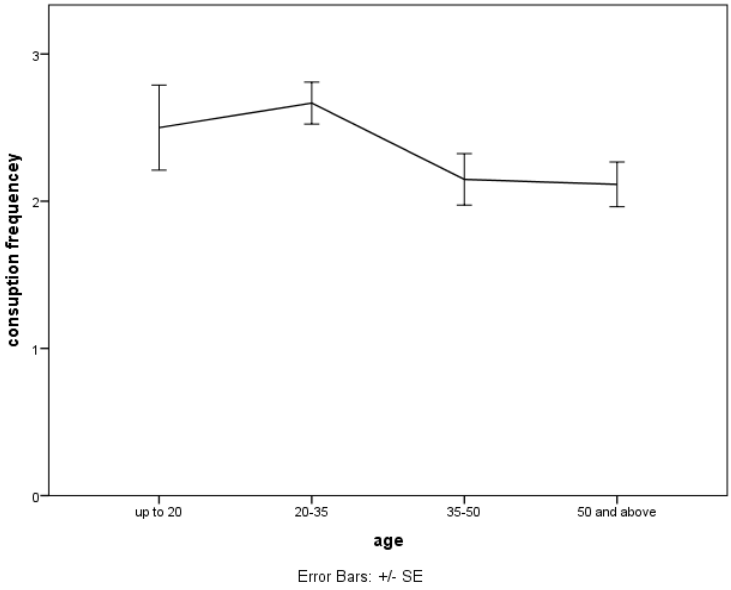
Consumption frequency

It is found that gender (p=0.018) and age (p=0.016) of respondents influence the honey consumption frequency. Namely, in average men consume honey less frequently than women, and generally younger respondents, up to 35

years of age, consume honey less frequently than the older ones. There was statistically significant influence of education (p=0.966).



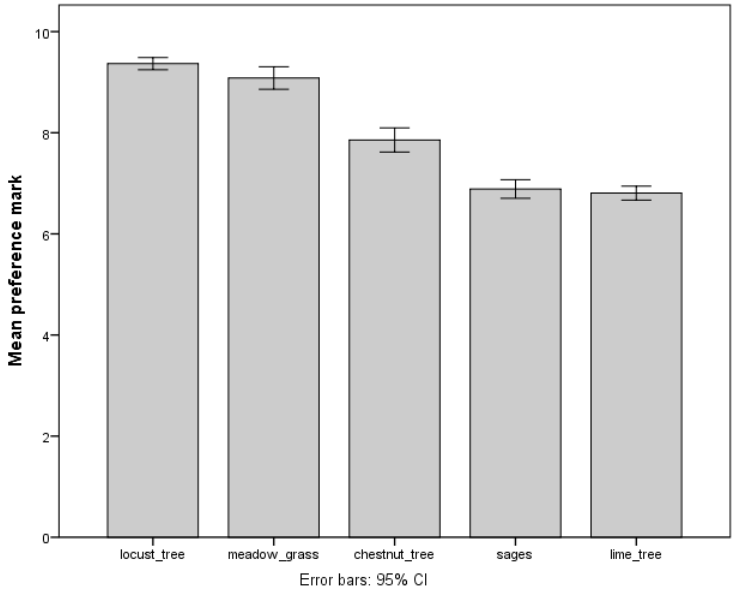
Graph 4. Consumption frequency with regard to gender of surveyed population (1-daily, 2-weekly, 3-mothly, 4-yearly)



Graph 5. Honey consumption frequency in various age categories

Honey type

The respondents were asked which floral source of honey they preferred choosing between black locust tree (*Robinia pseudoacacia* L.), meadow grasses, chestnut tree (*Castanea sativa* Mill.), sage (*Salvia officinalis* L.) or lime tree (*Tillia* sp.). The resulting answers revealed statistically significant difference ($p<0.001$) in preferences between the given floral sources (Graph 6). Respondents mostly preferred black locust honey. Meadow honey is the second best choice which slightly, but significantly ($p=0.044$) differed from the black locust honey. There were no statistically significant ($p>0.050$) influences of gender, education or age of respondents.



Graph 6. Preferences of floral source of honey

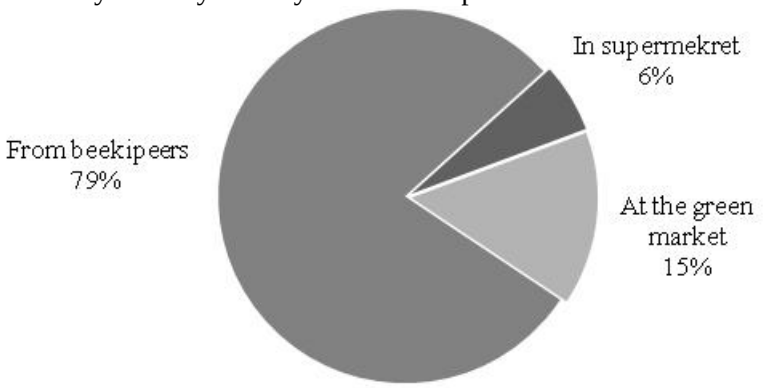
Honey price and place of purchase

When it comes to the price of honey at which consumers buy honey, between 32% of respondents said that a buying price is 6 €, 31% of respondents stated that it is a price of 5 €, followed by 28% who give the 7.5 € for honey. Comparing this data with the year of 2013, when the honey price was 4.5 € within the territory of Novi Grad, it can be seen that there was a price increase of honey in the previous period.

Although honey price varies between 4 and 7.5 € there was no difference between different respondent's origin and socio-economic status categories, or subsequent interactions. This can be explained with the honey price being not so important in comparison to quality.

The largest number of respondents, 79% buys honey directly from beekeepers. 15% of the surveyed buys it in the market, while 6% of them buy it

in a supermarket (Graph 7). Respondents claim to have more confidence in the quality of honey that buy directly from beekeepers than in the store.

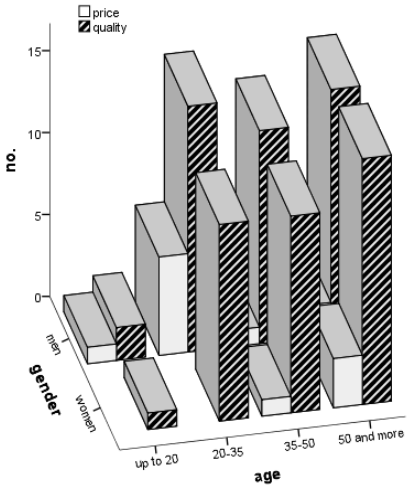


Graph 7: Place of purchase of honey

Respondents who buy honey directly from beekeepers were asked how they come into a contact with them. 73% of respondents declared that they reach beekeepers by recommendation of other consumers, 18% of respondents said they had come to beekeepers by chance, only one 1% of the respondents said that they heard about some the beekeepers through the advertisements.

Honey quality and price perception

In general, 86% of the subjects put the quality above the price. Weather the respondents prefer the quality or the price of the honey is deemed significantly influenced by age and gender interaction (p=0.05).



Graph 8. Comparison of number of subjects declaring priority of price or quality of honey with regard to gender and age categories

Namely, younger men up to 35 years prefer price over quality more than young women, though both categories generally prefer quality above the price. Education of the respondents did not have statistically significant influence ($p = 0.24$). None of the respondents put the design features before the quality or the price of honey

Frequency of honey consumption

Data from this survey, according to which 16% of participants consumed honey on a daily basis, 47% several times a week, compared with the results of similar surveys indicates that consumers from the area of Novi Grad consume honey quite often, almost as often as consumers in Poland and Croatia, significantly more often than in Hungary. According to Arvan-Vanyi et al. (2011) consumers in Hungary consume honey occasionally (56.9%), and after that monthly (25.4%) or weekly (9.3%) and 8.4% never. Women in Poland more frequently eat honey than men, and 61.1% women consumed honey more than once a week. Majority of women (45.2%) and men (38.6%) consumed honey every week (Pidek, 2001). According to survey of Špoljarić (2010) in Croatia, 38% of consumers consume honey on a daily basis, and another 18% several times a week. Since the survey has shown that the average consumption of honey in Novi Grad is 1.33 kg per month, this data puts them in the group of consumers that eat a lot of honey. Saner et. al. (2007) reported that in Turkey, an average of 0.82 kg of honey is consumed per capita per year. According to Ismaile et. al. (2014) in Saudi Arabia annual honey consumption per capita is high 4.5 kg, despite of high price per kg, due to relatively high annual average income. By the age, the consumption of honey in Novi Grad increases. Same was found by Pocol (2011) in Romania where old age people (46-60 year) consume honey the most frequently. Males in Novi Grad consume honey much more frequently than females, while for example, in Romania, the opposite behavior was found where women consume honey with a higher frequency than men (Pocol, 2011).

Type of honey

It can be concluded that the type of honey preferred by consumers largely depends on its local offer. In the studied case, most consumers use meadow honey (42%) and acacia (44%), but also chestnut honey (12%), which is precisely characteristic for this micro-region. Specifics of flora influence the type of honey production and consumption, so that in some other regions in Bosnia and Herzegovina (such as Trebinje region) most consumers prefer sage honey (55%) and heather honey (32%) (Ostojić et al, 2011). According to Arvan-Vanyi et al. (2011) consumers in Hungary mostly prefer acacia honey (80%) and poly-flower honey (30%), as their second choice. In Poland, men most prefer multi-flower honey and women honey of lime (Pidek, 2001). Similar but more diverse preferences have honey consumers on Province of Vojvodina, who mostly buy acacia honey (23%) and poli-floral honey (20%) and 18.8% don't buy honey at all (Ćirić et al, 2015). So for example in Congo, according to dominant

beekeeping method, most consumers have an initial preference for forest honey (64%), 7% have preferences for honey from beekeepers (Gyau et al, 2014). In Croatia, according to a study of Špoljarić (2010), meadows (36%), acacia (27%) and chestnut honey (16%) are bought almost exclusively.

Honey price

According to statistics, the price of honey in Bosnia and Herzegovina is in the range of 5-9 €/kg. In the study area consumers indicated that they are buying honey in the range of 4-10 €. The average price of honey obtained through the survey is 6.3 €. Otović and Miličić (2015) have come to an interesting conclusion that the financial status of consumers is not critical and has a very little impact on commitment when buying honey. Špoljarić (2010) found that there are significant differences in the price of honey depending on the place of purchase, for example acacia honey in supermarkets is 45 HRK (HRK=Croatian kuna), and on the farm it is 30 HRK, which affects the place of purchase and consumers mainly buy directly from the producers. Saner et al. (2007), based on the example of Turkey, drew attention to the consumer price of honey that can be up to three times higher than producer price due to the inclusion of intermediaries. Honey producers are aware that the consumer price of honey is very important and that it, along with the quality, makes the highest rank among their preferences (Otović and Miličić, 2014).

Place of honey purchase

Even 73% of consumers of honey from the area of Novi Grad buy directly from producers because it gives them the highest guarantee of origin and quality of honey, and a slightly lower price (because they do not pay transaction costs and value added tax). Directly through buying honey from beekeepers, or the market is most often present way of getting the honey and in other countries. According to Ismaile et. al. (2014) most consumers buy local honey direct from honey producers and in specialized honey stores and supermarkets. In Vojvodina, consumers buy honey mainly by small marketing channels, directly from beekeepers (40.6%) or at the green markets (31.6%) (Ćirić et. al., 2015). Most honey in Romania is purchased in the marketplaces (33%) and much less in supermarkets (8%) and honey stores (7%) (Pacol, 2007). In Hungary honey has been mostly bought in hypermarkets, and after that direct from producers or at green markets (Arvan-Vanyi et al., 2011). Otović and Miličić (2014) conducted a study of the market of honey in the municipality of Vrbas (Serbia). 62% of respondents said that their products are sold on green markets, and 42% are sold on the farm. Most consumers in Croatia purchase directly, 51% directly from the producer, but still 23% buy it on the market (Špoljarić, 2010).

Factors of honey purchase

Consumers have declared that when they purchase honey, one of the most important characteristics is the healing characteristic of certain type of honey.

The second most important characteristic of the respondents is the taste of honey, while the third place takes the price of honey. The size and design of packaging is listed by respondents as the least important item when buying honey. According to Byau et al. (2014) the price has the strongest influence on honey choice, packaging and colour, taste and origin have moderate influence. For consumers of Saudi Arabia, taste is the factor number 1 (66.8%) of quality of honey (Ismail et. al., 2014). In Poland women consume more honey than men (Pidek, 2001). From the perspective of producers and consumers of honey in Serbia for a decision on honey purchase, the most important factor is quality, price and recommendations of friends (Otović and Miličić, 2014). Decision about buying honey in Croatia is mostly influenced by the quality and origin of honey, and its taste (Špoljarić, 2010).

CONCLUSIONS

Based on the study, it can be concluded that the quality of honey is generally the most important factor when buying honey, consumers put it even ahead of cost of honey, which was found to vary considerably, and i.e. There is lower quality of honey offered to consumers so they can choose between the quality and price. This leads to the conclusion that consumers, in the first place, when it comes to selection and consumption of honey first think of health and a healthy diet. Results indicate that older people are more aware of the benefits of honey in the diet than young people. Younger men pay more attention to the price of honey in relation to women, but it can be explained by the fact that they consume it less than women.

Honey preferred by consumers is meadow and acacia, and less of chestnut, which is characteristic type of surveyed area. Other specific types of honey would be bought by the consumers, but they are not available to them due to low supply and high prices, especially Salvia honey.

Also, it is important to point out that the design, size and shape of the package are not important in relation to the quality of honey. The reason for this is that most of the respondents purchase it directly from beekeepers. It also further supports the conclusion that the quality of honey is the most important characteristic to the average consumer that do not want to buy easily accessible and even cheaper products in shops and asking for the best possible quality that the buyer believes can be provided by purchasing directly from the producers.

It is obvious that the average consumer of honey does not believe in the quality of honey that is offered in stores. This raises a whole range of activities to sellers of honey, which they could and should deal with. First of all, it is a reliable system of certification that would be acceptable and easily accessible to average consumer. This system is supposed to convince consumers of having stable and high quality of their honey, and even in the case of voluntary quality schemes. There are also activities on promotion and marketing, which should be directed towards direct contact marketing.

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REFERENCES

Agency for Statistics of Bosnia and Herzegovina (2016, 2015, 2014, 2013, 2012, 2011, 2010): *First Release, Number of livestock, poultry and livestock products*, No. 11, 10, 9, 8, 7, 6 and 5.

ÁrvánéVányi, G., Csapó, Z. & Kárpáti, L. (2011): Evaluation of Consumers' Honey Purchase Habits in Hungary. *Journal of Food Products Marketing*, 17: 227–240, DOI: 10.1080/10454446.2011.548293.

Gyau, A., Akalakou, C., Degrane, A. & Biloso, A. (2014): Determinants of Consumer Preferences for Honey in the Democratic Republic of Congo. *Journal of Food Products Marketing*, 20:5, 476-490. DOI: 10.1080/10454446.2013.807405.

Dimitrievska-Stojković, E., Angjelska, A., Stojković, G., Uzunov, R., Stojanovska-Dimzoska, B. & Hajrulai-Musliu Z. (2016): Monitoring of multi-class pesticicides in honey samples from Macedonia by uktra high performance liquid cromatography – Tandem quadrupole mass spectometry, *Agriculture and Forestry*, Vol. 62 (2) (243-252);

Ismaiel, S., Al Kahtani, S., Adgaba, N., Al-Ghamdi, A. A. & Zulail, A. (2014): Factors That Affects Consumption Patterns And Market Demands for Honey in the Kingdom of Saudi Arabia. *Food and Nutrition Science*, 5, 1725-1737, DOI: http://dx.doi.org/10.4236/fns.2014.517186.

Jacimovic, V., Radovic, M., Bogavac, M. & Bozovic, D. (2012): Impact of honey bees (*Apis mellifera L.*) on pollination and zield of cultivated plumb varietes, *Agriculture and Forestry*, Vol. 58 (2) (155-157);

Milošević, M., Pavlović, Z., Šobajić, S., Đorđević, B., Đurić, S. (2013): Kvalitet meda u braničevskom i podunavskom okrugu, *Hrana i ishrana*, Vol. 54 (1) (19-23) (in Serbian, abstract in English).

Ostojić, A., Vaško, Ž. & Maksimović, R. (2011): Utjecaj obujma proizvodnje na izbor kanala prodaje meda /The influence of the production volume on the honey sales channels/. *Proceedings 46th Croatian and 6th International Symposium on Agriculture*, Opatija, Croatia, 324-327 (in Croatian, abstract in English).

Otović, S. & Miličić, Ž. (2014): Marketing istraživanje: Proizvodnja meda kao vid zapošljavanja na selu /Marketing survey: Honey production as a way of employment of women in the country/. *Agroekonomika*, Vol. 43, No. 63-64, 110-124 (in Serbian, abstract in English).

Otović, S. & Miličić, Ž. (2015): Zainteresovanost potrošača prilikom kupovine meda i proizvoda od meda /Consumer interest when buying honey and honey products/. *Agroekonomika*, Vol. 44, No. 65, 123-135 (in Serbian, abstract in English).

Pocol, C. B. (2011): Modeling the honey consumption behavior in Romania by using socio-demographic determinants. *African Journal of Agricultural Survey*, Vol. 6(17), 4069-4080.

Pacol, C. B. & Marghitas, L. A. (2007): Market study about honey consumption in Romania, Bulletin USAMV-CN, 63-64.

Pidek, A. (2001): Youth preferences in honey consumption. *Journal of Apiculture Science*, Vol. 45, 115-119.

Saner, G., Yercan, M., Engideniz, S., Karaturhan, B. & Cukur, F. (2007): Alternative Marketing Strategies for Honey and Other Bee Products in Turkey. *Journal of Agricultural & Food Information*, 8:4, 65-74. Špoljarić, J. (2010): Istraživanje o konzumaciji meda za potrebe prodaje u pčelarstvu /Research on the honey consumption for need of sales management of bee products/. Članak broj 10-09, Sveučilište u Zagrebu, Ekonomski fakultet, Zagreb (in Croatian, abstract in English).

Špoljarić, J. (2010): Istraživanje o konzumaciji meda za potrebe prodaje u pčelarstvu /Research on the honey consumption for need of sales management of bee products/. Članak broj 10-09, Sveučilište u Zagrebu, Ekonomski fakultet, Zagreb (in Croatian, abstract in English).

Ćirić, M., Ignjatijević, S. & Cvijanović, D. (2015): Survey of honey consumers' behavior in Province of Vojvodina. *Economics of Agriculture*, 3, 627-644, UDC: 366.14:638.16(497.113).

DOI: 10.17707/AgricultForest.63.2.07

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PRODUCTIVITY OF SPRING FORAGE PEA *(PISUM SATIVUM)* IN DIVERGENT AGROECOLOGICAL CONDITIONS

SUMMARY

Spring forage pea varieties represent a broad source of genes for the desirable properties, especially those related to abiotic and biotic stress, that can be inserted into the existing varieties. Studies showed that a variety-line-hybrid had the greater impact on the expression of production and morphological traits in relation to the vegetation period. The factor of years did not had the same impact on the expression of each production and morphological traits.

Statistically significant impact on the plant height had the genotype and genotype x year interaction. It was noticed that this phenotypical characteristic vary in a wide range between 71.00 cm in Baccara genotype to 146 cm in Saša genotype.

The genotype and genotype x year interaction had a statistically significant impact on the pod length. Studies showed that the genotype and G x Y interaction had a statistically significant impact on the seed yield per plant. Baccara genotype had a statistically significant higher average seed yield per plant compared to the line L-CC.

The correlative relation of production and morphological traits has manifested in varying degrees. Strong positive correlation was recorded between the grain weight per plant and pod length (r = 0.53 **), then between the grain weight and pod weight per plant (r = 0.87 **), and grain weight and pod length (r = 0.47*).

The wide mutual variability of the most important characteristic may be extremely beneficial for the existing varieties, due to the narrow genetic base.

Keywords: *Fabaceae*, spring forage pea, plant breeding, production and morphological traits, correlation.

INTRODUCTION

Annual legumes are important, both in the terms of yield and quality as well as the protection of agroecological system: improvement of the soil physical, chemical and biological properties, fertilizer costs reduction, nutrient leaching prevention, moisture storage, erosion prevention, reduction of pesticide use and environmental protection; preservation of soil and water quality and thus

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human health. Despite a prominent character the annual forage plants are grown on relatively small areas in the agroecological conditions of Bosnia and Herzegovina. Annual forage plants have a long cultivation tradition in Bosnia and Herzegovina, where they represent an indispensable supply source of the quality forage and concentrated animal feed for livestock production.

The most important annual forage legumes are forage pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.), followed by broad bean (*Vicia faba* L.), hairy vetch (*Vicia villosa* Roth), Pannonian vetch (*Vicia pannonica* Crantz), ervil (*Vicia ervilia* (L.) Willd.) and grass pea (*Lathyrus sativus* L.), then narbon vetch (*Vicia narbonensis* L.), white lupine (*Lupinus albus* L.), blue lupine (*Lupinus angus - tifolius* L.) and cowpea (*Vigna unguiculata* (L.) Walp.), with high potential for intensive cultivation (Mihajlović et al., 2006b). Annual forage legumes have a multiple purpose and are grown for green fodder, hay, fodder meal, grain and straw, with the note that beans and other legumes can also be used for grazing. In the production great losses of pea yield are occurring due to biotic and abiotic stresses, which requires to direct selection towards the creation of varieties with an improved stress tolerance mechanism (Saha and Vandemark, 2012). The breeding of forage pea includes three basic directions: breeding for fodder yield; breeding for seed yield and for the yield of fodder and seed. Methods of breeding for forage pea yield are the pedigree method, bulk method and backcross method. Success of a breeder in the new pea varieties creation, that aims to increase seed yield, depends a lot on the starting material variability as well on the success in the improvement of quantitative and qualitative properties and their mutual interaction (Tiwari and Lavanya, 2012). For the successful breeding work and the creation of new varieties, one of the requirement is the starting material variability. However, if the selection goes in the direction towards certain traits, and if these traits have a high variability and heritability, it is important that they are in the preferred correlative relation with seed yield (Lakić et al., 2013).

Breeding for the fodder yield is the oldest direction in pea breeding that is aimed on the creation of a winter and spring varieties of the great potential for high-quality and stable yields of green fodder and hay, with the possibility to be used as green manure. The most important forage pea agronomic traits are the tall plants, moderate stem number, increased proportion of leaf in plant mass and satisfactory seed yield. Winter forage pea variety Pešter from Novi Sad is characterized by the fodder yield of 45 to 50 t ha^{-1} and about 10 t ha^{-1} of dry fodder matter (Mihajilović et al., 2007b). Pea breeding for the seed yield is usually focused on the creation of varieties with a good potential for high and stable yields of grain that is rich in proteins and have the reduced anti-nutritional ingredients content. In this breeding direction, the most significant characteristics are considered to be a shortened growing period, uniformly ripening, increased tolerance to lodging, increase in the number of knuckles in lower stem part and the introduction of genes for afila leaf type (af Tl). Biological nitrogen fixation efficiency depends on the strain type of nitrogen fixing bacterias, varieties,

followed by the interaction between different strains and varieties, environmental factors and technological practices. After photosynthesis, biological nitrogen fixation in leguminous plants can be considered the most important biological process on earth. The ability of nitrogen fixation is underutilized by the developing countries, but also by developed countries, as reflected on the excessive nitrogen fertilizers dependence in agriculture (Abi-Ghanem et al. 2014). In the European Union of the total cultivated land in different countries, fodder legumes are making 1-7%, which is certainly small compared to non-EU countries with 15-25%. One way to increase the areas in Europe is to intensify the work on selection in terms of the genotype creation that is tolerant to pedoclimatic or stress conditions, particularly diseases. As one of the measures for increasing the areas under annual fodder legumes in EU is the production subsidization per area unit (Mihajilović et al., 2009). In order to intensify livestock production and to obtain high yields with good quality, special attention should be given to the selection of appropriate assortment with short growing season, as well as to sowing, and above all to the seed quantity and sowing period. During the preliminary tests, NS spring pea cultivars Partner, Kristal and Dukat, had the average yields of about 5 t ha^{-1}, with a prominent early maturity (Mihajilović et al., 2007b). However, the yield and quality achieved in micro trials points to the fact that this plant species is unduly neglected.

The morphological description and general assessment of future parents is as important as its interaction with the environment (Popović et al., 2012a, 2012b, 2016a). The climate change impacts on agriculture are the result of a series of complex interactions with other environmental, social, economic and political factors and are mainly related to the biological effects on crop yields, as well as the resulting impacts on outcomes including prices, production, and consumption (Nelson et al., 2009). Therefore, the objective of this study was to determine the morphological diversity of pea genotypes, and to valorize the value of morphological characteristics and analyzes based on them, or to examine the productivity of facultative pea varieties.

MATERIALS AND METHODS

Parental varieties and their hybrids are shown on the experimental field of the PI Agricultural Institute of Republic of Srpska in Delibašino Selo during the years 2014 and 2015. The sowing was done in March. For the sowing 80 viable seeds per m^2 was used. Plot size was 2.5 m^2 (2 x 1.25m). Row spacing of 12.5 cm x 8 cm. Depth of sowing 4-5 cm. The number of repetitions (blocks) is 4. The basic fertilization during the preparation for sowing amounted to 350 kg/ha of NPK 8:24:24. Fertilization with 100 kg/ha of KAN 27% N in the phase of plant growth and development. Treatment against weeds (with herbicide) after sowing and before germination (Zanat - active ingredient pendimethalin). The dose of 5 l/ha - in 5 liters of water 50 ml of herbicide Zenit for 100 m^2. Treatment with Galbenon herbicide (a.i. bentazon 480 g/l) 3.5 l/ha (in pea upgrowth 8- 10 cm). Treatment with insecticides against pests (weevil) at the blooming and flowering

stage - systemic insecticide (Decis - a.i. deltamethrin 25 g/l, Perfekthion - a.i. dimethoate) at a concentration of 0.15-0.20%.

Nitrogen fertilization lead to a significant increase in dry matter yield, however, it reduced the value of the achieved yield and increase in the yield per unit of added nitrogen (Bijelić et al, 2016).

Parallel to the spring sowing all hybrids (crossbreeds) of F3 and F4 generations are sown in the first week of October in 2014 and 2015 as winter peas. In this study data are processed for F3 and F4 generation of spring forage pea. Of the each variety/hybrid 10 plants per replication were annually analyzed.

For breeding the following population of spring fodder pea were used:

1. Saša – variety created in the Agricultural Institute of Republic of Srpska in 1990. Designated for the combined use of fodder and grain.

2. Baccara – French variety of spring forage pea intended for the seed production.

3. Line L-CC – forage pea line originating from North America. It has pink-purple flowers. Line L-CC is facultative.

This study is processing the following hybrids (crossbreeds):

1. Line L-CC x Baccara; 2. Baccara x Line L-CC; 3. Saša x Line L-CC; 4. Baccara x Saša

Analysis of the following morphological and productive parameters was conducted: 1. plant height (cm); 2. legume length (cm); 3. legume weight without grain (g); 4. grain weight (g); seed yield per plant (g).

The original experimental data are processed, analyzed and evaluated using the following mathematical and statistical methods: factor impact on the plant characteristics (variance analysis) and correlation analysis of plant characteristics with the use of the statistical package STATISTICA 12 for Windows. Applied mathematical and statistical methods for the experimental data processing, analysis and evaluation of the research results enabled the correct understanding of all results. Significance was calculated based on LSD test for probability levels 0.05% and 0.01%. Relative dependence was defined by method of correlation analysis and stability tested traits determined by the coefficients of variation (%).

RESULTS AND DISCUSSION

Soil conditions

At the experimental field of the Agricultural Institute of Republic of Srpska in Delibašino Selo, where the experiment was established, dominant soil type was a valley-brown soil on alluvial substrate of the river Vrbas. By the mechanical composition soil belongs to the group of a clay loam. The surface structure layer of this soil type is crumbly. The results of chemical analysis of arable soil layer on which the experiment was set up are shown in Table 1.

In terms of pH, the soil is alkaline, and by humus content it belongs in soil types with low humus content (1.9%). The availability of easily accessible phosphorus is good (20.3 mg/100g), while the presence of potassium in the soil is

moderately with 16.1 mg/100 g of soil. Based on these results it can be concluded that the soil intended for the experiment is suitable for the cultivation of forage pea.

Table 1. Results of chemical analysis of arable soil layer

Depth (cm)	Humus %	pH in H_2O	pH in KCl	P_2O_5 mg/100g of soil	K_2O mg/100g of soil
0-30	1.9	7.9	6.9	20.3	16.1

Meteorological conditions

Weather conditions are unpredictable and variable (Popović, 2010, 2015a). Meteorological data recorded high variability during year (Bran et al., 2008, Popović et al., 2012a; 2015b; 2016a; 2016b; 2017; Glamočlija et al, 2015; Jankovic et al, 2015; 2016; Sikora et al, 2015; 2016; Marišovà et al, 2017). For the analysis of weather conditions the data from meteorological station in Banja Luka were used (Table 2). Winter period in the both years of testing was warmer compared to the multi-year average. The average multi-year temperature was 11.1°C, and during the vegetation period 13.4°C. The total precipitation amount in the vegetation period (III-VI) during the years from 1961-2014 was 376.0 l/m^2. The average temperatures in both years of testing, during the vegetation period, were higher compared to the multi-year average. In the first year of testing the precipitation amount during the vegetation period was higher by 244.0 l/m^2 compared to the multi-year average. During the vegetation period in the year 2015 rainfall amounts to 311.2 l/m^2, which is for 64.8 l/m^2 less precipitations compared to the multi-year average.

Table 2. Average temperatures (°C) and precipitations (l/m^2) for Banja Luka, B&H

Year	Temperature/ precipitation	Month												Average total
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2014	Temperature (°C)	5.6	6.5	9.6	13.1	15.8	20.3	21.7	20.6	16.4	13.5	8.9	4.0	13.0
	Precipitation (l/m^2)	52	74	91	214	218	97	139	276	284	117	42	83	1,687
2015	Temperature (°C)	3.4	2.4	7.3	11.8	17.4	20.9	25.2	24.0	18.3	11.5	7.1	3.2	12.7
	Precipitation (l/m^2)	111.2	91.1	79.0	54.1	117.6	60.5	20.5	22.8	75.0	142.7	85.7	8.1	868.3
1961-2014	Temperature (°C)	0.2	2.0	6.7	11.3	16.1	19.6	21.3	20.8	16.3	11.3	6.4	1.5	11.1
	Precipitation (l/m^2)	70	64	78	90	98	110	91	86	96	80	93	91	1.043

Research showed that on the tested parameters a statistically significant impact had the genotype and hybrids, and genotype x year interaction.

Plant height

It was noticed that this phenotypic characteristic vary in a wide range between 71.00 cm in genotype Baccara to 146 cm in genotype Saša (Tab. 3).

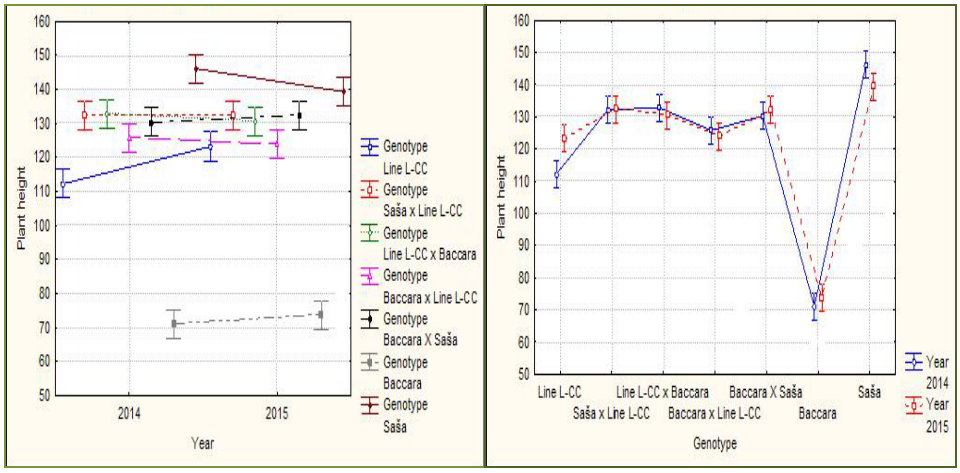
Table 3. Morphological and productive traits of tested pea varieties

No.	Variety-line-hybrid	Year	Plant height (cm)	Pod lenght (cm)	Pod mass per plant (g)	Grain weight (g)	Seed yield per plant (g)
1.	Baccara	2014	71.00	6.62	1.56	1.29	8.02
		2015	73.70	6.58	1.45	1.25	7.35
		Average	72.35	6.60	1.50	1.27	7.68*
2.	Saša	2014	146.00	6.47	0.99	0.81	6.69
		2015	139.00	6.35	1.16	0.96	6.23
		Average	142.70	6.41	1.08	0.88	6.46
3.	Line L-CC	2014	112.20	6.13	1.00	0.82	4.70
		2015	123.20	6.26	1.24	1.01	5.31
		Average	117.70	6.19	1.12	0.92	5.01-
4.	Line L-CC x Baccara	2014	132.80	7.55	1.71	1.39	6.97
		2015	130.60	7.00	1.43	1.16	6.31
		Average	131.70	7.28	1.57	1.28	6.64
5.	Baccara x Line L-CC	2014	125.80	6.41	1.28	1.16	7.09
		2015	124.00	6.33	1.26	1.09	7.14
		Average	124.90	6.37	1.27	1.26	7.12
6.	Saša x Line L-CC	2014	132.20	6.54	1.29	1.08	6.20
		2015	132.40	7.07	1.37	1.10	6.07
		Average	132.30	6.81	1.33	1.09	6.14
7.	Baccara x Saša	2014	130.30	7.20	1.57	1.09	6.84
		2015	132.20	6.75	1.35	1.08	6.62
		Average	131.25	6.98	1.46	1.09	6.73
Average		2014	121.48	6.70	1.34	1.09	6.65
		2015	122.20	6.62	1.32	1.095	6.43
		X	121.84	6.66	1.33	1.09	6.54

Parameter		Genotype	Year	G x Y
Plant height	LSD 0.5	4.163	2.221	5.898
	0.1	5.450	2.917	7.722
Pod lenght	0.5	0.323	0.169	0.449
	0.1	0.423	0.229	0.588
Pod mass per plant	0.5	0.152	0.081	0.214
	0.1	0.198	0.106	0.281
Grain weight	0.5	0.139	0.073	0.194
	0.1	0.182	0.096	0.253
Seed yield per plant	0.5	0.679*	0.367*	0.956
	0.1	0.889	0.401	1.252

The genotype and genotype x year interaction had a statistically significant effect on the plant height (Table 3).

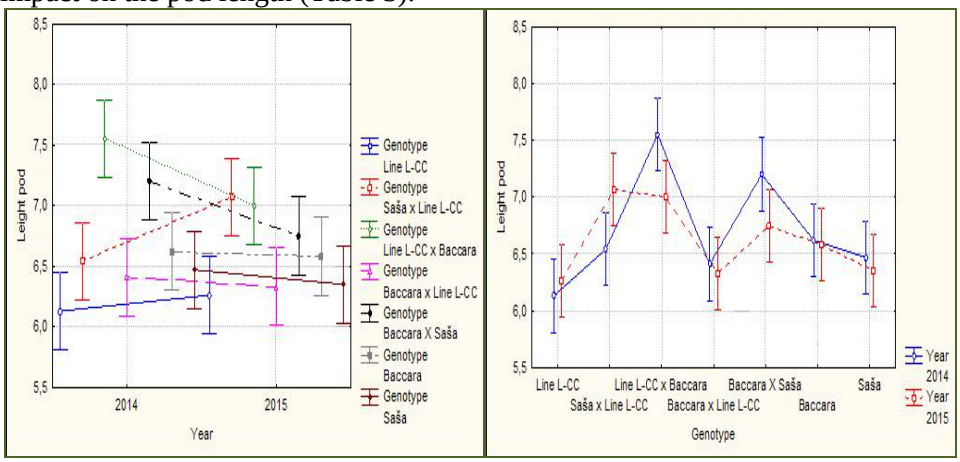
In the year 2015 pea plants were higher (122.2 cm) compared to the year 2014 (121.5 cm), but the difference was not statistically significant. Saša genotype had the greatest plant height in 2014 (146 cm), while the lowest plants had Baccara genotype (71 cm).



Graph 1. The impact of genotype x year interaction on forage pea plant height

Saša genotype had a statistically significant higher average plant height (142 cm) compared to other tested genotypes, while a statistically significant lower plants had the genotype Baccara (72.4 cm) in regard to other tested genotypes and hybrids (Table 3, Graph 1).

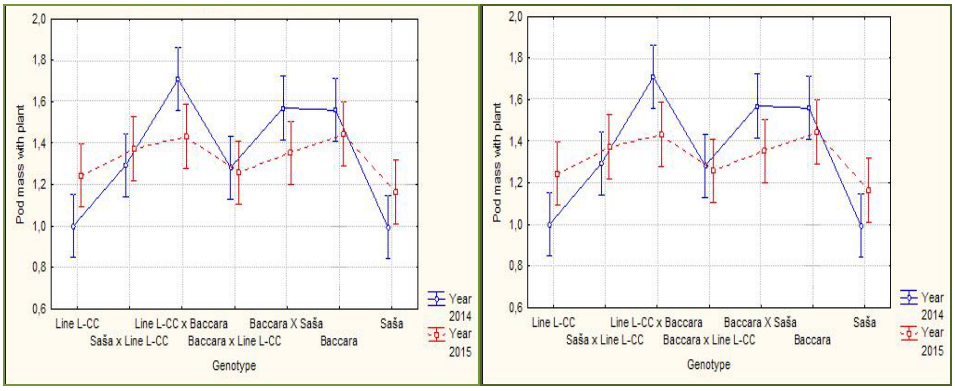
Pod length. Genotype x year interaction had a statistically significant impact on the pod length (Table 3).



Graph 2. The impact of genotype x year interaction on forage pea pod lenght

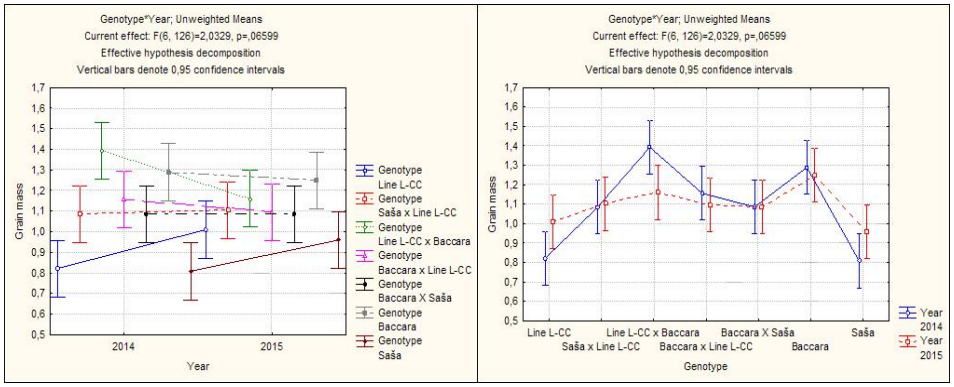
In the year 2014 plants had the longer pods (6.70 cm) compared to the year 2015, but the difference was not statistically significant. The highest average pod length had the hybrid Line L-CC x Baccara (7.55 cm) in 2014. The hybrid Line L-CCxBaccara had on average a statistically significant greater pod length compared to other tested genotypes, except hybrid Baccara x Saša (Tab.3, Graph 2).

Pod mass per plant was a statistically significant dependent on the genotype and genotype x year interaction (Table 3). Over the years there was no statistically significant difference in the average pod mass. The highest average pod mass had the hybrid Line L-CC x Baccara (1.71 g) in 2014. The hybrid Line L-CC x Baccara had a statistically significant greater average pod mass compared to the Line L-CC, hybrid Saša x Line CC and genotype Saša (Table 3, Graph 3).



Graph 3. The impact of G x Y interaction on forage pea pod mass per plant

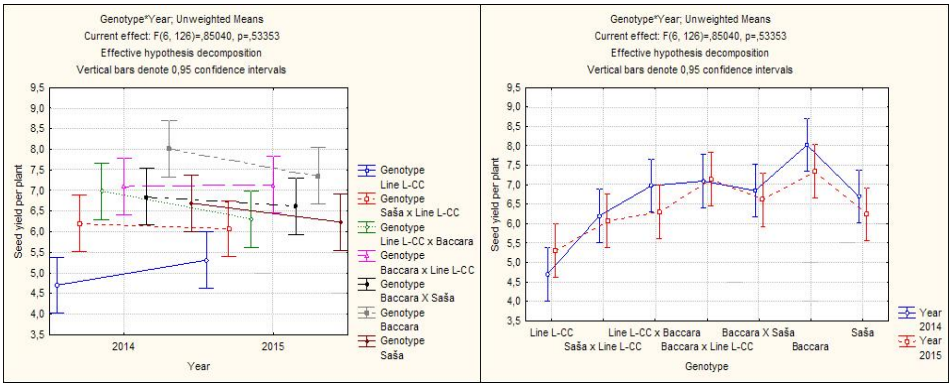
Grain weight was a statistically significant dependent on the genotype and genotype x year interaction (Table 3).



Graph 4. The impact of genotype x year interaction on forage pea grain weight

Over the years there was no statistically significant difference in the average grain weight. The highest average grain weight had the hybrid Line L-CC x Baccara (1.39 g) in 2014. The hybrid Line L-CC x Baccara and Baccara genotype had a statistically significant higher average grain weight compared to other tested genotypes (Table 3, Graph 4).

Seed yield is beside quality the most important indicator of the variety value (Mihajlović et al. 2009). Studies showed that the genotype and interaction of tested factors had a statistically significant impact on the seed yield per plant (Table 3). In the year 2014 a larger average grain yield per plant was achieved (6.65 g) compared to the year 2015, but the difference was not statistically significant. The highest average seed yield per plant had the genotype Baccara (8.02 g) in 2014. Baccara genotype had a statistically significant higher average seed yield per plant (7.68 g) compared to the Line L-CC, while the Line L-CC had a significantly significant lower seed yield per plant (5.01 g) compared to other tested genotypes (Table 3, Graph 5).



Graph 5. The impact of GxY interaction on forage pea seed yield per plant

Correlations of tested parameters

Correlation analysis results of tested parameters are given in Table 4. For the morphological and production traits correlation analysis shows a very different result values of the correlation connection between production and morphological traits. Strong positive correlation dependence was recorded between the grain weight per plant and pod length (r = 0.53 **), then between the grain weight and pod mass per plant (r = 0.87 **), and grain weight and pod length (r = 0.47 *), Table 4.

Seed yield per plant was in a significant positive correlation with the grain weight (r = 0.29 *), pod length (r = 0.24 *), pod mass per plant (r = 0.23 *), and in the negative correlation with plant height (Table 4). The positive insignificant correlation was achieved between plant height and pod length (r = 0.08), Table 4.

Spring forage pea varieties represent a broad source of genes for the desirable properties, especially those related to abiotic and biotic stress, that can

be inserted into the existing varieties. The wide mutual variability of the most important characteristics can be very useful for the existing varieties. For the demonstration of production and morphological traits in some cases the variety-line-hybrid have a greater impact, and in other cases the vegetation period. The factor of years did not had the same impact on the expression of each production and morphological traits. Togay et al., (2008) stated that the number of pods per plant and pod mass are in a positive high significant correlation with the seed yield.

Table 4. Correlations between tested parameters

Variable	Pod lenght	Grain weight	Pod mass per plant	Plant height	Seed yield per plant
Pod lenght	1.00	0.47*	0.53**	0.08 ^{ns}	0.24*
Grain weight	0.47*	1.00	0.87**	-0.26*	0.29*
Pod mass per plant	0.53**	0.87**	1.00	-0.20^{ns}	0.23*
Plant height	0.08	-0.26*	-0.20 ^{ns}	1.00	-0.20^{ns}
Seed yield per plant	0.24*	0.29*	0.23*	-0.20^{ns}	1.00
ns – not significant; *and ** significant at $p<0.05$ and $p<0.01$;					

Morphological markers are used for the description of a newly created varieties characteristics in order to conduct testing of distinctiveness, uniformity and stability (DUS). The main disadvantages of morphological markers are their paucity and the impact of environmental factors, as well as the development phase of plant. However, despite these limitations morphological markers were and still are highly useful in the plant breeding.

Thus, for example, resistance to lodging is a quantitative characteristic of pea, and it is related to the morphological characteristics such as root, stem hardness, height, leaf type, but as such it can be masked by an impact of the environment. In the modern pea breeding the choice of parents is based on a molecular level based diversity.

CONCLUSION

According to the results of conducted research, the following can be concluded:

For the demonstration of production and morphological traits in some cases the variety-line-hybrid has a greater impact, and in other cases the vegetation period. The factor of years did not have the same impact on the expression of each production and morphological traits.

The genotype and genotype x year interaction had a statistically significant impact on the plant height. Saša genotype had the greatest plant height in 2014 (146 cm), while the lowest plants had Baccara genotype (71 cm).

Genotype x year interaction had a statistically significant impact on the pod length. The hybrid Line L-CC x Baccara had on average a statistically

significant greater pod length compared to all tested genotypes, except hybrid Baccara x Saša.

Pod mass per plant was a statistically significant dependent on the genotype and genotype x year interaction. The hybrid Line L-CC x Baccara had a statistically significant greater average pod mass compared to the Line L-CC, hybrid Saša x Line CC and genotype Saša.

Grain weight was a statistically significant dependent on the genotype and genotype x year interaction. The hybrid Line L-CC x Baccara and Baccara genotype had a statistically significant higher average grain weight compared to all tested genotypes.

Studies showed that the genotype and interaction of tested factors had a statistically significant impact on the seed yield per plant. Baccara genotype had a statistically significant higher average seed yield per plant compared to the Line L-CC.

The correlation link between morphological and production traits has manifested in the different time and level. For the dependent variable characteristic of total seed number per plant the independent variable characteristics (plant height, pod length, pod weight without grain, grain weight and seed weight per plant) provided a high determination in the years of research and in all seven tested pea genotypes-lines-hybrids.

Strong positive correlation dependence was recorded between the grain weight per plant and pod length (r = 0.53 **), then between the grain weight and pod mass per plant (r = 0.87 **), and grain weight and pod length (r = 0.47 *).

ACKNOWLEDGEMENT

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REFERENCES

Abi-Ghanem, R., Bodah, E. T., Wood, M., Braunwart , K. 2013. Potential Breeding for High Nitrogen Fixation in Pisum sativum L.: Germplasm Phenotypic Characterization and Genetic Investigation. American Journal of Plant Sciences, Vol.4 No.8, 1597-1600.

Al Lawati, A.H, Pierce, C.A, Murray, L.W, Ray, I.M., 2010. Combining ability and heterosis for forage yield among elite alfalfa core collection accessions with different fall dormancy responses. Crop Sci. 50: 150-158.

Bijelić, Z., Tomić, Z., Mandić, V., Ružić-Muslić, D., Krnjaja, V., Vučković, S., Simić, A., 2016. The efficiency of nitrogen from fertilizer in lucerne cultivated as a pure sward or as a mixture with grasses. Romanian Agricultural Research, No. 33, 227-234.

Bran, M., Dobre, I., Stefan, M. Bobac, D., Papuc, C.M., 2008. Long-term development of agriculture in micro-area „Dobrotfor-Pojorâta". Romanian Agricultural Research, 25: 97-105.

Ikanović, J., Popović, V., Janković,S., Živanović, Lj., Rakić, S., Dončić, D. (2014): Khorasan wheat population researching (*Triticum Turgidum*,sp. *Turanicum*

(McKEY) in the minimum tillage conditions**.** Genetika, Belgrade, 46 (1), p.105-115.

Jankovic, S., Ikanovic, J., Popovic, V., Rakic, S., Pavlovic, S., Ugrenovic, V., Simic, D, Doncic, D. (2015): Morphological and productive traits of spelt wheat – Triticum spelta L. "Agriculture and Forestry", Podgorica, Vol. 61, 2, 173-182.

Jankovic, S., Popovic, V., Ikanovic, J., Rakic, S., Kuzevski, J., Gavrilovic, M., 2016. Productivity traits of rye (Secale cereale), Khorasan wheat (Triticum turgidum, ssp. Taranicum MCKEY) and quinoa (Chenopodium quinoa Willd) grown on degraded soil. Nardi Fundulea, Romanian Agricultural Research, No. 33.

Lakić, Ž, Sokolović, D., Babić, B., Vojin, S., Ikanović, J., Veljovic, J., Balalić, I. 2013.. Genetic variabilyty of seed yield and seed components of autochthonous *Lolium perenne* L. populations. Genetika, Vol. 45. No. 2, 553-556.

Marišovà, E., Milovanović, J., Đorđević, S., Jerekovà, Z., Dražić, G., Hauptvogl, M., Prčik, M., Mariš, M., Kortla, M., Fandel, P., Ilkova, Z., Gaduš, J., Popović, V., Ikanović, J., Živanović, Lj., Radojević, U., Kovàčik, M., Mandalovà, K., 2016. Agro-energy for sustainable agriculture and rural development. Monograph. Good practices from Slovakia-Serbia bilateral cooperation. 1-291.

Mihailovic, B., 2008. Marketing. Faculty of Economy, Podgorica, Montenegro.

Mihailović, V., Mikić, A., Ćupina, B., Katić, S., Karagić, Đ., Pataki, I., Erić, P. 2006. Yield and for age yield com po nents in win ter vetch cultivars. Grass land Sci ence in Eu rope, 11: 255-257. 204

Mihailović, V., Mikić, A., Katić, S., Milić, D., Vasiljević, S., Pataki, I.,Ćupina, B. 2007. Directions and recent achieve ments in breeding annual forage and grain legumes in Serbia. Proceedings of the COST Action 852 Final Meeting Quality Legume-Based Forage Systems for Contrasting Environments, Gumpenstein, Austria, 27-30.

Mihailović, V., Pataki, I., Mikić, A., Katić, S.,Vasiljević, S., Karagić, Đ., Milić, D. 2009. A new generation of NS forage crop cultivars. Institute of Forage and Vegetable Crops, Novi Sad, 46, 199-205.

Nelson, G.C, Rosegrant, M.W., Koo, J., Robertson, R., Sulser, T., Zhu, T., Ringler, Claudia, Msangi, S., Palazzo, A., Batka, M., Magalhaes, M., Valmonte-Santos, R., Ewing, Mandy and Lee, D., 2009. Climate Change Impact on Agriculture and Costs of Adaptation. International Food Policy Research Institute, Food Policy Report, Washington, 30 p. DOI: 10.2499/0896295354

Popovic, V., 2010. Influence of agro-technical and agro-ecological practices on seed production of wheat, maize and soybean. Doctoral thesis, University of Belgrade, Faculty of Agriculture in Zemun, 20-35.

Popovic, V., Vidic, M., Jockovic, Dj., Ikanovic, J., Jaksic S., Cvijanović G., 2012a. Variability and correlations between yield components of soybean [Glycine max (L.) Merr.]**.** Genetika, Belgrade, Vol. 44, No.1, 33-45.

Popovic, V., Jaksic, S,, Glamoclija, DJ., Djekic, V., Grahovac, N., Mickovski Stefanovic, V. 2012b. Variability and correlations between soybean yield and quality components. Romanian Agricultural Research, Romania. No. 29, 131-138.

Popovic, V., Miladinovic, J., Vidic, M., Vučkovic, S., Ikanovic, J., Drazic, G., Djekic, V., Filipovic, V., 2015a. Determining genetic potential and quality components of NS soybean cultivars under different agroecological conditions. Romanian Agricultural Research, 32: 35-42. DII 2067-5720 RAR 2015-32.

Popović, V., 2015b. The concept, classification and importance of biological resources in agriculture. Editor. Milovanovic & Đorđević: Conservation and enhancement of biological resources in the service of ecoremediation**.** Monograph. Futura. Belgrade. ISBN 978-86-86859-41-9; 1-407. 29-51.

Popovic, V., Tatic, M., Sikora, V., Ikanovic, J., Drazic, G., Djukic, V., Mihailovic, B., Filipovic, V., Dozet, G., Jovanovic, Lj., Stevanovic, P., 2016a. Variability of yield and chemical composition in soybean genotypes grown under different agroecological conditions of Serbia. Romanian Agricultural Research, No. 33, 29-39. DII 2067-5720 RAR 2016-167.

Popović, V., Vidic, M., Ikanovic, J., Djekic, V., Filipovic, V., Tabakovic, M., Veselic, J. (2016b): Soybean oil yield as affected by the growing locality in agro-climatic divergent years. "Agriculture and Forestry" - Podgorica, Vol.62, 1, 217-225.

Popovic, V., Sikora, V., Ugrenovic, V., Filipovic V. (2017). Status of buckwheat (*Fagopyrum esculentum*) production in the worldwide and in the Republic of Serbia Book. Chapter 9. In. Rural Communities in the Global Economy. Beyond The Classical Rural Economy Paradigms Authors. NICOLAE I., DE LOS RIOS I. AND VASILE A.J. NOVA SCIENCE PUBLISHERS, USA. 178-198.

Statistica 12.0, StatSoft. University Licence, Institute of Field and Veget.Crops, Serbia.

Saha, G.C., Vandemark, G.J., 2012. Evaluation of Expression Stability of Candidate References Genes among Green and Yellow Pea Cultivars (*Pisum sativum* L.) Subjected to Abiotic and Biotic Stress. American J. Plant Sciences, 3, 2, 235-242.

Sikora V., Berenji J., Popović Vera, Brdar Jokanović M., Maksimović L. (2015): Accumulation and distribution of NPK in above ground parts of grain sorghum and maize in intensive production. Vol. 61. Issue 1: 223-230.

Sikora, V., Popovic, V., Zoric, M., Latkovic, D., Filipovic, V., Tatic, M., Ikanovic, J. (2016). An agro-technological characterization of south-eastern european broomcorn landraces. Pak. J. Agri. Sci., Vol. 53 (3), 567-576;

Tiwari, G., Lavanya, G. R., 2012. Genetic Variability, Character Association and Component Analysis in F4 Generation of Forage pea (Pisum sativum var.arvense L.). Karnataka Journal of Agricultural Sciences, 25, 173-175.

Togay, N., Togay, Y., Yildirim, B., Dogan, Y., (2008). Relationships between yield and some yield components in pea (*Pisum sativum* ssp arvense L.) genotypes by using correlation and path analysis. African J. of Biotechnology, 7(23): 4285-4287.

DOI: 10.17707/AgricultForest.63.2.08

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MONITORING THE POPULATION DEVELOPMENT AND THE INFESTATION DENSITY OF TOMATO MOTH (*TUTA ABSOLUTA* MEYRICK) (LEP.: GELECHIIDAE) IN GREENHOUSES

SUMMARY

The adult population development and infestation ratio of Tomato Moth (*Tuta absoluta* Meyrick) were studied in Çumra (Konya) district in three tomato greenhouses in 2011 and 2012. Two sexual pheromone traps were placed in greenhouses to monitor population development. Infestation density of tomato leaf and fruit was determined on 100 tomato plants by visual examination method. The number of adults in the traps was examined and recorded weekly. Additionally, as the mass trapping technique, ferolite traps were used to control this pest. Four generations of *T. absoluta* were determined in greenhouses and maximum adult numbers on pheromone traps in first, second and third greenhouse were found as 640, 626 and 154, respectively. While infestation ratio of tomato leaves in each greenhouses were determined as 70%, 80% and 25%, fruit infestation ratios were 23, 25 and 5%, respectively. The maximum number of adult Tomato Moth in ferolite traps in the first and second greenhouse was as 1250 and 1525, respectively. Study results suggested that ferolite traps were found to be successfully used for mass trapping of the pest by decreasing adult population density.

Keywords: *Tuta absoluta*, pheromone, ferolite, greenhouse

INTRODUCTION

Tomato is one of the most widely grown vegetables in Turkey. It's grown both in field and greenhouse. In recent years, it's grown successfully in greenhouses especially located in Sugar Factory and some other places in Central Anatolia along with the greenhouses in Aegean and Mediterranean Regions.

According to data of 2011, tomato was grown a total of 136.276 t which include 91.936 t for table and 44.430 t for sauceboat in 17.343 ha field in Konya province. It was grown a total of 9.587 tomatoes which include 4.637 t for table and 4.950 t for sauceboat in 3.068 ha fields in Çumra district (Anonymous, 2011).

There are many pests that damage the leaves and the fruits of the tomato and one of them is Tomato Moth (*Tuta absoluta* Meyrick),in Lepidoptera order.

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

The pest, first reported in South America, damaged the tomato plants in different parts of the world (Barrientos et al., 1998; Estay, 2000). Although the larvae of the pest feed on all above-ground parts of the tomato plant, they mostly prefer the leaves and the fruits. Various researchers mentioned the importance of the pest all around the world (González-Cabrera et al., 2010; Roditakiset al., 2010; Seplyarskyet al., 2010).

Tuta absoluta have led to yield loss and decrease the market value of tomato in recent years (Seplyarskyet al., 2010; Roditakiset al., 2010).

It was reported that the pest damaged the tomato in İzmir (Kılıç, 2010), Antalya (Erler et al., 2010), Mersin (Karut et al., 2011) and it caused damage to tomato (Ünlü, 2011) and potato (Unlu, 2012) in Konya. In this study, we studied population development of the pest with pheromone traps, infestation density on tomato leaves and fruits and ferolite traps for the control of the pest in greenhouses in Çumra district, Konya province.

MATERIALS AND METHODS

The study was carried out in Çumra Sugar Mill tomato greenhouses during the production seasonsof2011-2012 (period October 2011 to July 2012). Pheromone traps were used to monitor the adult population development and ferolite traps were used for mass trapping.

Experiment area

The study was conducted in three greenhouses in Çumra Sugar Mill. Two of them, 0.5 ha each, were hydroponic (soilless) tomato greenhouses (G1 and G2) and the third one, 0.2 ha in size, was terreous greenhouse (G3). One of the hydroponic tomato greenhouses was covered with glass and the other one with polycarbonate. The terreous greenhouse was covered with plastic.

Adult Population Development

Two pheromone traps were established for each greenhouse (greenhouse 1, 2 and 3) to monitor adult population. The pheromone capsules of the traps were replaced every two months; sticky sheets were replaced every 3-4 weeks. The trapped adults were counted and recorded, weekly.

Mass-trapping of Adults with Ferolite traps

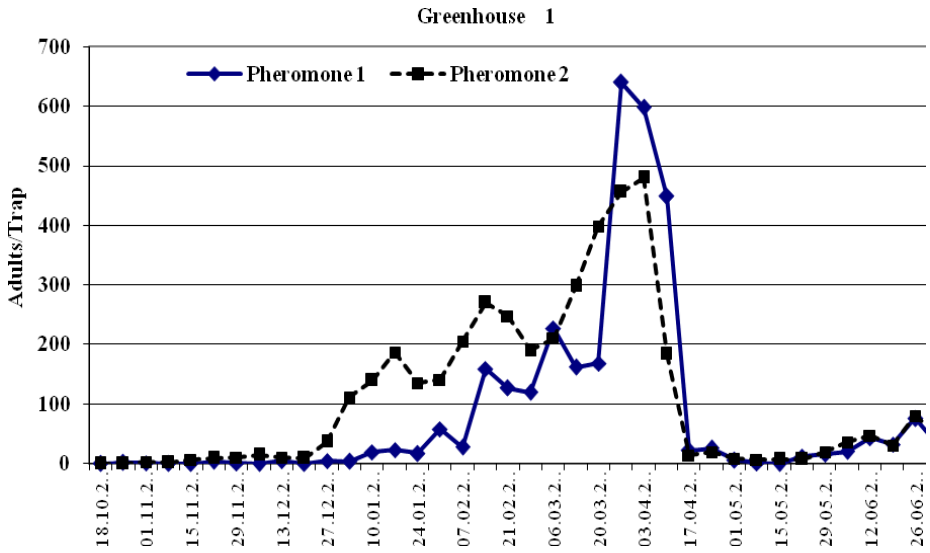
Ferolite traps, a combination of light and pheromone traps, were used for tomato moth mass-trapping. The power source of the traps maintained by a charged battery via solar panel on the top of the trap during the day time and thus the lamp of the trap can be on during the night time. In addition, there is a detergent water area in the base of the trap in order to catch the adult moths. One ferolite trap was established in each of greenhouse 1 and 2. The capsule of the traps was replaced with the new ones every two months; water was added weekly if needed. The trapped adults were recorded weekly.

Determination of the Infestation Density of the Tomato Moth

In order to determine the damage of the tomato moth on the leaves and fruits; 100 tomato plants were selected randomly in each greenhouse, every part of selected plants were monitored. Larvae and larvae damages were recorded.

RESULTS AND DISCUSSION

The weekly adult population development of the pest was monitored in Çumra Sugar Mill tomato greenhouses, October 2011 to July 2012, by pheromone traps. The results of the G1 were shown in the Graph 1.



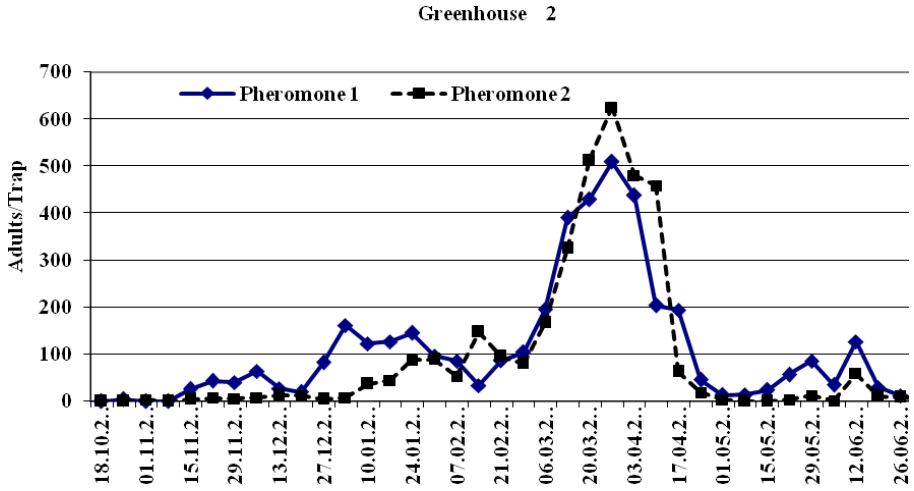
Graph 1. The adult population development of tomato moth in pheromone traps in G1

First *T. absoluta* adults were captured by pheromone traps on October 25th, 2011 in G1. The number of adults was less than twenty (5-15 adults/week) until December 20th 2011, density started to increase in the last week of December (27 December 2011) and reached the maximum (640 adults/week) on March 27th2012. The population decreased during the last week of March and a new peak (47 adults/week) occurred on 12thof June. The numbers of trapped adults decrease in mid-June. As long as all the conditions are favorable, larvae do not enter diapause and produced 10-12 generations per year (EPPO, 2005). It have been detected that the pest population peaked 4-5 times in G1 throughout the tomato production season.

The results of the greenhouse 2 (G2), were shown in Graph 2.

First *T.absoluta* adults were captured by pheromone traps on 25thOctober 2011 in G2, similar to G1. There were no moths in the traps until November 08th, 2011. Adults appear and their density started to increase(26 adults/week) on November 15th, 2011. The first peak of the pest population (64 adults/week) was

detected on December 06th, 2011, the second peak (162 adults/week), with a more adults moth was on January 03th, 2012.The pest population density fluctuated throughout the season and reached the highest level (626 adults/week) on March 27th, 2012. After this date, the number of adults rapidly decreased making two peaks (86-127 adults/week) on May 29th and June 12th.The production cycle end's with the dismantling of the plants in greenhouse.



Graph 2. The adult population development of tomato moth in pheromone traps in G2

The results of the terreous greenhouse 3 (G3) were shown in the Graph 3.

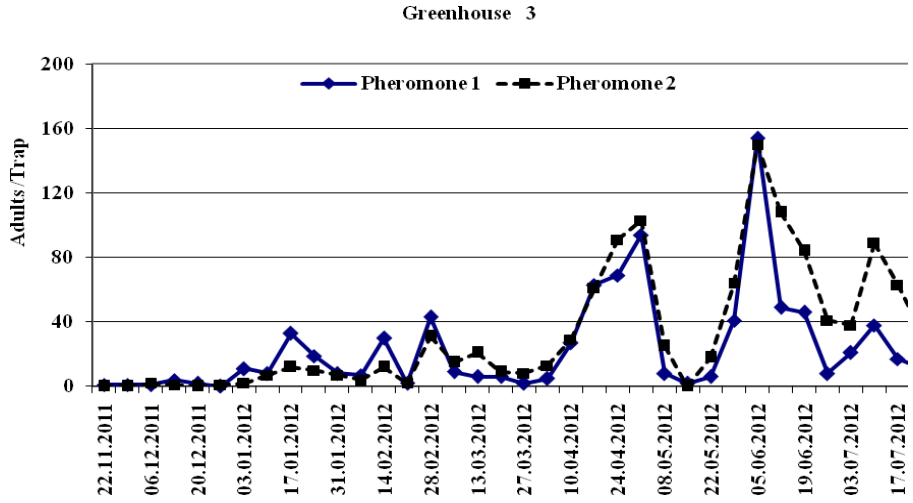
Tuta absoluta adults were captured by pheromone traps on November 22th, 2011 in G3. However the population of the pest had been low approximately for five months, the first peak of the pest (103 adults/week) was on May 01st, 2012.After this date the number of adults decreased and detected only 1 adult/week on May 15th, 2012. The pest peaked approximately in every four weeks and reached the highest level (154 adults/week) on June 05th, 2012 and the population of the pest declined steadily towards the end of the production season.

The number of adults trapped in ferolite traps is shown in Graph 4.

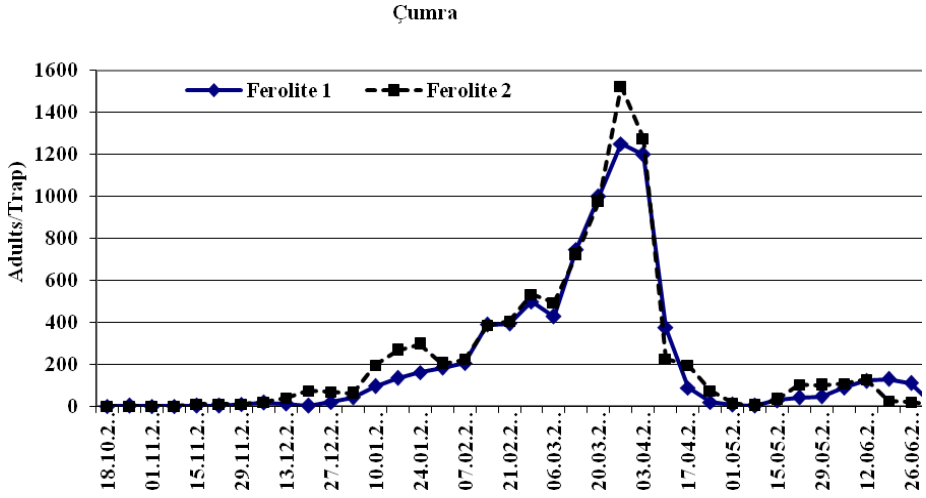
The first *T. absoluta* adults were captured by Ferolite traps in G1 and G2 on October 25th, 2011. The numbers of adults in the traps generally increased from November 15th2011 to March 27th2012, with a peak (1525adults/week) on March 27th 2012.After this date, the number of adults rapidly decreased in the ferolite traps and the decrease in the population continued until 15 May 2012. The final peak of the moth was (129 adults/week) on June 19th, 2012 and the population of the pest declined gradually towards the end of June.

Also, cultural control (pruning the infected leaves) led to decrease in the pest populations in the traps. The pest population continued in low numbers from late May (May 22nd 2012) until early July (July 03rd 2012). Due to the lack of the economic efficiency of the insecticide applications and coming to the end of

spring production season in late May and early July, the greenhouse management stopped the insecticide application and it was considered that this contributed to an increase in the pest population.



Graph 3. The adult population development of tomato moth in pheromone traps in G3



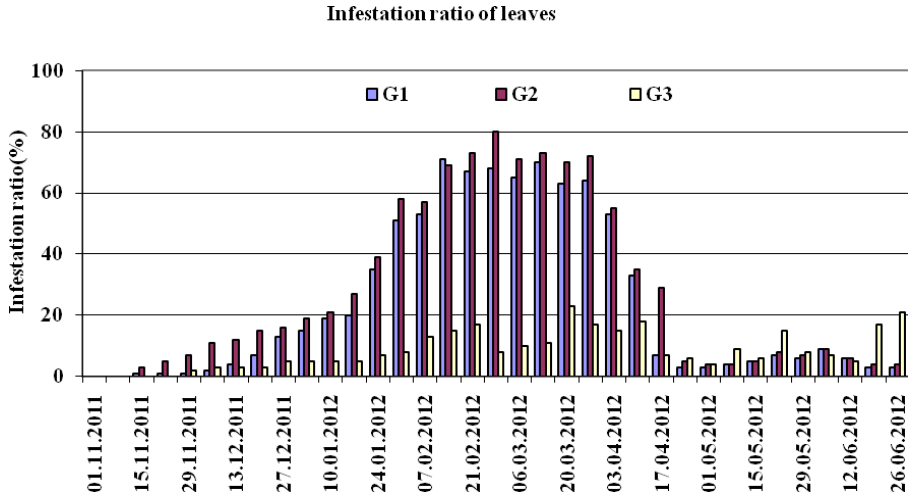
Graph 4. The adult population development of tomato moth in Ferolite traps in tomato greenhouses

Filho et al. (2000b), caught 869 adults per pheromone traps for three nights in succession in field trials, Ferrara et al. (2001), caught 201 male adults with standard pheromone dose and it's up to 1200 adults when high pheromone dose

applied. In this study, it was found that the highest number of adults caught in all three greenhouses was 473 adults per week.

It was found that ferolite traps were able to capture both male and female adults and capture more adult than pheromone traps, thus, they were judged as more effective than pheromone traps. Also, the numbers of adults in ferolite traps were twice or more than sex pheromone traps because ferolite traps acted as both light traps and pheromone traps

In order to determine the damage of the tomato moth on the leaves and fruits; tomato plants were selected randomly in each greenhouse, leaves and fruits of the tomato plants were checked and larvae and larvae damages were recorded. The infestation ratio of the leaves is shown in Graph 5.

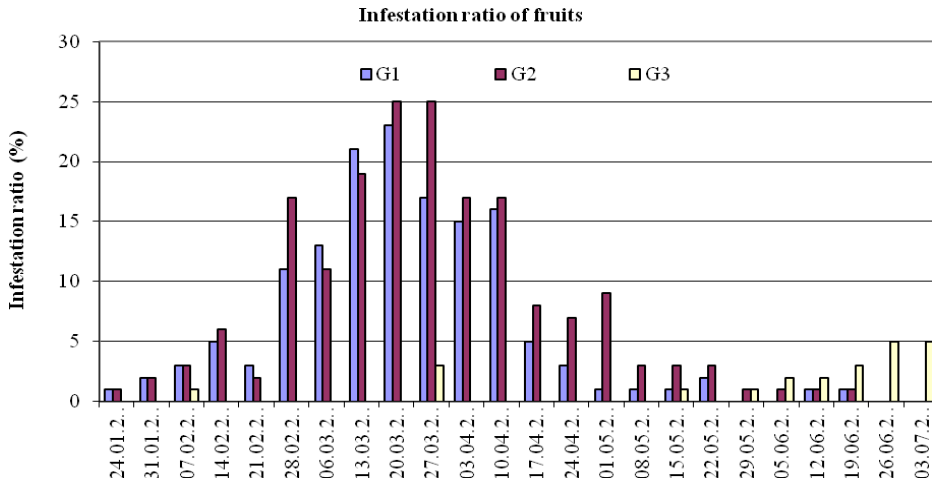


Graph 5. The infestation ratio of tomato moth on the leaves of tomatoes in greenhouses

The larvae and the damages of the pest were detected on November 15th, 2011 in G1 and G2, on November 29th, 2011 in G3on the lower leaves of the plants. The infestation ratio started to increase on December 06th, 2011.Due to the prune of the lower leaves of the tomato plants, on January 03rd, 2012 in G1 and January 17th, 2012 in G2, the pest shifted to the middle leaves of the plants. The infestation rate was under 50% in both greenhouses until January 24th, 2012, than started to increase over 50% since January 31th, 2012.In G3, the infestation ratio increased over 50% on March 20th, 2012. The pupa was observed on the leaves and branches of tomato plants in greenhouses on February 21th, 2012. The infestation ratio peaked; on February 28th, 2012 in G1 and G2, and on March 20th, 2012 in G3.The infestation ratio of the pest decreased less than 50% on April 10th, 2012 in all greenhouses and continued to decline except G3. Towards the end of the production season, while the infestation ratio decreased in G1 and

G2, it was observed that the infestation amount increased, reached the highest level and peaked on July 03rd, 2012 in G3.

Data of the infested fruits were shown in Graph 6.



Graph 6. The infestation ratio of tomato moth on the fruits in greenhouses

The pest was determined on ripe and under ripe fruit in G1 and G2 on January 24th, 2012, in G3 on February 07th, 2012. The pupa was observed on the fruits on February 14th, 2012 and larvae damages were found on the sepals. The infestation intensity of the fruits was increased due to pruning in G1 and G2 on February 21th, 2012.It was observed that the highest infestation rate of G1 and G2 on March 20th, 2012 were 23 and 25%, respectively. Throughout the production season, the highest infestation rate in G3 was detected as 5% on 26 June 2012.

The highest infestation rate of the leaves was in G2. Fruit infestation rate was found high in G2 like infestation ratio of leaves. This can be resulted from pruning the leaves due to the infestation or for better plant growth. So, the pest able to pass from leaves to fruit. Karut et al. (2001) found that the highest infested tomato fruit ratio per plant was recorded as 38.4% in tomato greenhouse in Mersin province. In this study, it was found that the highest infestation ratio in tomato leaves and fruit was 80 and 25%, respectively.

It was determined that the number of adults was fewer in the terreous greenhouse (G3) than the other two hydroponic (soilless) tomato greenhouses (G1 and G2). Various types of vegetables located in (e.g. pepper and cucumber) and late planting of tomato seedlings can cause fewer numbers of adults in G3 and this led to decrease in the infestation ratio of leaves and fruits.

CONCLUSION

Study results revealed that tomato moth damaged the tomato plants in all greenhouses located in Çumra where the study was conducted. In the control of

the pest, establishing the pheromone or ferolite traps from the planting of tomato seedling better than would be better able to give us knowledge about the damage and early populations of the tomato moth. When the pest population was extremely high, increasing the number of traps for monitoring would also provide suppression on the number of the pest. It was advised that because of high temperatures in greenhouses during the growing season, the pheromone capsules which are replaced every five-six weeks for the efficiency of the traps.

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REFERENCES

Anonymous, 2011. Türkiye İstatistik Kurumu, Bitkisel Üretim İstatistikleri 10.07.2012

Barrientos, ZR, Apablaza, HJ, Norero SA, Estay PP, 1998. Temparatura base y constante térmica de desarrollo de la popilla del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). Ciencia e Investigación Agraria 25:133-137.

EPPO, 2005. Bulletin OEPP/EPPO Bulletin 35, 434–435. https://www.eppo.int/QUARANTINE/data_sheets/insects/DS_Tuta_absoluta.pdf

Erler, F., M. Can, M. Erdoğan, A. Ö. Ateş and T. Pradier, 2010. Domates güvesi, *Tuta absoluta* Antalya'da. http://www.bioglobal.com.tr/tr.i88.domates-guvesi-tuta-absoluta-antalya-da

Estay, P., 2000. Polilla del Tomate *Tuta absoluta* (Meyrick). Impresos CGS Ltda. Available online at:

http://www.inia.cl/medios/Decargas/CRI/Platina/Informativos/Informativo9.pdf. Accessed 21 August 2007

Ferrara, FAA, EF Vilela, GN Jham, AE Eiras, MC Picanço, AB Attygalle, A Stavos, RTS Frighetto and J Meinwald, 2001. Evaluation of Synthetic Major Component of the Sex Pheromone of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Journal of Chemical Ecology, 27: 907-917.

Filho, MM, EF Vileda, GN Jham, A Attygalle, A Svatoś, J Meinwald, 2000. Initial Studies of Mating Disruption of the Tomato Moth, *Tuta absoluta* (Lepidoptera: Gelechiidae) Using Synthetic Sex Pheromone. Journal of Brasil Cemical Society, 11:621-628.

Gonzalez-Cabrera J, O Molla, H Monton and A Urbaneja, 2010. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). BioControl,

Karut, K, Kazak, C, Döker, İ and Ulusoy, MR, 2011. Pest status and prevalence of Tomato moth *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) in tomato growing greenhouses of Mersin. *Türkiye Entomoloji Dergisi*, 35: 339-347, (2011).

Kılıç, T., First record of *Tuta absoluta* in Turkey. *Phytoparasitica*, 38:243-244, (2010).

Roditakis E, D. Papachristos, NE Roditakis, 2010. Current Status of the tomato leafminer *Tuta absoluta* in Greece. Bulletin OEPP/EPPO Bulletin 40, 163-166.

Seplyarsky V, M Weiss, A Haberman, 2010. *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), a new invasive species in Israel. *Phytoparasitica*, DOI: 10.1007/s12600-010-0115-7

Ünlü, L., 2011. Domates Güvesi, *Tuta absoluta* (Meyrick)'nın Konya İlinde Örtüaltında Yetiştirilen Domateslerdeki Varlığı ve Popülasyon Değişimi. *S.Ü. Ziraat Fakültesi Dergisi* 25 (4): 27-29, (2011).

Unlu, L., 2012. Potato: A New Host Plant of *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) in Turkey. *Pakistan J. Zool*, 44, , 1183-1184.

DOI: 10.17707/AgricultForest.63.2.09

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SCREENING SUGAR BEET GENOTYPES IN DROUGHT STRESS CONDITION USING TOLERANCE INDICES

SUMMARY

Drought is one of the major problems affecting crops production, including sugar beet. In order to identify drought tolerant sugar beet genotypes, an experiment with forty three sugar beet genotypes was conducted during 2016 in West Azerbaijan Province of Iran, using a complete randomized block design with three replications, under normal and water-stressed states. Analysis of variance revealed variability among studied genotypes in response to moisture conditions. Genotypes "HSF-861" and "HSF-844" produced the highest root yield and genotypes "F-205051" and "HSF-883" produced the highest white sugar yield under well-watered and water-stressed states, respectively. Assessing genotypes according to some selection indices lead to introduce promising genotypes (Group A) for root yield ("HSF-844", "HSF-859", "HSF-861", "HSF-883" and "32434-91") and white sugar yield ("HSF-841", "HSF-844", "HSF-847", "HSF-861"). In this study, genotype "HSF-844" possessed acceptable root and white sugar yield in both states simultaneously. Classification of studied genotypes using calculated tolerance indices, located them into three (root yield data) and five (white sugar yield data) groups. These differentiate groups relies on the existence of genetic variability in studied sugar beet germplasm. Hence, sugar beet breeders could effectively use selected parental lines from this germplasm for further research works like genetic analysis of drought tolerance and hybrid breeding programs.

Keywords: Drought tolerance, genetic variability, sugar beet

INTRODUCTION

Drought stress is one of the several environmental factors that greatly limiting crop production and plant distribution worldwide. Sugar beet (*Beta vulgaris* L.) supplies about a quarter of the world's sugar demand (Draycott, 2006). Assuming its origin from the indigenous Mediterranean *B. maritima*, sugar beet is a relatively young crop possessing a narrow genetic base (Van Geyt et al., 1990). In sugar beet, drought causes yield reductions about 10-30% in

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central and western Europe (Ober, 2001; Pidgeon et al., 2001; Jones et al., 2003), which increase in arid and semiarid regions (Sadeghian et al., 2000), especially where precipitation is low. Results indicated that root yield was more by 20% in 100% water requirement treatment compared with 50% treatment but sugar percentage in drier conditions was achieved more than wet conditions.

Identification of drought-tolerant sugar beet germplasm and determination of their genetic variability level in order to improvement of sugar beet varieties is considered as one of the most important strategies.

Albeit, improved tolerance to drought has been a goal but unfortunately, success in breeding for drought tolerance has been limited because (I) it is controlled by several genes, and their simultaneous selection is difficult (Richards, 1996; Yeo, 1998; Flowers et al., 2000), (II) tremendous effort is required to eliminate undesirable genes tightly linked to favorable ones, that are also incorporated during breeding (Richards, 1996) and (III) there is a lack of efficient selection procedures particularly under field conditions (Ribaut et al., 1997; Kirigwi et al., 2004).

Thus, the indices which provide a measure of stress based on yield loss under stress conditions in comparison to normal conditions have been used for screening stress tolerant genotypes (Adamczewska et al., 2009). These indices are either based on drought resistance or susceptibility of genotypes. Various quantitative criteria have been proposed for selection of genotypes based on their yield performance in stress and non-stress environments. Based on these indicators genotypes are compared in normal and stress conditions (Cieslik et al., 2009). Accordingly, geometric mean (Fernandez, 1992), mean productivity (Rosielle and Hamblin, 1981), harmonic mean (Jafari et al., 2009), stress susceptibility index (Fischer and Maurer, 1978), yield stability index (Bouslama and Schapaugh, 1984), yield index (Gavuzzi et al., 1997), stress tolerance index (Fernandez, 1992) and tolerance index (Rosielle and Hamblin, 1981) were introduced.

Genotypes can be categorized into four groups based on their yield in stress and non-stress environments: genotypes express uniform superiority in both stress and non-stress environments (Group A); genotypes perform favorably only in non-stress environments (Group B); genotypes yield relatively higher only in stress environments (Group C); and genotypes perform poorly in both stress and non-stress environments (Group D). The optimal selection criterion should distinguish Group A from the other three groups (Fernandez, 1992). Clarke et al., (1992) showed that yield-based SSI index did not differentiate between potentially drought resistant genotypes and those that possessed low overall yield potential. Similar limitations were reported by White and Singh (1991). Selection through TOL chooses genotypes with low Yp but with high Ys (group C) hence, TOL cannot distinguish between group C and group A (Fernandez, 1992). MP is mean yield for genotype in two stress and non-stress conditions. MP can select genotypes with high Yp but with relatively low Ys (group B) and it fails to distinguish group A from group B. By decreasing TOL

and increasing MP, the relative tolerance increases (Rosielle and Hamblin, 1981; Fernandez, 1992). There are several studies about drought screening in crops such as wheat (Sio-Se Mardeh et al., 2006), sunflower (Darvishzadeh et al., 2010), rice (Ouk et al., 2006) and so on. While, there is narrow studies about utilization of above mentioned tolerance indices in evaluation of sugar beet reaction into stress states. For instance, Vahidi et al. (2013), were proved that drought stress could significantly influenced white sugar yield of a studied sugar beet germplasm and therefore, application of tolerance indices for screening and identification of desirable genotype was recommended. Considering Korshid (2016), tolerance indices including STI, GMP, MP and YI could effectively screen sugar beet genotypes against salinity stress. About drought stress, there is narrow study about utilization of tolerance selection indices in sugar beet. However, literature review depicted a sharp contrast between the root and the shoot in their response to water deficit. Abdollahian-Noghabi and Froud-Williams (1998), also noted a drastic reduction in the leaf area and a smaller decrease in the taproot growth of sugar beet when subjected to drought stress. In another study (Mahmoodi et al., 2008), revealed that irrigation treatments had a significant effect on sugar yield and its quality and optimum soil water content for root yield is 70% of field capacity.

Hence, this project was aimed for evaluation of several tolerance indices in screening of studied sugar beet germplasm against drought stress and identification of drought tolerant genotypes of sugar beet based on root and white sugar yield.

MATERIALS AND METHODS

Plant material and experimental methodology

This research was conducted at Agricultural Research Station of Miandoabm located in West Azerbaijan province of Iran. This research station with a latitude of 36º 58´ N, longitude of 46º 90´ E and altitude of 1314m has possess silty-loam soil. This area has the Feric temperature regime (the average annual temperature of soil is 8-15 ºC) and Xeric moisture regime (semi-arid). In this study, 43 sugar beet half-sib families were evaluated in two (normal water and water-stressed states) separate randomized complete block design with three replications in field state.

Seedbed preparation practices such as plowing, disking and leveling were uniformly applied. Potassium and phosphorous fertilizers were applied at the time of seedbed preparation and nitrogen fertilizer was applied as topdressing. The between- and within-row spacing was 50 and 15 cm, respectively. Each plot consisted of three rows of 8m length. Cultural practices including irrigation and control of diseases and pests were applied when needed. After plant establishment (4-6-leaf stage), furrow irrigation was applied on the basis of cumulative evaporation from the class A evaporation pan. The inlet and outlet irrigation water was measured by using WSC flumes. Plants were harvested at

maturity, and then root yield accompanied with white sugar yield were measured for all genotypes in each replication. Drought tolerance indices were calculated using the equations cited in Table 1.

Table 1. List of drought tolerance indices used for evaluation of the reaction of sugar beet genotypes to drought conditions

Drought tolerance indices	Equation	Reference
Stress Susceptibility Index	$SSI = \frac{1-(\frac{Y_S}{Y_P})}{1-(\frac{\overline{Y}_S}{\overline{Y}_P})}$	Fischer and Maurer, 1978
Geometric Mean Productivity	$GMP = \sqrt{(Y_S)(Y_P)}$	Fernandez, 1992 and Kristin *et al.*, 1997
Mean Productivity	$MP = \frac{Y_S + Y_P}{2}$	Rosielle and Hambling, 1981
Harmonic Mean	$HM = \frac{2(Y_P \ . \ Y_S)}{Y_P + Y_S}$	Jafari et al. 2009
Tolerance index	$TOL = Y_P - Y_S$	Rosielle and Hambling, 1981
Stress Tolerance Index	$STI = \frac{(Y_S)(Y_P)}{(\overline{Y}_P)^2}$	Fernandez, 1992
Yield Index	$YI = \frac{Y_S}{\overline{Y}_S}$	Gavuzzi *et al.*, 1997
Yield Stability Index	$YSI = \frac{Y_S}{Y_P}$	Bouslama and Schapaugh, 1984

Y_S and Y_P are stress and optimal (potential) yield of a given genotype, respectively. $\overline{Y}_S$ and $\overline{Y}_P$ are average yield of all genotypes under stress and optimal conditions, respectively.

Statistical analysis

The data were analyzed using the general linear model (GLM) procedure in the SAS software (SAS Institute Inc., Cary, NC, USA). Correlations between root and white sugar yield in each of the water regimes with drought tolerance indices were determined using SPSS 18.0. Multivariate statistical analysis comprising three dimensional plots as well as cluster analysis were performed using the STASTICA ver. 7.0 software.

RESULTS AND DISCUSSION

Genetic variability for root and white sugar yield

Results revealed that there are significant differences among studied sugar beet genotypes for both root and white sugar yield traits under stress and non-stress conditions and their related tolerance indices (data not shown). Similar to finding of Abdollahian-Noghabi et al. (2011) and Korshid (2016), variability of yield in both stressed and non-stressed environments can imply the existence of useful resource for selection of drought tolerant genotypes through classical breeding methods.

In this study, the maximum and minimum value of root yield under normal condition (Yp) were belonged to genotypes "HSF-861" and "HSF-877" with value of 84.66 and 39 $t.ha^{-1}$, respectively (Table 2). Results showed that root yield under stress condition (Ys) varied from 19.50 (HSF-862) to 61.33 (HSF-844) $t.ha^{-1}$. Regarding Table 2, genotype "HSF-844" had the maximum values of several tolerance indices including MP, GMP, HM, YSI, SSI, STI, YI (Table 2) accompanied with high Yp (root yield under normal state) value (75.67 $t.ha^{-1}$) and therefore could be consider as drought tolerant genotype (Table 2).

Such root yield response to water stress was also reported by Fabiro et al. (2003), Mahmoodi et al. (2008), and Ober and Rajabi (2011). Generally, water deficit reduces plant growth as a result of first; inhibition of leaf growth and development due to the lower water availability and turgor pressure, and secondly; photosynthesis decrease due to the stomata closure (Smirnoff, 1995; Clover, 1997).

In this experiment, the maximum value of white sugar yield under normal condition (Yp) was belonged to genotype "F-205051" with value of 8.69 $t.ha^{-1}$ (Table 3) and the minimum value (2.02 $t.ha^{-1}$) was obtained for genotype "HSF-877". White sugar yield under stress condition (Ys) was varied from 1.67 (HSF-854) to 4.60 (HSF-883) $t.ha^{-1}$ (Table 3). In controversy, Last et al. (1983) stated that genotypes produced high root yield in both normal and water stress conditions, could resulted high white sugar yield regardless of water state, because of no reduction in sugar content.

Regarding root yield data and according to Fischer and Maurer index (SSI) (1978), the genotype "HSF-862" with high SSI value was found to be the most susceptible genotypes whereas genotype "HSF-844 "with low value were found to be tolerant to drought stress (Table 2).

The less numerical rate of SSI indicates less stress susceptibility and more drought stress tolerance of a genotype. Yadav and Bhatnagar (2001) suggested the use of SSI in combination with yield value under stressed condition for identifying drought tolerant/susceptible genotypes. As shown in Table 2, selection based on SSI index identified genotypes with relatively high Yp and Ys (for example: genotype "HSF-844") and this is not in agreement with Sio-Se Mardeh et al. (2006) and Clarke et al. (1992) reported that SSI index does not differentiate between potentially drought resistant genotypes.

Table 2. Average root yield of sugar beet genotypes under optimal and stress conditions, and calculated different drought tolerance indices

Genotype	Yp	Ys	TOL	MP	GMP	HM	YSI	SSI	STI	YI
HSF - 841	71.22	48.89	22.33	60.06	58.86	57.70	0.70	0.74	0.79	1.25
HSF - 842	65.22	37.89	27.33	51.56	49.43	47.42	0.59	1.01	0.56	0.97
HSF - 843	72.56	39.89	32.67	56.22	53.65	51.22	0.56	1.09	0.68	1.02
HSF - 844	75.67	61.33	14.33	68.50	68.07	67.64	0.80	0.48	1.07	1.56
HSF - 846	57.33	32.78	24.56	45.06	43.30	41.61	0.56	1.08	0.47	0.84
HSF - 847	74.00	35.56	38.44	54.78	49.83	45.82	0.52	1.18	0.59	0.91
HSF - 848	72.00	42.67	29.33	57.33	54.61	52.15	0.63	0.92	0.68	1.09
HSF - 849	64.78	33.89	30.89	49.33	46.45	43.79	0.55	1.11	0.51	0.86
HSF - 850	70.11	38.89	31.22	54.50	51.28	48.41	0.58	1.04	0.62	0.99
HSF - 851	61.22	40.00	21.22	50.61	49.45	48.32	0.67	0.81	0.60	1.02
HSF - 852	66.00	38.89	27.11	52.44	50.04	47.84	0.63	0.89	0.58	0.99
HSF - 854	66.22	25.00	41.22	45.61	39.70	34.90	0.40	1.46	0.37	0.64
HSF - 855	75.67	41.11	34.56	58.39	55.45	52.70	0.56	1.07	0.70	1.05
HSF - 856	73.67	42.89	30.78	58.28	55.91	53.68	0.59	1.00	0.72	1.09
HSF - 857	59.11	32.22	26.89	45.67	43.53	41.52	0.55	1.11	0.46	0.82
HSF - 859	83.67	53.17	30.50	68.42	66.61	64.86	0.63	0.91	1.08	1.35
HSF - 860	65.11	40.56	24.56	52.83	51.31	49.84	0.63	0.91	0.60	1.03
HSF - 861	84.67	46.33	38.33	65.50	61.92	58.62	0.56	1.08	0.91	1.18
HSF - 862	65.67	19.50	46.17	42.58	35.70	29.97	0.30	1.71	0.29	0.50
HSF - 864	52.89	38.33	14.56	45.61	44.99	44.38	0.73	0.66	0.46	0.98
HSF - 865	67.89	41.67	26.22	54.78	53.16	51.59	0.61	0.96	0.68	1.06
HSF - 866	69.44	45.67	23.78	57.56	56.11	54.72	0.66	0.83	0.72	1.16
HSF - 867	58.00	35.89	22.11	46.94	45.60	44.31	0.62	0.94	0.49	0.91
HSF - 868	67.89	42.78	25.11	55.33	53.04	50.98	0.63	0.90	0.67	1.09
HSF - 869	63.00	39.44	23.56	51.22	49.44	47.79	0.62	0.93	0.57	1.00
HSF - 870	52.00	27.33	24.67	39.67	37.63	35.71	0.53	1.16	0.33	0.70
HSF - 871	64.56	44.67	19.89	54.61	53.69	52.79	0.69	0.75	0.66	1.14
HSF - 872	69.00	37.22	31.78	53.11	50.18	47.50	0.58	1.02	0.60	0.95
HSF - 873	65.78	37.44	28.33	51.61	49.35	47.23	0.56	1.09	0.58	0.95
HSF - 875	65.78	34.00	31.78	49.89	47.01	44.35	0.51	1.21	0.53	0.87
HSF - 876	66.78	52.17	14.61	59.47	58.88	58.31	0.79	0.52	0.79	1.33
HSF - 877	39.00	26.33	12.67	32.67	31.91	31.19	0.67	0.82	0.24	0.67
HSF - 881	73.00	42.22	30.78	57.61	54.72	52.08	0.62	0.93	0.68	1.08
HSF - 882	69.89	35.22	34.67	52.56	49.15	46.04	0.52	1.18	0.56	0.90
HSF - 883	70.67	54.00	16.67	62.33	61.61	60.90	0.76	0.59	0.88	1.38
HSF - 884	82.33	38.56	43.78	60.44	56.16	52.22	0.47	1.31	0.73	0.98
HSF - 885	56.33	32.78	23.56	44.56	42.87	41.26	0.57	1.05	0.43	0.84
110	63.44	36.11	27.33	49.78	47.63	45.61	0.56	1.07	0.54	0.92
191	46.78	27.22	19.56	37.00	35.59	34.24	0.58	1.03	0.29	0.69
31265	61.89	33.89	28.00	47.89	45.27	42.92	0.55	1.09	0.47	0.86
32434-91	75.78	53.11	22.67	64.44	63.10	61.80	0.72	0.68	0.91	1.35
32926-92	55.33	35.22	20.11	45.28	44.01	42.80	0.64	0.89	0.45	0.90
F - 20505	71.44	45.22	26.22	58.33	56.69	55.10	0.64	0.89	0.75	1.15
$LSD_{5\%}$	19.74	16.55	22.19	14.47	14.77	15.65	0.23	0.59	0.36	0.43

Table 3. Average white sugar yield of sugar beet genotypes under optimal and stress conditions, and calculated different drought tolerance indices

	Yp	Ys	TOL	MP	GMP	HM	YSI	SSI	STI	YI
HSF - 841	5.33	4.31	1.02	4.82	4.75	4.69	0.85	0.46	0.99	1.35
HSF - 842	3.01	3.43	-0.43	3.22	3.07	2.92	1.34	-1.03	0.41	1.08
HSF - 843	4.45	4.44	0.01	4.45	4.44	4.42	1.00	0.01	0.90	1.39
HSF - 844	6.63	4.42	2.21	5.52	5.41	5.30	0.66	1.02	1.31	1.38
HSF - 846	3.40	3.74	-0.34	3.57	3.54	3.51	1.12	-0.36	0.60	1.17
HSF - 847	6.77	3.85	2.93	5.31	5.10	4.89	0.59	1.23	1.30	1.21
HSF - 848	5.15	3.59	1.57	4.37	4.23	4.10	0.74	0.78	0.78	1.12
HSF - 849	4.37	2.45	1.92	3.41	3.17	2.96	0.70	0.92	0.46	0.77
HSF - 850	4.15	2.69	1.46	3.42	3.33	3.25	0.64	1.08	0.51	0.84
HSF - 851	4.96	1.93	3.03	3.45	3.04	2.70	0.44	1.69	0.42	0.61
HSF - 852	4.64	3.48	1.16	4.06	3.80	3.58	0.91	0.26	0.63	1.09
HSF - 854	5.90	1.66	4.24	3.78	2.85	2.29	0.33	2.04	0.38	0.52
HSF - 855	4.11	3.83	0.28	3.97	3.73	3.52	1.26	-0.79	0.62	1.20
HSF - 856	4.34	3.65	0.70	4.00	3.93	3.87	0.89	0.34	0.68	1.14
HSF - 857	5.40	2.26	3.14	3.83	3.43	3.10	0.41	1.78	0.57	0.71
HSF - 859	4.63	3.75	0.88	4.19	4.13	4.07	0.79	0.63	0.81	1.18
HSF - 860	4.11	2.47	1.64	3.29	3.16	3.04	0.58	1.28	0.47	0.78
HSF - 861	5.74	4.08	1.66	4.91	4.79	4.68	0.68	0.96	1.10	1.28
HSF - 862	3.45	1.82	1.63	2.64	2.48	2.34	0.56	1.34	0.27	0.57
HSF - 864	3.15	3.84	-0.69	3.50	3.29	3.13	2.08	-3.28	0.51	1.20
HSF - 865	6.01	2.72	3.29	4.36	3.71	3.29	0.47	1.60	0.79	0.85
HSF - 866	5.10	3.05	2.05	4.08	3.81	3.57	0.75	0.76	0.64	0.96
HSF - 867	5.44	3.09	2.34	4.26	4.04	3.83	0.61	1.20	0.71	0.97
HSF - 868	5.22	3.26	1.96	4.24	3.86	3.55	0.79	0.64	0.66	1.02
HSF - 869	4.14	2.91	1.22	3.52	3.38	3.25	0.77	0.70	0.50	0.91
HSF - 870	3.77	3.10	0.67	3.43	3.34	3.26	0.85	0.46	0.50	0.97
HSF - 871	5.38	3.24	2.14	4.31	4.15	3.99	0.62	1.14	0.76	1.02
HSF - 872	4.80	2.62	2.18	3.71	3.55	3.39	0.55	1.37	0.55	0.82
HSF - 873	4.14	3.75	0.39	3.95	3.57	3.25	0.95	0.14	0.64	1.18
HSF - 875	6.91	2.39	4.52	4.65	4.01	3.48	0.37	1.90	0.70	0.75
HSF - 876	4.06	2.40	1.66	3.23	2.99	2.77	0.73	0.81	0.39	0.75
HSF - 877	2.02	2.58	-0.55	2.30	2.28	2.26	1.27	-0.83	0.24	0.81
HSF - 881	5.75	3.94	1.81	4.85	4.51	4.23	0.80	0.62	0.89	1.23
HSF - 882	4.70	2.67	2.03	3.68	3.52	3.37	0.59	1.24	0.55	0.84
HSF - 883	5.13	4.56	0.57	4.85	4.78	4.71	0.91	0.27	1.00	1.43
HSF - 884	5.47	3.00	2.47	4.23	3.93	3.66	0.72	0.86	0.72	0.94
HSF - 885	3.64	3.13	0.51	3.39	3.31	3.23	0.92	0.26	0.48	0.98
110	5.13	3.32	1.80	4.22	4.11	4.00	0.61	1.17	0.84	1.04
191	3.61	2.43	1.18	3.02	2.93	2.85	0.73	0.81	0.39	0.76
31265	4.46	3.69	0.77	4.07	4.04	4.01	0.82	0.56	0.74	1.16
32434-91	4.60	3.53	1.07	4.07	4.00	3.93	0.80	0.61	0.70	1.11
32926-92	4.11	3.64	0.46	3.87	3.83	3.78	0.89	0.33	0.66	1.14
F - 20505	8.69	2.48	6.21	5.59	4.64	3.86	0.29	2.16	0.98	0.78
$LSD_{5\%}$	2.73	1.94	3.38	1.66	1.59	1.607	0.82	2.51	0.56	0.60

Considering TOL index, a genotype would be more tolerant if it has less TOL value. Based on TOL, the genotype "HSF-877" with lowest values was considered as tolerant genotypes, whereas the genotype 'HSF-862" with the highest TOL value was considered as susceptible (Table 2). Fernandez (1992) manifested that, TOL index was efficient in improving yield under stressed condition and the selected genotypes performed poorly under non-stressed condition.

Yield stability index (YSI) also was calculated for a given genotypes using root yield under stressed and non-stressed conditions. The genotypes with high YSI is expected to have high yield under stressed and low yield under non-stressed conditions. The lowest and highest of YSI were observed for genotypes "HSF-862" and "HSF-844", respectively (Table 2). Fernandez (1992) proposed that, high yield and stress tolerant genotypes can be discriminated based on STI index. A high STI demonstrates a high tolerance and the best advantage of STI is its ability to separate group A genotypes from other genotypes. Based on the STI index, the genotype "HSF-844" had the highest value and considered as tolerant genotype with high yield stability in the both conditions (Table 2). In this study, the results of GMP, MP, HM and YI indices in selection of tolerant genotypes were similar to STI index (Table 2).

Regarding white sugar yield data, the genotype "F-20505" with high SSI value was found to be the most susceptible genotypes, whereas genotype "HSF-864" with low value was found to be tolerant to drought stress (Table 3). Based on TOL index, the genotypes "HSF-864" and "F-20505" with lowest and highest value was considered as tolerant and susceptible genotypes, respectively (Table 3). The lowest YSI was observed for genotype "F-20505" and the highest was observed for genotype "HSF-864" (Table 3). Paralleled with finding of YSI index, this is inferable from Table 3 that genotype "F-20505" have maximum value of white sugar yield in normal condition while it possessed minimum value under stress state.

According to STI, HM and GMP indices, the genotype "HSF-844" had the highest value and considered as tolerant genotype with high yield stability in the both conditions (Table 3). From YI index view, genotype "HSF-854" was identified as drought tolerant genotype, whereas it have not acceptable white sugar yield under stress condition. Sadeghian et al. (2000) believes that sugar beet genotypes can be categorized into four groups according to their performance in drought and favorable conditions: 1. Genotypes with high productivity in both conditions, 2. Genotypes with higher yield in non-stress environment, 3.genotypes with a relatively high yield in stress environment and 4.genotypes with a poor yield in both conditions. Genotypes with high productivity in both stress and non-stress conditions are useful for breeding purposes.

Correlation between root and white sugar yield with drought tolerance indices

Correlation coefficients were used to identify the best criterion for identification and screening of drought tolerant genotypes. According to

literature (Farshadfar and Sutka, 2002; Darvishzadeh et al., 2010), a suitable index must to have a significant correlation with yield in both stressed and non-stressed conditions. Correlation coefficients between both root yield (Yp and Ys) with studied tolerance indices (Table 4) revealed that indices including MP, GMP, HM, YSI, STI and YI could effectively use in screening of drought tolerant sugar beet genotypes. TOL index was strongly associated with YSI and YI indices and hence they could make similar ranking of genotypes. In this study, STI, YSI, HM, and GMP indices had significant correlations with each other in most cases (Table 4).

About white sugar yield (Table 4), both Yp and Ys possessed significant correlations with studied indices exception of YI. This is similar to findings of average white sugar yield (Table 3) which manifested that genotype "HSF-854" has not acceptable white sugar yield under stress state despite of having highest YI index. Considering Table 4, in most cases there is not any significant relation among studied indices and so, they could produce variable ranking of drought tolerant genotypes. Similar to our findings, Hesadi et al. (2015) investigated the response of five promising sugar beet genotypes against drought stress using tolerance indices and reported strong correlation between GMP, STI, MP and HM indices with white sugar yield in both normal and stress conditions.

Table 4. Correlation between different drought tolerance indices and white sugar yield (above of diagonal) and root yield (below of diagonal) of sugar beet genotypes under optimal and stress conditions

	Yp	**Ys**	**TOL**	**MP**	**GMP**	**HM**	**YSI**	**SSI**	**STI**	**YI**
Yp	1.00	0.02	0.85**	0.85**	0.68**	0.52**	-0.65**	0.65**	0.69**	0.02
Ys	0.69**	1.00	-0.51**	0.54**	0.72**	0.81**	0.50**	-0.50**	0.69**	1.00**
TOL	0.47**	-0.28	1.00	0.45**	0.21	0.01	-0.82**	0.82**	0.23	-0.51**
MP	0.94**	0.89**	0.15	1.00	0.95**	0.86**	-0.29	0.29	0.94**	0.54**
GMP	0.89**	0.94**	0.02	0.99**	1.00	0.97**	-0.15	0.15	0.98**	0.72**
HM	0.82**	0.97**	-0.09	0.96**	0.99**	1.00	–0.04	0.04	0.95**	0.81**
YSI	0.30*	0.80**	-0.67**	0.58**	0.66**	0.72**	1.00	1.00**	-0.17	0.50**
SSI	0.17	-0.58**	0.84**	-0.16	-0.28	-0.39**	-0.70**	1.00	0.17	-0.50**
STI	0.75**	0.93**	-0.03	0.89**	0.92**	0.93**	0.54**	-0.445**	1.00	0.69**
YI	0.68**	1.00**	-0.28	0.89**	0.94**	0.97**	0.80**	-0.58**	0.93**	1.00

Multivariate analysis

To identify the relationship among Yp, Ys with their significant tolerance indices, three-dimensional graphs for each one were also employed. These graphs showed the ability of these indices to detect Fernandez (1992) groups (Figure 3). By using these indices and Yp and Ys variables, three dimensional diagrams could partition the genotypes in four groups: (1) Genotypes producing high yield under both water stress and non-stress environments (group A), (2) genotypes with high yield under either non-stress (group B) or (3) stress (group C) environments and (4) genotypes with poor performance under both stress and non-stress environments (group D).

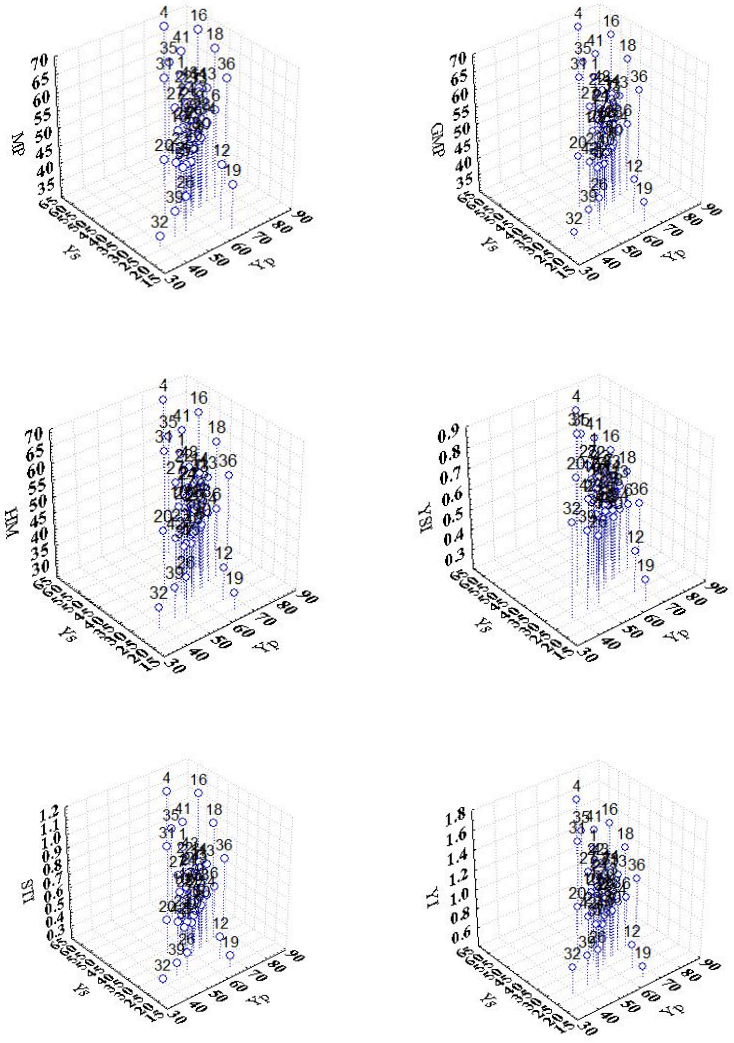

Figure 1. Tree dimension scheme of potential root yield (YP), stress root yield (YS) and geometric mean productivity (GMP), harmonic mean (HM), mean productivity (MP), stress tolerance index (STI) for sugar beet genotypes. Genotype codes: see Table 2.

Accordingly, data from root yield (Figure 1) revealed that genotypes such as G04 "HSF-844", G16 "HSF-859", G18 "HSF-861", G35 "HSF-883" and G41"32434-91" are promising sugar beet genotypes (Group A) which have suitable root yield in both conditions. Also, among studied genotypes, G26 "HSF-870", G32 "HSF-877", and G39 "191" having low root yield in both conditions (group C). Three dimensional plots of white sugar yield data showed

that the response of white sugar yield against drought stress is varied from the response of root yield (Figure 2).

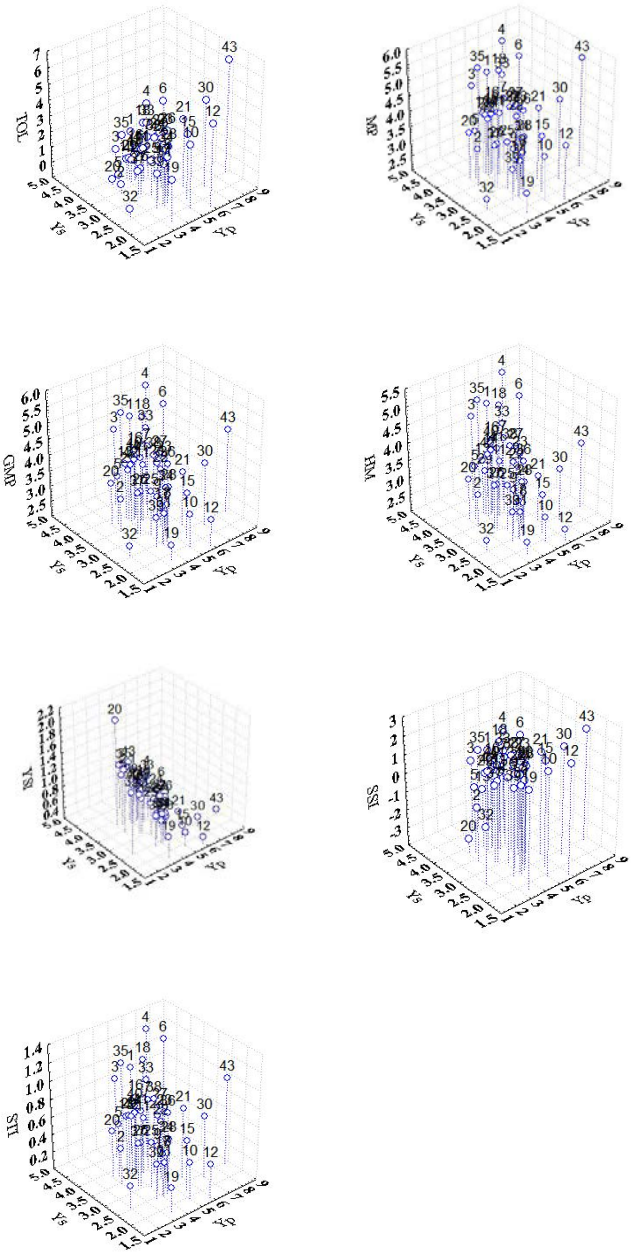

Figure 2. Tree dimension scheme of potential white sugar yield (YP), stress white sugar yield (YS) and geometric mean productivity (GMP), harmonic mean (HM), mean productivity (MP), stress tolerance index (STI) for sugar beet genotypes. Genotype codes: see Table 2.

Considering Figure 2, genotypes such as G01 "HSF-841", G04 "HSF-844", G06 "HSF-847", G18 "HSF-861" could be calculated as group A genotypes whereas G19 "HSF-862", G32 "HSF-877", G39 "" with low white sugar yield under both states divided into group C. Albeit, genotypes G12 "HSF-854", G15 "HSF-857", G21 "HSF-865", G30 "HSF-875" and G43 "F-20505" possessed high level of white sugar yield in normal state but they have not any suitable white sugar yield under stress condition.

The cluster analysis was done using UPGMA algorithm to classification and study the variation between sugar beet genotypes based on drought tolerance indices calculated using root and white sugar yield. Classification based on drought tolerance indices calculated via root yield in both conditions (Figure 3), grouped the studied genotypes into three main groups which involved 15 (group I), 17 (group II) and 11 (group III) genotypes, respectively, which each group could divided into several subgroups.

In this study, group I was comprised genotypes that had high root yield in both conditions (group A of Fernandez's classification) (Figure 1 and Figure 3).

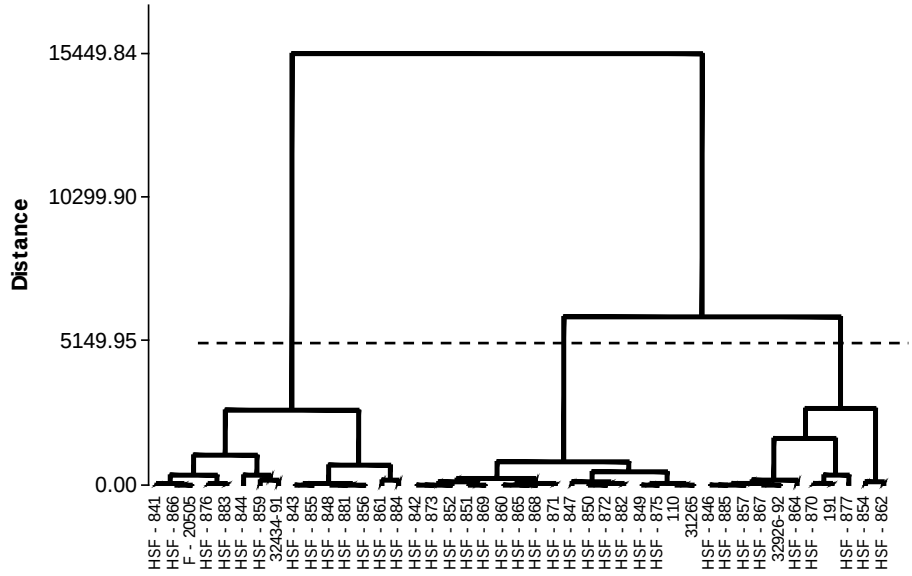

Figure 3. Cluster analysis of sugar beet genotypes based on drought tolerance indices and root yield in both normal and stress conditions.

Regarding tolerance indices calculated by means of white sugar yield in both conditions, the studied sugar beet genotypes located in five main groups involved 7 (group I), 14 (group II), 3 (group III), 5 (group IV) and 14 (group V) genotypes (Figure 4). Similar to finding of root yield based classification (Figure 3), group A of Fernandez's classification also fined by classification via white sugar yield. Here, in consistent with findings of Darvishzadeh et al. (2010), the

genotypes classification based on cluster analysis was paralleled with output of three dimensional plots. Therefore, by using genotypes that are located in separate groups and have maximum genetic distance, it is possible to analyze genetic basis of these drought tolerance indices in sugar beet.

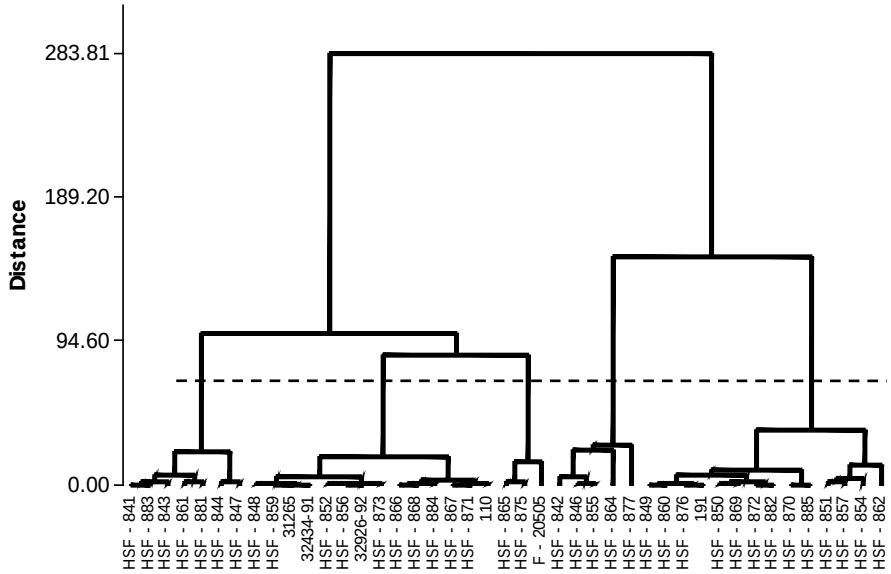

Figure 4. Cluster analysis of sugar beet genotypes based on drought tolerance indices and white sugar yield in both normal and stress conditions.

CONCLUSIONS

To sum up, in studied sugar beet germplasm, genotypes "HSF-861" and "HSF-844" having maximum root yield in non-stress and stress conditions, respectively. Here, the maximum values of white sugar yield in non-stress and stress conditions was belonged to genotype "F-205051" as well as "HSF-883". So, it is resulted that genotypes produced high root yield in both normal and stress conditions, could not resulted high white sugar yield regardless of water condition. Also, in this studied germplasm, genotype "HSF-877" had the minimum value of root and white sugar yield in normal condition. Regarding GMP, MP, HM, YI and STI indices, genotype "HSF-844" was introduced as tolerant genotype which produced acceptable root yield in both conditions. Based on white sugar yield trait, STI, HM and GMP indices also proposed "HSF-844" as tolerant genotype. It is concluded that indices are including STI, MP, GMP, HM and YSI owing significant correlation with Yp and Ys and hence, proposed for selection of a sugar beet genotype with stable and high root and white sugar yield in stressed and non-stressed conditions. From three dimensional view, genotypes such as "HSF-844", "HSF-859", "HSF-861", "HSF-883" and "32434-91" are promising sugar beet genotypes (Group A) which have suitable root yield

in both states. Whereas, genotypes such as "HSF-841", "HSF-844", "HSF-847", "HSF-861" could be calculated as group A which have suitable white sugar yield in both states. Based on studied tolerance indices, the studied sugar beet germplasm classified into differentiate groups and these identified distant groups could effectively used in sugar beet breeding programs like selection of parents for confirmation of mapping population as well as in hybrid breeding programs.

REFERENCES

Abdollahian-Noghabi M., FroudWilliams RJ. 1998. Effect of moisture stress and re-watering on growth and dry matter partitioning in three cultivars of sugar beet. Asp Applied Biol., 52: 71–78.

Abdollahian-Noghabi M., Radaei-al-Amoli Z., Akbari GA., Sadat-Nuri SA. 2011. Effect of severe water stress on morphological, quantitative and qualitative characteristics of 20 sugar beet genotypes. Iran J Field Crop Sci., 42: 453-464.

Adamczewska SK., Uklanska CM. 2009. Effect of nitrogen fertilization on yield and quality of endive. Veg Crops Res Bull., 70: 193-201.

Bouslama M., Schapaugh WT. 1984. Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. Crop Sci., 24: 933-937.

Cieslik E., Topolska K., Pisulewska PM. 2009. Effect of inulin- type fructans on body weight gain and selected biochemical parameters at calcium hypo-alimentation in rats. Pol J Food Nutr., 59: 163-168.

Clarke JM., Depauw RM., Townleysmith TF. 1992. Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci., 32: 723–728.

Clover GRG. 1997. Effects of Beet Yellows Virus and Drought on Growth of Sugar Beet. Ph. D. Thesis, University of Nottingham.

Darvishzadeh R., Pirzad A., Hatami Maleki H., Poormohammad Kiani S., Sarrafi A. 2010. Evaluation of the reaction of sunflower inbred lines and their F1 hybrids to drought conditions using various stress tolerance indices. Span J Agric Res., 8:1037-1046.

Draycott AP. 2006. Introduction. In: "Sugar Beet", (Ed.): Draycott, A. P.. Oxford, lackwell Publishing Ltd, UK, PP. 1-8.

Esmaeili MA., 2011. Evaluation of the effects of water stress and different levels of nitrogen on sugar beet (*Beta Vulgaris*). Intl J Biol., 3: 89-93.

Fabiro C., Martin de Santa Olalla F., Lopez A., Dominguez R. 2003. Production and quality of the sugar beet (*Beta vulgaris* L.) cultivated under controlled deficit irrigation conditions in a semi-arid climate. Agric Water Manage., 62: 215-227.

Farshadfar E., Sutka J. 2002. Screening drought tolerance criteria in maize. Acta Agron Hungarica, 50: 411-416.

Fernandez GCJ. 1992. Effective selection criteria for assessing plant stress tolerance. In: Proceeding of a symposium, Taiwan, 13-18 August. pp. 257-27.

Flowers TJ., Koyama ML., Flowers SA., Sudhakar C., Singh KP., Yeo A R. 2000. QTL: their place in engineering tolerance of rice to salinity. J Exp Bot., 51: 99-106.

Gavuzzi P., Rizza F., Palumbo M., Campaline RG., Ricciardi GL., Borghi B. 1997. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Can J Plant Sci., 77: 523-531.

Hesadi P., Fathollah Taleghani D., Shiranirad A., Daneshian J., Jaliliyan A. 2015. Selection for Drought Tolerance in Sugar Beet Genotypes (*Beta vulgaris* L.). Biol Forum, 7: 1189-1204.

Jafari A., Paknejada F., Jami Al-Ahmadi M. 2009. Evaluation of selection indices for drought tolerance of corn (*Zea mays* L.) hybrids. Int J Plant Prod., 3: 33-38.

Jones PD., Lister DH., Jaggard KW., Pidgeon JD. 2003. Future climate change impact on the productivity of sugar beet (*Beta vulgaris* L.). Eur Clim Change, 58: 93–108.

Kirigwi FM., Van Ginkel M., Trethowan R., Sears RG., Rajaram S. Paulsen GM. 2004. Evaluation of selection strategies for wheat adaptation across water regimes. Euphytica, 135: 361-371.

Korshid A. 2016. Biplot analysis of salinity tolerance indices in sugar beet breeding lines. Adv Plants Agric Res., 5: 174.

Last PJ., Draycott AP., Messem AB., Webb DJ. 1983. Effects of Nitrogen Fertilizer and Irrigation on sugar beet at Broom's Barn (1973-1978). J Agric Sci., 101:185-205

Mahmoodi R., Maralian H., Aghabarati A. 2008. Effects of limited irrigation on root yield and quality of sugar beet (*Beta vulgaris* L.). Afr J Biotech., 7: 4475-4478.

Ober ES. 2001. The search for drought tolerance in sugar beet. Brit Sugar Beet Rev., 69: 40–43.

Ober ES., Rajabi A. 2011.Abiotic Stress in Sugar Beet. Sugar Tech (September and December 2010) 12: 294-298.

Ouk M., Basnayake J., Tsubo M., Fukai S., Fischer K., Cooper M., Nesbitt H. 2006. Use of drought response index for identification of drought tolerant genotypes in rainfed lowland rice. Field Crops Res., 99: 48-58.

Pidgeon JD., Werker AR., Jaggard KW., Richter GM., Lister DH., Jones PD. 2001. Climatic impact on the productivity of sugar beet (*Beta vulgaris* L.) in Europe (1961–1995). Agr Forest Meteorol., 109:27–37

Ribaut JM., Jiang C., Gonzalez-De-Leon D., Edmeades GO., Hoisington DA. 1997. Identification of quantitative trait loci under drought conditions in tropical maize: 2. Yield components and marker-assisted selection strategies. Theor Appl Genet., 94: 887-896.

Richards RA. 1996. Defining selection criteria to improve yield under drought. Plant Grow Reg., 20: 157-166.

Rosielle AA., Hambling J. 1981. Theoretical aspects of selection for yield in stress and non stress environments. Crop Sci., 21: 943-946.

Sadeghian SY., Fazli H., Mohammadian R., Taleghani DF., Mesbah M. 2000. Genetic variation for drought stress in sugar beet. J Sugar Beet Res., 37: 55–77

Sio-Se Mardeh A., Ahmadi A., Poustini K., Mohammadi V. 2006. Evaluation of drought resistance indices under various environmental conditions. Field Crops Res., 98: 222–229.

Smirnoff N. 1995. Metabolic Flexibility in Relation to The Environment. In: N. Smirnoff (ed.). Environment and Plant Metabolism: Flexibility and Acclimation. Oxford: BIOS, Scientific Publishers Limited, pp1-16.

Vahidi H., Rajabi A., Haj Seyed Hadi M., Fathollah Talegani D., Azadi A. 2013. Screening of sugar beet (*Beta vulgaris* l.) genotypes for drought tolerance. Int J Agric Crop Sci., 6: 1104-1113.

Van Geyt JPC., Lange W., Oleo M., De Bock TSM. 1990. Natural variation within the genus Beta and its possible use for breeding sugar beet: A Review. Euphytica, 49: 57-76.

White JW., Singh SP. 1991. Breeding for adaptation to drought. In: A. van Schoonhoven and O. Voysest (Eds.), Common Beans. Research for Crop Improvement, pp. 501–560. CAB Int CIAT, Colombia.

Yadav OP., Bhatnagar SK. 2001. Evaluation of indices for identification of pearl millet cultivars adapted to stress and non-stress conditions. Field Crop Res., 70: 201-208.

Yeo A. 1998. Molecular biology of salt tolerance in context of whole-plant physiology. J Exp Bot., 49: 915-929.

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IMPACT OF EXPORT PLANT PRODUCTS IN BRANDING OF THE COUNTRY

SUMMARY

This paper examines the effects of plant products export in branding of the country. Branding of the country begins by defining the strategy of promoting products, especially export brands, since the development of high quality local brands and their presence on the foreign market improves the image of the country. According to the Anholt Nation Brands Index, which shows maps of the individuals responses to their perception along different dimensions in one index number, one dimension of the Anholt`s hexagon is and export regarding customer`s perceptions and stereotypes about the products from that country.

An econometric model was used in order to determine the export of plant products from 40 countries (the data presented in the table for the UN). The observed period was from 2006 to 2015, including 2006 and 2015. The test article consisted 500 plant products divided into 100 sub-categories, marked with 4-digit and 10 categories marked with two figures. The data presented in the table are: the share of exports of plant products in total exports, RCA, domestic credit to private sector (% of GDP), employment in agriculture (% of total employment), fertilizer consumption (kilograms per hectare of arable land), foreign direct investment, net inflows (% of GDP), GDP growth (annual %), GDP per capita growth (annual %), general government fil consumption expenditure (% of GDP), gross capital formation (% of GDP), rural population (% of total population), time to export (days), trade (% of GDP).

The result of this analysis, with the use of mentioned econometric model implies that if we increase the percentage of the population in rural areas to 1%, the export (plant products) of the country in total export will be reduced by 0.027%. Given that increased use of fertilizers per hectare of cultivated land by 1% share of the country's exports will increase by 0.009%.

The export, regarding plant product, is adequate component for branding of country. But, for nation’s recognition and for better positioning in the minds of consumers, attention should not be paid to increase the number of inhabitants in rural areas, but the improvement of technology, knowledge and application of research which will result with the growth plant product’s export in total exports.

Keywords: branding, plant production, export, model, country, organic product.

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INTRODUCTION

Philip Kotler (1980) also referred to marketing as satisfying needs and wants through an exchange process. Likewise, Kyle (2009) refers to the Merriam Webster's marketing definition as the process or technique of promoting, selling, and distributing a product or service. In these three traditional definitions, marketing is seen as a process. But, in the 21st century we can find slightly different definitions for marketing. Davis (2012) defines marketing, at a minimum, as "developing, building, and sustaining a positive reputation for a given offering so that it attracts support from members of a marketplace". In fact, another new definition of marketing points to the brand, to positioning as well as to differentiation, since building the brand is the key.[2]

The American Marketing Association (AMA) defines brand as a "name, term, design, symbol or any other features that identifies one seller's good or service as distinct from those of other sellers", that is, a combination of characteristics intended to identify the goods and services of one seller or group of sellers and to differentiate them from those of competition.[3]

In the last few years there has been a change from place marketing to place branding. Many pages have been written on place branding but one of the most prolific writers on "nation branding" is Anholt (1998, 2003 & 2007 among others). Olins (2002) argues that branding a country is similar to branding a product. It is true that a country is considered more complex than a product since it usually involves many more variables. The changes in national identity and its elements and branding strategy can be compared (Ibid). It is clear that there are fundamental differences between the brand and the nation brand commercial products (Fan, 2006). For example, one nation cannot advertise its coast just as the coast, because the coasts are many, but only through a cultural experience of peoples who live on these coasts, which offers something different from all the others. The nation does not provide services and does not offer tangible products, on the contrary, it includes a number of associations and factors such as: the place (geography, tourist attractions), natural resources and local products, people (race, ethnicity), history, culture, language, political and economic systems, social institutions, infrastructure, famous people (faces), image or image.

As a measure of these soft factors – perceptions and stereotypes – we use the Anholt Nation Brands Index and its components. (Anholt, 2005). This index maps the answers of individuals on their perceptions along different dimensions into a single index number. Individuals are asked about their perceptions of other countries, which may be summarized by the following dimensions:

Tourism: the country's attractiveness from a tourism point of view

[2] A presentation by Triodos Bank (2010) is available at www.slideshare.net/bmmaShare/triodos-bank1980-kotlers-marketing-30-2010 (Accessed 16 May 2017).

[3] Definition available at www.marketingpower.com/_layouts/dictionary.aspx?dLetter=B (Accessed 19 May 2017)

Exports: their perceptions and stereotypes about the products from the specific country

Governance: their perceptions as regards the government in that country

Investment and Immigration: their personal willingness to work in that country and their perceptions about social and economic conditions in that country,

People: stereotypes about the people from the respective country as employees,

Culture: perceptions about the country's achievements in terms of culture, history and sports.

In the theory, there is difference between the brand image and identity of the nation. The brand image of the nation is a set of beliefs, opinions and impressions that people have of a country, a result of the information and experience that have. This information is gathered from early childhood, through education, media and information from the immediate surroundings. Buying products and countries, the level of economic development, people, etc. are also ways of gaining impressions and to construct an image. National identity is a complex set of elements that includes the nation's identity: its history, culture, legal and political system, geography, and its visual elements, such as flags and buildings that are symbols. These elements make it unique and distinctive (Veljković, 2010). On the identity of a nation affected by the following factors: export brands, promotion in terms of tourism, trade, attracting investments and experts, internal and external policies, the promotion of culture and customs, the people who go abroad and represent their culture, landscape and architecture of the country, media presence, state participation in international organizations, links with other countries, sport and entertainment. Kotler & Gertner (2002) examine how the image of a country influences the opinion about that country and its products as well as the capacity for attracting tourists, and they state that strategic place marketing mainly refers to "the enhancement of a country's position in the global market-place".

Overall, in this era of globalization, branding is often necessary. The reasons are manifold, and may be the following: tourism, attract investment, geopolitical influence, selling products in domestic and foreign markets, membership in international organizations and alliances, attract talent. First of all, the global market is the interest of every country to be more competitive and to present itself as a good environment for economic growth.

Architecture brand is crucial for product branding and the branding of the nation. "The architecture of the brand is a kind of structure that organizes the portfolio of brands in the company. It defines the role of the brand, hierarchical levels and relationships between brands within the company" (Veljković, 2010). In order to create a brand architecture that can adapt to the needs of the nation, was created NBAR model (*Nation Brand Architecture*). This model represents the structure and organization of the portfolio of brands nationwide, which investigates relations and the role of brands.

In this work, emphasis will be placed on research to export (in this case plant products) as one of the elements Anholt Nation Brands Index affect the branding of the nation and on this basis, creating the perception of consumers in a particular country and the positioning of that country in the minds of consumers. Several studies have been conducted to increase the yield and quality of fruits or vegetables (Kacjan Maršić et al., 2009; Usenik et al., 2009; Veberič et al., 2010). In this respect, organic production, in this case, herbal products, are increasingly gaining in importance.

Organic food production is not the only, but it is the most far-reaching of currently applied measures to increase the sustainability of food production (Thøgersen, J., 2009). For example, Denmark stands out as the country with the highest increase in demand of these products per capita worldwide (Wier, M., and Calvery, C., 2002). However, share of organic products consumption in overall food consumption still remains at a relatively low level (Wier, M., Mette, 2003).

According to Padel, S., & Foster, C., (2005) most consumers associate organic at first with vegetables and fruit as well as with a healthy diet. Organic food consumption is part of a way of life (Schifferstein, H., and Peter AM Oude Ophuis, 1998). Food consumption in most developed countries has attained a saturation point in quantity terms, and consumer food choices are broader than in the past. In this saturated market environment, distribution channels, marketing activities, diversification strategies, and food quality are increasingly important. In addition, consumers have become more concerned about the nutrition, health, and quality of food they eat (Gil et al., 2000). Income and organic knowledge positively influences the final decision to buy organic food products (Gracia, A., & de Magistris, T., 2013).

MATERIALS AND METHODS

An econometric model was used in order to determine the export of plant products from certain countries. The observed period was from 2006 to 2015, including 2006 and 2015. The test article consisted 500 plant products divided into 100 sub-categories, marked with 4-digit and 10 categories marked with two figures. Herbal products that are observed are: live trees & other plants, edible vegetables, ed. fruits & nuts, peel of citrus/melons, coffee, tea, mate & spices, cereals, milling industry products, oil seeds/misc. grains/med. plants/straw, lac, gums, resins, etc. vegetable plaiting materials, animal or vegetable fats, oils & waxes.

The sampling included the following countries: Albania,Azerbaijan, Austria,Armenia, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia,Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Luxembourg, Rep. of Moldova, Montenegro,Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland, Ukraine,FYR of Macedonia and United Kingdom

The econometric model that was tested according following:

$$lshare=a+b1*lrural+b2*lfertilizer$$

$$lshare = 0.0735 - 0.0272 *lrural + 0.00919*lfertilizer , R^2adj=0.1542$$

Table 1. Variables of the econometric model

	(RE)
VARIABLES	lshare
lrural	-0.0272* (0.0161)
lfertilizer	0.00919** (0.00441)
Constant	0.0735 (0.0616)
R-sq	0.1542
Observations	167
Number of Reporters	40

Robust standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1

lshare the natural logarithm of the two-year average share isozymes of plant products in total exports of 40 countries of Europe. Data on exports of plant products consists taken from the database of the UN.[4] Include categories of HS-06 to HS-15 in the two-digit level of aggregation.

lrural the natural logarithm of the two-year average percentage of the population who live in rural areas. The data were obtained from the WDI database[5]. lfertilizer the natural logarithm of two years of average use of fertilizers (kg per hectare of cultivated land). The data were obtained from the WDI database.

RESULTS AND DISCUSSION

The model is considered as one of the most common ways of measuring comparative advantages (revealed comparative advantage-RCA), defined by B.Balasse (1965). The concept of comparative advantage explains the ability or the possibility of some countries with their exports to compete with the same products with the rest of the world. It is estimated as the ratio of export products of the country and exports of all products of the country to the same proportion of world exports in the reporting period. What is RCA higher, the more pronounced the comparative advantage of a certain sector of the country is

[4] http://data.un.org/Explorer.aspx, (Accessed 23.April, 2017).
[5] http://databank.worldbank.org/data/home.aspx, (Accessed 24.April, 2017).

increasing. The data presented in the table for the UN listed 40 countries are: the share of exports of plant products in total exports, RCA, domestic credit to private sector (% of GDP), employment in agriculture (% of total employment), fertilizer consumption (kilograms per hectare of arable land), foreign direct investment, net inflows (% of GDP), GDP growth (annual %), GDP per capita growth (annual %), general government fil consumption expenditure (% of GDP), gross capital formation (% of GDP), rural population (% of total population), time to export (days), trade (% of GDP).

Table 2: Used indicators on example of Montenegro from 2006 to 2015

	Share	**RCA**	**Domestic credite to private sector (% of GDP)**	**Employment in agriculture (% of total)**	**Fertilizer consumption (kilograms per hectare of arable land)**	**Foreign direct investment ((% of GDP)**	**GDP growth (annual %)**
2006	8,5E-05	0,729	36,302		18,701	23,071	8,566
2007	6,63E-05	0,588	80,260	8,7	15,632	25,553	10,657
2008	7,8E-05	0,794	87,019	7,6	14,791	21,574	6,922
2009	8,74E-05	1,084	76,537	6,5	11,329	37,410	-5,656
2100	9,4E-05	1,168	66,471	6,2	14,354	18,322	2,463
2011	9,34E-05	0,952	55,294	5,6	12,549	12,257	3,228
2012	0,000103	1,375	55,060	5,7	12,412	15,127	-2,723
2013	0,000102	1,351	53,048	4,5	324,743	10,001	3,548
2014	0,000106	1,567	51,386	5,7		10,829	1,783
2015	7,12E-05	1,149	50,555			17,525	3,374
	GDP per capita growth (annual %)	**General government fil consumption expenditure (% of GDP),**	**Gross capital formation (% of GDP)**	**Rural population (% of total population)**	**Time to export (days)**	**Trade (% of GDP)**	
2006	8,431	26,991	25,444	37,62	14	128,470	
2007	10,505	20,121	33,805	37,44	14	131,092	
2008	6,733	22,624	40,671	37,261	14	133,478	
2009	-5,859	22,188	27,121	37,082	14	97,539	
2100	2,276	22,093	21,769	36,904	14	99,780	
2011	3,120	21,062	19,328	36,726	14	106,658	
2012	-2,805	21,094	20,585	36,543	14	111,766	
2013	3,447	19,476	19,606	36,357	14	102,771	
2014	1,685	19,372	20,221	36,168	14	100,127	
2015	3,278	17,601	20,511	35,974	14	104,354	

If we perceive Montenegro only, the table shows that the share of exports of plant products in total exports is extremely low. RCA varies from 0,729, what was 2006 to 1,149 in 2015, with minor fluctuations in the years within this period. When it comes to domestic credit of private sector, since 2008, when it stood at around 87% this number is in constant decline. Employment in agriculture is steadily declining, from 8.7% in 2007 to 4.4 in 2013, to be increased in 2014 to 5.7%. When it comes to fertilizer consumption (kilograms per hectare of arable land), since 2006, when it was 18,701, is in permanent decline, and in 2013 it grew up to 324.743. FDI were the highest in 2009, when it was amounted to 37.4% of GDP, and it decreased steadily until 2014, when it was 10.829%, and then increased to 17,525% of GDP. If we look at GDP growth (annual %) since 2007 when it stood at 10.657%, in 2009 and 2012, I had even negative (-5.656 and -2.723%), that in 2013 increase to 3,548 in 2014 and fell again to 1,783. When it comes rural population (% of total population), this figure is about the same in the observed period-about 36% and the time to export is always 14 days.

The following table shows the lshare I lrural for the period 2006-2015 for the Western Balkans.

Table 3: Ishare and Irural indicators for the period 2006-2015 for the Western Balksans

	1	**2**	**3**	**4**	**5**
	lshare	lshare	lshare	lshare	lshare
Albania	0,000247	0,000224	0,000249	0,00031	0,000487
BiH	0,000519	0,000612	0,000735	0,000693	0,001037
Croatia	0,001616	0,001784	0,001829	0,001968	0,002366
Montenegro	0,000075	0,000082	0,0000937	0,000102	0,000088
Macedonia	0,001046	0,000938	0,00115	0,000943	0,001067
Serbia	0,006085	0,006478	0,008471	0,008094	0,009306
	1	**2**	**3**	**4**	**5**
	lrural	lrural	lrural	lrural	lrural
Albania	3,963505	3,92131	3,877328	3,831756	3,786278
BiH	4,124381	4,124356	4,122956	4,120142	4,115918
Croatia	3,790646	3,780376	3,76888	3,756153	3,742183
Montenegro	3,651437	3,642089	3,632706	3,623007	3,612835
Macedonia	3,777291	3,782188	3,784712	3,784871	3,782677
Serbia	3,830238	3,825713	3,823563	3,821223	3,817624

1-the average for 2006 and 2007
2-the average for 2008 and 2009
3-average in 2010-2011
4-average 2012-2013
5 average for 2014 and 2015

If we perceive lshare of Albania, as the natural logarithm of the two-year average share plant products export, we can notice that the exports fell in the period 2008-2009 and after that grew during the whole period. On the other hand,

lrural as the natural logarithm of two-year average percentage of the population living in rural areas is in constant decline in the reporting period. When it comes to Bosnia and Herzegovina, lshare grew during the entire period, excluding the average of 2012-2013, a lrural has been in constant decline. If we take Croatia for an example, the share of exports of plant products has steadily increased, while the percentage of population living in rural areas was in steady decline. In Montenegro lshare was increasing steadily until the period 2014-2015, when it began to fall, while lrural was in steady decline. The situation is similar in Serbia, while in Macedonia the share of exports of plant products decreased in the period 2008-2009, while in the next period it grew, but then again, I began to fall. In the period 2014-2015 it grew again.

The result of this analysis, with the use of mentioned econometric model implies that if we increase the percentage of the population in rural areas to 1%, the export (plant products) of the country in total export will be reduced by 0.027%. If the increased use of fertilizers per hectare of cultivated land by 1% share of the country's exports will increase by 0.009%. Changes in variables lrural and lfertilizer explained 15.42% change in the share of exports of vegetable land.

In order to export, in our case, mentioned plant product, is adequate component for branding (according Anholt Brand Nation Index) and recognition of the nation and its better positioning in the minds of consumers. A special attention should not be paid to increase the number of inhabitants in rural areas, but the improvement of technology, knowledge and application of research which will be a function of the growth of exports of plant products in total exports. Special attention should be paid to the production of organic products because organic food buyers considered themselves more responsible for their own health and were more likely to undertake preventive health action than the general population.

However, taking into account the overall consumption of agricultural-food products in Montenegro, import of agricultural-food products had a more dynamic growth than export (Jovanović, M and Despotović, A., 2014). The situation in Montenegro is generally characterized by continuous lack of co-operation between food producers and tourism industry in particular, which could be improved at the level of the regulator (state) and producers (Jovanović, M., 2004). In this context, Anholt's hexagon with export, tourism, governance, investment, people and culture in synergy has a very great importance to branding and recognizable image of country.

CONCLUSIONS

Marketing and branding are directly associated with country branding targets customers. This is related to the position of a country in the world, and in the potential consumer's mind. As Kotler & Keller (2006) state, 'positioning' is the act of designing the company's offering and image to occupy a distinctive place in the mind of the target market. A country brand could be described as the

total sum of all perceptions about a country or the nation in the mind of international stakeholder (Fan 2006). In a more complex approach, country branding is influenced by the country image, reputation and positioning – if the case (Gilmore 2002), country branding being in some cases similar to corporate branding implying the usage of similar branding techniques.

Nation branding isn't the holy grail of economic development, but it can provide a distinct advantage when it is aligned with a well-defined economic strategy and supported by public policy. Countries around the world now started to realize that nation branding works as a catalyst for growth. In this context, a good image of the country can be very beneficial to affect the sales of products outside the national borders and the growth of export brands.

When it comes to herbal products, research results that indicate that if we increase the percentage of the population in rural areas to 1%, the export (plant products) of the country in total export will be reduced by 0.027%. If the increased use of fertilizers per hectare of cultivated land by 1% share of the country's exports will increase by 0.009%. In this context, it should take into account not only the percentage of people living in rural areas or the use of fertilizers per hectare of cultivated land, but also on investments in technology, knowledge and application of research, especially in the field of organic and healthy plant products that consumers offer added value, which multiplicative positively affect the recognition, exclusivity and reputation of the country.

REFERENCES

Anholt, S.(2009), *Places: identity, image and reputation*, London, Palgrave Macmillan

Anholt, S.(2005), *Brand new justice: how branding places and products can help the developing world*, Oxford, Elsevier Butterworth- Heinemann

Anholt, S.(2005), Anholt Nation Brand Index: How Does the World See America?, *Journal of Advertising*, September: 298.

Davis, J.A. (2012) 2nd ed. *The Olympic Games Effect. How Sports Marketing Builds Strong Brands*.Singapore: John Wiley & Sons: 30.

Fan, Y.(2006), Nation Branding: what is being branded?, *Journal of Vacation Marketing*, 12(1): 5-14.

Gil, J. M., Gracia, A., & Sanchez, M. (2000). Market segmentation and willingness to pay for organic products in Spain. *The International Food and Agribusiness Management Review*, *3*(2): 207-226.

Gracia, A., & de Magistris, T. (2013). Organic food product purchase behaviour: a pilot study for urban consumers in the South of Italy. *Spanish Journal of Agricultural Research*, *5*(4): 439-451.

Jovanović, M., Despotović Aleksandra, (2014). Analysis of position of agro-food trade in Montenegro's foreign trade, *Agriculture & Forestry*, vol. 60. ISSUE 1, Podgorica, UDC (UDK) 631.11(497.16): 235-243.

Jovanović, M.(2004), Food Market from the tourism consumption aspect. *Agriculture & Forestry*, vol. 50. Issue 1-2, Podgorica, UDK: 339.133: 127-137.

Kacjan Maršić N., Šircelj H., Kastelec D. (2009). Lipophilic antioxidants and some carpometric characteristics of fruits of ten processing tomato varieties, grown in different climatic conditions. *Journal of agricultural and food chemistry* 58: 390-397.

Kotler, P. (1980) ,*Marketing Management: Analysis, planning and control*. Englewood Cliffs, N.J.

Kotler, P. and Gertner, D.(2002), Country as a brand, product and beyond: a place marketing and brand management perspective, *Journal of Brand Management*, 9 (4): 253.

Kotler, P. & K.L. Keller (2006) 12ed. *Marketing Management*. Englewood Cliffs, N.J.: Prentice-Hall

Kyle, B. (2009) *The Definition of Marketing. Has it changed?* Available at http://www.websitemarketingplan.com/marketingmanagement/marketing_change.htm (Accessed May 2017).

Olins, W. (2002) Branding the nation – The Historial Context. *Journal of Brand Management, 9 (4-5):* 241-8.

Padel, S., & Foster, C. (2005). Exploring the gap between attitudes and behaviour: Understanding why consumers buy or do not buy organic food. *British food journal*, *107*(8): 606-625.

Schifferstein, Hendrik NJ, and Peter AM Oude Ophuis (1998), Health-related determinants of organic food consumption in the Netherlands. *Food quality and Preference* 9.3: 119-133.

Thøgersen, John, 2009. Consumer decision-making with regard to organic food products. *Traditional food production and rural sustainable development: A European challenge*: 173-192.

Usenik V., Štampar F., Veberič R. (2009). Anthocyanins and fruit colour in plums (*Prunus domestica* L.) during ripening. *Food chemistry* 114: 529-534. Sinkovic *et al:* 336.

Veberič R., Jurhar J., Mikulič-Petkovšek M., Štampar F., Schmitzer V. (2010). Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). *Food Chemistry* 119: 477-483.

Veljković, S.(2010), *Brand Management*, Čugura print, Faculty of Economics, Belgrade: 121- 343.

Wier, M. and C. Calverley, 2002. Market Perspectives for Organic Foods in Europe, *British Food Journal*, Vol.104: 45-62.

Wier, M. et al. 2003. Consumer preferences for organic foods. *Organic agriculture: Sustainability, markets and policies*: 257-271.

www.slideshare.net/bmmaShare/triodos-bank1980-kotlers-marketing-30-2010 (Accessed 16 May 2017)

www.marketingpower.com/_layouts/dictionary.aspx?dLetter=B (Accessed 19 May 2017)

http://data.un.org/Explorer.aspx, (Accessed 23.April, 2017).

http://databank.worldbank.org/data/home.aspx, (Accessed 24.April, 2017).

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EFFECT OF DILUTED SEAWATER ON SEED GERMINATION AND SEEDLING GROWTH OF THREE LEGUMINOUS CROPS (PEA, CHICKPEA AND COMMON BEAN)

SUMMARY

Seawater intrusion into fresh water aquifers is due to natural processes or human activities, and consequently salinization results not only from the ground but also from irrigation water. In an attempt to evaluate the effect of seawater irrigation on seedling growth and germination, seeds of three leguminous crops (*Pisum sativum*, *Cicer arietinum* and *Phaseolus vulgaris*) were irrigated with Mediterranean seawater of different concentrations (0, 10, 30, 50 and 100%) for 8-day period. Various parameters such as germination kinetics, mean germination time, germination rate index, shoot and root length, fresh and dry weight and moisture content were analysed.

The results showed that these species were able to germinate at different salinity levels, except for 100% seawater which resulted in total inhibition of germination. Compared with control, seed germination in all species remained unaffected up to 30% of seawater treatments. Indeed, the final germination percentage was maintained between 90 and 100%. A solution of 50% seawater significantly reduced germination rate index and increased mean germination time. 10% of seawater increased shoot and root length in all species compared to the control. The saline conditions reduced the growth parameter such as fresh and dry shoot and root weights of the three-studied species. Shoot and root dry weight was stable by 10, 30 and 50% of seawater except for *P. vulgaris* seedlings. The decrease in moisture content begins from 30% of seawater solution compared to the control in *P. sativum* and *C. arietinum* seedlings. However, it was maintained stable for *P. vulgaris* compared to the control.

Keywords: Salt-tolerance, agriculture, moisture content, irrigation.

INTRODUCTION

Salinity is one of the major abiotic factors that limits plant growth and productivity in many regions of the world due to increasing use of poor quality of water for irrigation and soil salinization (Chen and Jiang 2010; D'Odorico *et al*., 2013; Shrivastava and Kumar, 2015). 20% of croplands in world contain high enough concentrations of salt to cause a salt stress for plants (Shelef *et al*., 2012). Considerable reduction of the plant growth is generally due to salt stress, except

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that these reductions vary from a species to the other one. Salinity tolerance of some cultivated legumes varieties turns out thus crucial for the country's economy.

The legume crops have a very important part of several research in agronomy (Williams *et al*., 2014; Popovic *et al.,* 2015; Popovic *et al.,* 2016), genetic (Smýkal *et al*., 2015), entomology (Söğüt *et al*., 2014; Duan *et al*., 2014), phytopathology (Khan *et al.*, 2014) and physiology (Neugschwandtner *et al*., 2015). Leguminous plants also maintain a very privileged relationship with the rhizosphere. Their agronomic interest results first of all from their ability to establish a mutualistic symbiosis with bacteria belonging to *Rhizobia* family for the utilization of atmospheric nitrogen as a nitrogen source (Giri and Joshi, 2010; Iantcheva *et al*., 2013). It is estimated that approximately 40-60 million metric tons of atmospheric nitrogen is fixed by cultivated legume plants annually (Robertson *et al*., 2013). Indeed, this symbiosis allows them to produce in abundance proteins even in the absence of nitrogenous fertilization (Pérez-Montaño *et al*., 2014). They play consequently a key role in the crop rotation (Nemecek *et al*., 2015).

The salinization results not only from the ground but also from irrigation water. Indeed, in the arid and semi-arid lands, the agricultural production requires irrigation especially with the shortage of rain (Chen *et al*., 2010). These water resources of irrigation come generally from groundwater and contain variable quantities of dissolved salts (Prasanth *et al*., 2012). In the Mediterranean countries as Algeria, the legume crops are often cultivated near the coastal regions where we attend an increase of the salt stress. Therefore, a vast use of irrigation waters calls up to the intrusion of seawater. Seawater intrusion is the movement of seawater into fresh water aquifers due to natural processes or human activities. Indeed, seawater intrusion is caused by decreases in groundwater levels or by rises in seawater levels (Werner *et al*., 2013). The use of poor quality water thus results in an increase of salinization level in the soil which can have negative effects on yield (Arslan, 2013). On the other hand, the available fresh water resources for agriculture declined regarding quantity and quality of both surface water and groundwater systems (Liu *et al*., 2016). Therefore, the use of lower quality water for irrigation purposes is inevitable to maintain economically viable crops. According to the dilution levels tested on some plants, seawater has proved even an excellent natural fertilizer and can contain several minerals very useful for the plant growth (Glenn *et al*., 1998; Tawfik *et al*., 2011; Ventura *et al*., 2015; Kheloufi et *al*., 2016a).

The plant adaptation in salt environment is crucial at the seedling stage for best species establishment. The first stage of development is thus the most vulnerable in this salt constraint because the passage of this one will determine the evolution of the cultivated species. Indeed, the salinity can affect the seedling by creating osmotic potential which prevent the imbibition of water, or by exercising toxic effects on the viability of the embryo (Chaves *et al*., 2009). The improvement of certain salt tolerant species is of a major importance. So, the

scientific research should concentrate on mechanisms implied in this salt tolerance. The aim of the present study was to investigate seed germination and seedling growth of three leguminous crops: "*Pisum sativum* (L.), *Cicer arietinum* (L.) and *Phaseolus vulgaris* (L.)" to gradient salinity. For this purpose, seeds and seedlings were subjected to various levels of seawater solutions, and an analysis of the parameters related to germination, growth and moisture content was made.

MATERIALS AND METHODS

Experimental material and salt treatments

Factorial combinations of three species and five levels were salinity test treatments. Seeds of pea (*P. sativum* var. *Arvense*), chickpea (*C. arietinum* var. *Temouchent*) and bean (*P. vulgaris* var. *Djadida*) were kindly provided by the Technical Institute of Field Crops (ITGC), Sidi bel abbes (Algeria). Seawater was provided from coastal site of Oran (Algeria) (35°40'36.82"N; 1°1'30.54"O). Different seawater concentrations of 0, 10, 30, 50 and 100% were created using distilled water. Three replicates of 15 seeds each were used for each treatment. Germination experiments were carried out in plastic boxes (5 cm height, 15 cm length and 8 cm width) with two-layer wet filter paper (Whatman No. 1) moistened with 25 ml of the appropriate solution of seawater or distilled water for 0% (Control).

The germination rate was recorded every 2-day for 8 day-period. First of all, the seeds were surface sterilized with 5% of sodium hypochlorite for 5 minutes to avoid fungal invasion, followed by washing with distilled water. Seeds were incubated under continuous dark at 23˚C ± 2˚C (Celsius) in controlled temperature room. The papers were changed with the same treatment each 2 day to prevent salt accumulation. The seeds were moistened with the appropriate solutions of seawater and kept wet throughout the experiment. Seeds were considered as germinated when the radicle had protruded 2 mm through the seed coat (Côme, 1970).

Germination parameters

In order to characterize salinity tolerance, several parameters were calculated:

Germination kinetics: for better apprehending the physiological significance of germination behaviour, the number of germinated seeds was counted every 2-days until the 8th day of the experiment.

Final germination percentage (FGP): this parameter constitutes the best identification means of salt concentration which presents the physiological limit of germination. It is expressed at the 8th day of the experiment by the following equation:

$$\text{FGP (\%)} = \frac{\text{Number of germinated seed}}{\text{Total Number of seed tested}} \times 100$$

Germination rate index (GRI): It reflects the percentage of germination on each day of the germination period (Maguire, 1962).

$$GRI\ (\%) = \sum \frac{\text{Number of Germinated Seeds}}{\text{Number of Days}}$$

Mean germination time (MGT): It represents the meantime, a seed lot requires to initiate and end germination (Orchard, 1977).

$$MGT\ (\text{days}) = \frac{\sum n \times D}{\sum n}$$

n: number of seeds newly germinated at time D; D: days from the beginning of the germination test; Σn: final germination.

Seedling growth parameters

After 8-day period, shoot length was immediately measured by caliper. Roots and shoots were separated and blotted. Fresh weight (FW) was immediately determined and then samples were dried in an oven at 80°C for 48h to obtain dry weight. Moisture content (MC) was estimated using the following formula as described by Wu *et al*. (2013):

$$MC\ (\%\ FW) = \frac{FW - DW}{FW} \times 100$$

The average of a water content of the plant is a very used method at present, to estimate the hydric state of the plant in the condition of hydric stress.

Statistical analysis

The experiment was made as a completely randomized design with 3 replicates of 15 seeds (n=3) for the germination parameters and with 15 replicates (n=15) for the seedlings growth parameters. The data were statistically treated by Fisher's analysis of variance (ANOVA). The Generalized Linear Model (GLM) was used in the kinetic of germination (Repeated Measures Analysis of Variance). Duncan's multiple range tests were performed to determine significant difference between means at a significance level of 5% using SAS software version 9.0 (SAS 2002).

RESULTS AND DISCUSSION

In this study, four concentrations (10, 30, 50 and 100 %) of seawater were used to irrigate three leguminous crops in order to evaluate the effect of seawater salinity in plant germination and growth.

Germination parameters

The effect of salinity on germination kinetics is presented on Figure 1. First of all, using high concentration of seawater (100 %) resulted in total inhibition of germination. The effect of the external salinity on the seed germination may be partially osmotic or ion toxicity which can alter physiological processes such as enzyme activation (El-Keblawy, 2004; Chinnusamy *et al*., 2005; Nichols *et al*., 2009). This toxic effect can lead to metabolic processes changes in seedlings and at the extreme case in the death of embryo by ion accumulation (El-Keblawy, 2004). The osmotic or toxic effect can be verified by salinity recovery test (Kheloufi *et al*., 2016b). A Two-way ANOVA indicated that germination kinetics of the three leguminous seeds was significantly affected by the factor species (F=3.73, p=0.0357), time (F=1839.52,

P<0.0001), seawater concentration (F=390.56, P<0.0001) and by their correlation (F=4.65, P<0.0001). Compared with control, seed germination in the three-species remained unaffected up to 30 % of seawater treatments. Indeed, the final germination percentage was maintained between 90 and 100 %. A further increase in salinity (50 % of seawater) has inhibited a few rates of seed germination in all the studied species with (86.7 %) in the case of *P. sativum*, following by (73.3 %) and (71.1 %) for *C. arietinum* and *P. vulgaris*, respectively. The decrease of FGP follows the increase of external osmotic pressure, what allocates the water absorption by seeds and/or to an ion accumulation (Na^+ and Cl^-) in the embryo (Debouba *et al*., 2006; Ghogdi *et al*., 2013).

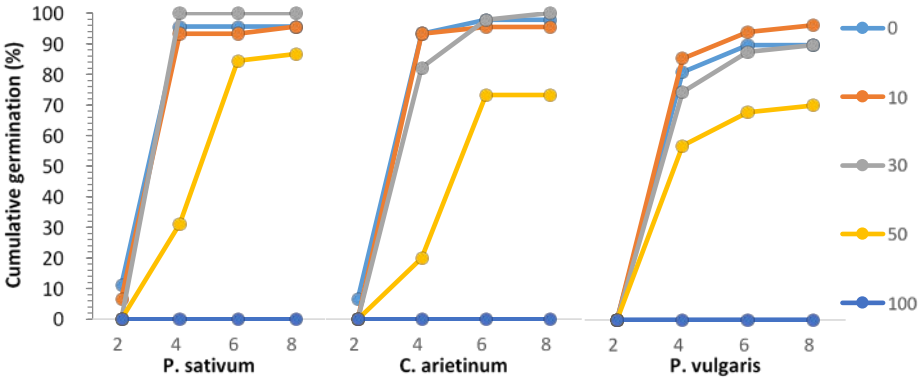

Figure 1. Effects of various seawater concentrations (0 to 100%) on the germination kinetics of three leguminous crops (*P. sativum*, *C. arietinum* and *P. vulgaris*) seeds over an 8-day period.

Final germination percentage was higher in non-saline controls and higher in *P. sativum* and *C. arietinum* seeds than *P. vulgaris* seeds in all treatments. Under salinity treatment, the highest rate of germination (100%) was recorded in P. sativum and *C. arietinum* seeds at 30% of seawater and the highest rate of 97.78% was obtained at 10 % of seawater in *P. vulgaris* seeds. In the three species, the germination was completely inhibited at 100% of seawater concentration. In non-saline control, highest germination was recorded at (3.77 days) in *P. sativum* following by MGT of (3.95 days) and (4.19 days) for *C. arietinum* and *P. vulgaris*, respectively (Table 1). The data presented on Figure 1 also showed marked differences in the timing of initiation and completion of germination. The MGT increased significantly (P<0.0001) by increasing the seawater concentration. Increase of salinity also delayed the germination at 50 % of seawater where the highest rate was reached at the 6th day with reduced values in *C. arietinum* seeds and at the 8th day for the seeds of *P. sativum* and *P. vulgaris* (Figure 1). Delay in germination by increased salt concentration may be explained by the lower osmotic potential of the solution (Nonogaki *et al.*, 2010).

Compared with control, a solution of 50 % seawater significantly reduced germination rate index (GRI) (p<0.05) and increased mean germination time

(MGT) (Tableau 1). It was shown that higher FGP, GRI, and lower MGT represent higher and faster seed germination (Panuccio *et al*., 2014; Kheloufi, 2017).

The results also showed that the three-leguminous species were able to germinate at different salinity concentration. Our results indicated that high salinity remarkably inhibited seed germination and delayed germination time in all studied species. Similar results were recorded in other crops such as fava bean (*Vicia faba*) (Yang *et al*., 2013), wheat (*Triticum aestivum*) (Hussain *et al*., 2013), and lentil (*Lens culinaris*) (Al-Quraan *et al*., 2014).

Table 1. Effects of different seawater (SW) concentrations on final germination percentage (FGP), mean germination time (MGT), germination rate index (GRI), shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), shoot moisture content (SMC), root length (RL), root fresh weight (RFW), root dry weight (RDW), root moisture content (RMC) in three leguminous species (SP) (*P. sativum*, *C. arietinum* and *P. vulgaris*) for 8 day-period.

SP	SW (%)	Germination			Seedling shoots growth				Seedling root growth			
		FGP (%)	MGT (day)	GRI (%)	SL (cm)	SFW (g)	SDW (g)	SMC (%)	RL (cm)	RFW (g)	RDW (g)	RMC (%)
P. sativum	0	95.6^{a}	3.77^{b}	57.3^{a}	4.75^{b}	0.29^{b}	0.14^{c}	50.8^{a}	5.12^{b}	0.41^{a}	0.15^{c}	62.7^{a}
	10	95.6^{a}	3.94^{b}	54.2^{a}	6.07^{a}	0.32^{a}	0.17^{a}	49.2^{a}	8.73^{a}	0.38^{b}	0.16^{a}	56.3^{b}
	30	100^{a}	4.00^{b}	54.2^{a}	3.84^{c}	0.23^{c}	0.16^{a}	29.5^{b}	3.66^{c}	0.24^{c}	0.15^{b}	37.8^{c}
	50	86.7^{a}	5.32^{a}	32.7^{b}	1.41^{d}	0.18^{d}	0.16^{b}	11.9^{c}	1.64^{d}	0.18^{d}	0.13^{d}	24.9^{d}
	100	0.00^{b}	-	0.00^{c}	0.00^{e}	0.00^{e}	0.00^{d}	-	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}
C. arietinum	0	97.8^{a}	3.95^{b}	55.2^{a}	5.30^{a}	0.34^{a}	0.08^{b}	73.2^{a}	8.17^{a}	0.41^{a}	0.10^{b}	73.9^{a}
	10	95.6^{a}	4.04^{b}	51.2^{a}	5.93^{a}	0.32^{ab}	0.10^{a}	66.6^{b}	6.81^{b}	0.43^{a}	0.11^{a}	73.7^{a}
	30	100^{a}	4.40^{b}	49.35^{a}	3.68^{b}	0.28^{b}	0.10^{a}	63.56^{b}	4.09^{c}	0.25^{b}	0.10^{b}	57.72^{b}
	50	73.3^{b}	5.45^{a}	26.4^{b}	0.00^{c}	0.00^{c}	0.00^{c}	-	0.58^{d}	0.14^{c}	0.09^{c}	33.0^{c}
	100	0.00^{c}	-	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{d}	-	0.00^{d}	0.00^{d}	0.00^{d}	-
P. vulgaris	0	91.1^{a}	4.19^{a}	47.1^{a}	16.6^{b}	1.03^{b}	0.11^{b}	88.4^{a}	6.14^{b}	0.18^{c}	0.04^{c}	75.8^{a}
	10	97.8^{a}	4.28^{a}	49.8^{a}	20.4^{a}	1.66^{a}	0.17^{a}	89.8^{a}	8.29^{a}	0.28^{b}	0.09^{a}	77.2^{a}
	30	91.1^{a}	4.39^{a}	45.1^{a}	5.76^{c}	0.40^{c}	0.08^{c}	80.5^{b}	7.43^{a}	0.34^{a}	0.08^{b}	67.2^{b}
	50	71.1^{b}	4.44^{a}	34.8^{b}	0.51^{d}	0.08^{d}	0.03^{d}	66.8^{c}	1.60^{c}	0.08^{d}	0.03^{d}	70.1^{b}
	100	0.00^{c}	-	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{e}	-	0.00^{d}	0.00^{e}	0.00^{e}	-

^{a,b,c,d,e} Means followed by the same letters in the same column and within the same age are not significantly different ($p < 0.05$). Values are the average of 3 replicates of 15 seeds for germination parameters and the average of 15 replicates for seedlings growth parameters.

Delayed germination in seed of all species compared to controls with the increase of seawater concentration is explained by the time required to the seed to set up mechanisms allowing to adjust their osmotic internal pressure (Farissi *et*

al., 2011). The seed reserve mobilization depends on the activation of hydrolytic enzymes (Tan-Wilson and Wilson, 2012).

Growth parameters

The Two-way ANOVA presented on Table 3 shows that species and treatment and their correlation (Treatment × Species) significantly (P<0.0001) affect all seedling growth parameters. Table 1 showed that high lengths were recorded in seedlings treated with 10% of seawater. 10% of seawater increased shoot length in all species compared to the control. Indeed, shoots length of *P. vulgaris* increased from (16.6 cm) to (20.43 cm), those of *P. sativum* from (4.75 cm) to (6.07 cm) and finally shoots of *C. arietinum* increased from (5.30 cm) to (5.93 cm) (table 1). At the same diluted solution of seawater, the root lengths also increased but only in *P. sativum* and *P. vulgaris* seedlings with mean values of (8.73 cm) and (8.29 cm), respectively. The root length decreased significantly (P<0.0001) with the increase of salinity until reached its low development at 50% of sweater with mean values close to one cm in the three-leguminous species. Increase the concentration of seawater up to 30% made significant delay in development by decreasing lengths by half in *P. sativum* and *C. arietinum*, and by four-times in *P. vulgaris* seedlings. At 50% of seawater, no shoots appeared in *C. arietinum*. On the other hand, the seedlings of *P. sativum* and *P. vulgaris* recorded low lengths with (1.41 cm) and (0.51 cm), respectively.

Table 2. Analysis of variance testing the effect of time after sowing (Time), species (SP) (*P. sativum*, *C. arietinum* and *P. vulgaris*) and seawater treatments (TRT) on the variation of germination parameters (germination kinetics, final germination percentage 'FGP', mean germination time 'MGT', germination rate index 'GRI').

Parameters	Sources of variation	*Df*	*F*	*P*
Germination Kinetics	Species 'Sp'	2	3.73	0.0357
	Time after sowing 'Time'	3	1 839.52	<0.0001
	Seawater concentration 'TRT'	4	390.56	<0.0001
	Sp × Time	6	1.28	0.2745
	Sp × TRT	8	1.73	0.1320
	Time × TRT	12	142.22	<0.0001
	Sp × Time × TRT	24	4.65	<0.0001
FGP	TRT	4	316.28	<0.0001
	Sp	2	2.18	0.1306
	TRT × Sp	8	1.13	0.3723
MGT	TRT	4	46.64	<0.0001
	Sp	2	2.67	0.0899
	TRT × Sp	8	8.09	<0.0001
GRI	TRT	4	346.90	<0.0001
	Sp	2	5.69	0.0081
	TRT × Sp	8	2.75	0.0208

Table 3. Two-way ANOVA testing the effect of species (SP) (*P. sativum*, *C. arietinum* and *P. vulgaris*) and seawater treatment (TRT) on the variation of seedlings growth parameters (shoot length 'SL', shoot fresh weight 'SFW', shoot dry weight 'SDW', shoot moisture content 'SMC', root length 'RL', root fresh weight 'RFW', root dry weight 'RDW', root moisture content 'RMC').

Parameters		Sources of variation	*Df*	*F*	*P*
Shoot	SL	TRT	4	663.53	<0.0001
		Sp	2	491.59	<0.0001
		TRT × Sp	8	153.07	<0.0001
	SFW	TRT	4	359.81	<0.0001
		Sp	2	376.64	<0.0001
		TRT × Sp	8	134.96	<0.0001
	SDW	TRT	4	922.34	<0.0001
		Sp	2	604.30	<0.0001
		TRT × Sp	8	120.39	<0.0001
	SMC	TRT	4	2 583.22	<0.0001
		Sp	2	1 603.99	<0.0001
		TRT × Sp	8	219.82	<0.0001
Root	RL	TRT	4	344.01	<0.0001
		Sp	2	11.06	<0.0001
		TRT × Sp	8	17.20	<0.0001
	RFW	TRT	4	469.08	<0.0001
		Sp	2	48.96	<0.0001
		TRT × Sp	8	35.37	<0.0001
	RDW	TRT	4	6 416.88	<0.0001
		Sp	2	6 433.16	<0.0001
		TRT × Sp	8	514.27	<0.0001
	RMC	TRT	4	230.30	<0.0001
		Sp	2	372.09	<0.0001
		TRT × Sp	8	60.48	<0.0001

The saline conditions reduced the growth parameter such as fresh and dry shoot and root weights of the three-studied species as have been similarly reported by several authors (Long *et al*., 2010; Ventura *et al*., 2011; Vibhuti *et al*., 2015; Petrović *et al*., 2016). The reduction of the dry weights due to increased salinity may be a result of a combination of osmotic and specific ion effects (Khan *et al*., 2015). One of the initial effects of salinity on plants is the reduction of growth rate (Munns *et al*., 1995). These results are in agreement with the findings of Hirich *et al*. (2014) who reported a significant decline in shoot length at high salinity levels.

Data presented in Table 1 shows that shoot and root fresh weight was decreased with different seawater concentrations except for 10% seawater solution. In the three species, there was gradually decreased of fresh weight as compared to the control plants. Using high concentration of seawater (50%)

resulted in the highest value of decline in root than shoot. Moreover, data in Table 1 showed that shoot and root dry weight was constant by 10, 30 and 50% of diluted seawater except for *P. vulgaris* seedlings. Reduction in FW at high salinity might be due to poor absorption of water from the growth medium due to physiological drought (Munns and Gillham, 2015).

Table 1 also shows the effect of salt concentration on moisture content. The decrease in moisture content begins from 30% of seawater solution compared to the control in *P. sativum* and *C. arietinum* seedlings. However, seedlings water content of *P. vulgaris* was maintained stable compared to the control. Another related trait important to plant function is the ability to maintain water content in tissues at optimal levels in the face of environmental stress. Plants under stress often lose some water from their tissues, which can have rapid and large effects on cell expansion, cell division, stomatal opening, abscisic acid accumulation, etc. (Hsiao and Xu, 2000). Most of these effects become evident with no change in turgor pressure, although water potential can become more negative due to osmotic potential becoming more negative (Negrão *et al*., 2016).

During germination stage, the radicle emergence would be controlled by the environment osmolarity, while the later growth of the seedling would be limited by the reserve mobilization and their transport towards the embryonic axis (Hager *et al*., 2014). The study of the effects of various concentrations of seawater on the seed germination showed that neither the FGP, nor the MGT and GRI are affected by the salt to a concentration 30% of seawater. But, in higher concentrations, they become sensitive. Reduction in seedlings growth was also recorded in response to increasing salt stress. In glycophytes species, salinity can reduce the growth of plants or damage the plants through osmotic effect (it causes water deficit); toxic effects of ions; and imbalance of the uptake of essential nutrients (Roy *et al*., 2014; Parihar *et al*., 2015).

CONCLUSIONS

The use of seawater in agriculture can offers an alternative substitute to fresh water but with respected dilution to each plant species. Our study showed that irrigation with seawater did not affect germination and seedlings growth of *P. sativum*, *C. arietinum* and *P. vulgaris* at 10 and 30% concentrations. So, at these levels, coastal underground water cannot be so harmful for these species establishment. However, further experiments are needed to evaluate the effect of saline water irrigation on yield and crop production.

REFERENCES

Al-Quraan NA, Al-Sharbati M, Dababneh Y and Al-Olabi M. 2014. Effect of temperature, salt and osmotic stresses on seed germination and chlorophyll contents in lentil (*Lens culinaris* Medik). Acta horticulturae, 1054:47-54.

Arslan H. 2013. Application of multivariate statistical techniques in the assessment of groundwater quality in seawater intrusion area in Bafra Plain,Turkey. Environmental Monitoring and Assessment, 185(3):2439-2452.

Chaves MM, Flexas J and Pinheiro C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of botany, 103(4):551-560.

Chen H and Jiang JG. 2010. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environmental Reviews, 18:309-319.

Chen W, Hou Z, Wu L, Liang Y and Wei C. 2010. Evaluating salinity distribution in soil irrigated with saline water in arid regions of northwest China. Agricultural water management, 97(12):2001-2008.

Chinnusamy V, Jagendorf A and Zhu JK. 2005. Understanding and improving salt tolerance in plants. Crop Science, 45(2):437-448.

Côme D. 1970. Obstacles to germination. Monographies of Plant physiology, 6.

D'Odorico P, Bhattachan A, Davis KF, Ravi S and Runyan CW. 2013. Global desertification: drivers and feedbacks. Advances in Water Resources, 51:326-344.

Debouba M, Gouia H, Suzuki A and Ghorbel MH. 2006. NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato (*Lycopersicon esculentum*) seedlings. Journal of plant physiology, 163(12):1247-1258.

Duan CX, Zhu ZD, Ren GX, Wang XM and Li DD. 2014. Resistance of faba bean and pea germplasm to *Callosobruchus chinensis* (Coleoptera: Bruchidae) and its relationship with quality components. Journal of economic entomology, 107(5):1992-1999.

Glenn EP, Brown JJ and O'Leary JW. 1998. Irrigating Crops with Seawater. Scientific American, pp 76-81

El-Keblawy A. 2004. Salinity effects on seed germination of the common desert range grass, *Panicum turgidum*. Seed Science and Technology, 32(3):873-878.

Farissi M, Bouizgaren A, Faghire M, Bargaz A and Ghoulam C. 2011. Agro-physiological responses of Moroccan alfalfa (*Medicago sativa* L.) populations to salt stress during germination and early seedling stages. Seed Science and Technology, 39(2):389-401.

Ghogdi E, Borzouei A, Jamali S and Pour N. 2013. Changes in root traits and some physiological characteristics of four wheat genotypes under salt stress. International Journal of Agriculture and Crop Sciences, 5(8):838.

Giri N and Joshi NC. 2010. Growth and yield response of chick pea (*Cicer arietinum*) to seed inoculation with Rhizobium sp. Nature and science, 8(9):232-236.

Hager AS, Mäkinen OE and Arendt EK. 2014. Amylolytic activities and starch reserve mobilization during the germination of quinoa. European Food Research and Technology, 239(4):621-627.

Hirich A, Jelloul A, Choukr-Allah R and Jacobsen SE. 2014. Saline water irrigation of quinoa and chickpea: seedling rate, stomatal conductance and yield responses. Journal of Agronomy and Crop Science, 200(5):378-389.

Hsiao TC and Xu LK. 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. Journal of Experimental Botany, 51(350):1595-1616.

Hussain S, Khaliq A, Matloob A, Wahid MA and Afzal I. 2013. Germination and growth response of three wheat cultivars to NaCl salinity. Soil and Environment, 32(1):36-43.

Iantcheva A, Mysore KS and Ratet P. 2013. Transformation of leguminous plants to study symbiotic interactions. International Journal of Developmental Biology, 57(6-7-8):577-586.

Khan MR, Ashraf S, Rasool F, Salati KM, Mohiddin FA and Haque Z. 2014. Field performance of Trichoderma species against wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*. Turkish Journal of Agriculture and Forestry, 38(4):447-454.

Khan MSA, Karim MA and Haque MM. 2015. Genotypic differences in growth and ions accumulation in soybean under NaCl salinity and water stress conditions. Bangladesh Agronomy Journal, 17(1):47-58.

Kheloufi A. 2017. Germination of seeds from two leguminous trees (*Acacia karroo* and *Gleditsia triacanthos*) following different pre-treatments. Seed Science and Technology, 45(1):1-4.

Kheloufi A, Chorfi A and Mansouri LM. 2016a. The Mediterranean seawater: the impact on the germination and the seedlings emergence in three Acacia species. Journal of Biodiversity and Environmental Sciences, 8(6):238-249.

Kheloufi A, Chorfi A and Mansouri LM. 2016b. Comparative effect of NaCl and $CaCl_2$ on seed germination of *Acacia saligna* L. and *Acacia decurrens* Willd. International Journal of Biosciences, 8:1-13.

Liu J, Liu Q and Yang H. 2016. Assessing water scarcity by simultaneously considering environmental flow requirements, water quantity, and water quality. Ecological Indicators, 60:434-441.

Long X, Huang Z, Zhang Z, Li Q, Zed R and Liu Z. 2010. Seawater stress differentially affects germination, growth, photosynthesis, and ion concentration in genotypes of Jerusalem artichoke (*Helianthus tuberosus* L.). Journal of plant growth regulation, 29(2):223-231.

Maguire JD. 1962. Speed of germination-aid in selection aid in evolution for seedling emergence and vigor. Crop Sci, 2:176-177.

Munns R and Gilliham M. 2015. Salinity tolerance of crops-what is the cost?. New Phytologist, 208(3):668-673.

Munns R, Schachtman DP and Condon AG. 1995. The significance of a two-phase growth response to salinity in wheat and barley. Functional Plant Biology, 22(4):561-569.

Negrão S, Schmöckel SM and Tester M. 2016. Evaluating physiological responses of plants to salinity stress. Annals of Botany, 119(1):1-11.

Nemecek T, Hayer F, Bonnin E, Carrouée B, Schneider A and Vivier C. 2015. Designing eco-efficient crop rotations using life cycle assessment of crop combinations. European Journal of Agronomy, 65:40-51.

Neugschwandtner R, Ziegler K, Kriegner S, Wagentristl H and Kaul HP. 2015. Nitrogen yield and nitrogen fixation of winter faba beans. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 65(7):658-666.

Nichols PGH, Malik AI, Stockdale M and Colmer TD. 2009. Salt tolerance and avoidance mechanisms at germination of annual pasture legumes: importance for adaptation to saline environments. Plant and Soil, 315(1-2):241.

Nonogaki H, Bassel GW and Bewley JD. 2010. Germination-still a mystery. Plant Science, 179(6):574-581.

Orchard T. 1977. Estimating the parameters of plant seedling emergence. Seed Science and Technology, 5:61-69.

Panuccio MR, Jacobsen SE, Akhtar SS and Muscolo A. 2014. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB Plants, 6: 1-18.

Parihar P, Singh S, Singh R, Singh VP, and Prasad SM. 2015. Effect of salinity stress on plants and its tolerance strategies: a review. Environmental Science and Pollution Research, 22(6):4056-4075.

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny MR, Jiménez-Guerrero I, López-Baena, FJ, Ollero and Cubo T. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiological Research, 169(5):325-336.

Petrović G, Jovičić D, Nikolić Z, Tamindžić G, Ignjatov M, Milošević D and Milošević B. 2016. Comparative study of drought and salt stress effects on germination and seedling growth of pea. Genetika, 48(1):373-381.

Popović V, Miladinović J, Vidić M, Ikanović J, Đekić V, Filipović V, Kolarić LJ, Jokanović BM and Čobanović L. 2015. Productive characteristics of soybean in agroecological conditions of *Sremska Mitrovica*, Serbia. Agriculture and Forestry, 61(1):67-75.

Popović V, Vidić M, Ikanović J, Filipović V, Đekić V, Tabaković M, Veselić J. 2016. Soybean oil yield as affected by the growing locality in agro-climatic divergent years. Agriculture and Forestry, 62(1):217-225.

Prasanth SS, Magesh NS, Jitheshlal KV, Chandrasekar N and Gangadhar K. 2012. Evaluation of groundwater quality and its suitability for drinking and agricultural use in the coastal stretch of Alappuzha District, Kerala, India. Applied Water Science, 2(3):165-175.

Robertson GP, Bruulsema TW, Gehl RJ, Kanter D, Mauzerall DL, Rotz CA and Williams CO. 2013. Nitrogen–climate interactions in US agriculture. Biogeochemistry, 114(1-3):41-70.

Roy SJ, Negrão S and Tester M. 2014. Salt resistant crop plants. Current Opinion in Biotechnology, 26:115-124.

Shelef O, Gross A and Rachmilevitch S. 2012. The use of *Bassia indica* for salt phytoremediation in constructed wetlands. Water Research, 46(13):3967-3976.

Shrivastava P and Kumar R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi journal of biological sciences, 22(2):123-131.

Smýkal P, Coyne CJ, Ambrose MJ, Maxted N, Schaefer H, Blair MW, Berger J, Greene SL, Nelson MN, Besharat N, Vymyslický T, Toker C, Saxena RK, Roorkiwal M, Pandey MK, Hu J, Li YH, Wang LX, Guo Y, Qiu LJ, Redden RJ and Varshney RK. 2015. Legume crops phylogeny and genetic diversity for science and breeding. Critical Reviews in Plant Sciences, 34(1-3):43-104.

Söğüt MA, Göze FG, Önal T, Devran Z and Tonguc M. 2014. Screening of common bean (*Phaseolus vulgaris* L.) cultivars against root-lesion nematode species. Turkish Journal of Agriculture and Forestry, 38(4):455-461.

Tan-Wilson AL and Wilson KA. 2012. Mobilization of seed protein reserves. Physiologia Plantarum, 145(1):140-153.

Tawfik MM, El Lateef EA, Amany AB and Hozayen M. 2011. Prospect of biofertilizer inoculation for increasing saline irrigation efficiency. Research Journal of Agriculture and Biological Sciences, 7(2):182-189.

Ventura Y, Eshel A, Pasternak D and Sagi M. 2015. The development of halophyte-based agriculture: past and present. Annals of botany, 115(3):529-540.

Ventura Y, Wuddineh WA, Myrzabayeva M, Alikulov Z, Khozin-Goldberg I, Shpigel M, Samocha TM and Sagi M. 2011. Effect of seawater concentration on the productivity and nutritional value of annual Salicornia and perennial Sarcocornia halophytes as leafy vegetable crops. Scientia Horticulturae, 128(3):189-196.

Vibhuti CS, Bargali K and Bargali SS. 2015. Seed germination and seedling growth parameters of rice (*Oryza sativa* L.) varieties as affected by salt and water stress. Indian Journal of Agricultural Sciences, 85(1):102-108.

Werner AD, Bakker M, Post VE, Vandenbohede A, Lu C, Ataie-Ashtiani B, Simmons CT and Barry DA. 2013. Seawater intrusion processes, investigation and management: recent advances and future challenges. Advances in Water Resources, 51:3-26.

Williams CM, King JR, Ross SM, Olson MA, Hoy CF and Lopetinsky KJ. 2014. Effects of three pulse crops on subsequent barley, canola, and wheat. Agronomy Journal, 106(2), 343-350.

Wu GQ, Liang N, Feng RJ, Zhang JJ. 2013. Evaluation of salinity tolerance in seedlings of sugar beet (*Beta vulgaris* L.) cultivars using proline, soluble sugars and cation accumulation criteria. Acta Physiologiae Plantarum, 35:2665-2674.

Yang R, Guo Q, and Gu Z. 2013. GABA shunt and polyamine degradation pathway on γ-aminobutyric acid accumulation in germinating fava bean (*Vicia faba* L.) under hypoxia. Food Chemistry, 136(1):152-159.

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ANALYSIS OF DENDROFLORA ON THE GREEN AREAS OF SOME KINDERGARTENS IN BELGRADE (SERBIA)

SUMMARY

Green areas of kindergartens and schools represent an important part of the system of a city's greenery and perform a range of functions like ecological, environmental, sanitary, aesthetic, educational, recreational, etc. These areas may have some woody species which are of an invasive or allergenic character. Allergenic woody species may negatively influence the children's health, or provoke certain allergic reactions. Since children spend most of their time in kindergartens, the research of the presence of allergenic species in these areas is very important. Invasive woody species could threaten biodiversity and disturb aesthetics of the green kindergarten's area by sudden and quick spreading. The research has been carried out on the green areas of four kindergartens in Belgrade. This research consists of the survey of the main elements of tree growth, the assessment of vitality and decorativeness of each tree as well as the establishment of the number of allergens and invasive species on every researched area. The obtained data have been processed in the statistical program SPSS Statistics 17.0. The results have shown the presence of 41 plant species with the total number of 359 trees on those 4 researched areas. One invasive species (*Robinia pseudoacacaia* L.) with the total number of 38 trees (10.6%) is present. 12 allergenic species with 133 trees have been ascertained, which is 37.0% of the total number. The species *Betula pendula* Roth., which pollen has very conspicuous allergenic characteristics, is present with 17 trees, and the species of the genus *Tilia* spp., with moderately to strongly conspicuous characteristics of pollen, with 21 tree.

Keywords: allergenic plants, dendroflora, invasive species, green areas of kindergartens

INTRODUCTION

Urban forests and green areas can improve environmental conditions and quality of life in cities (Pretzsch et al. 2015). In urban environment, urban forests and green areas interconnect ecosystems and are an indicator of sustainability (Jankovska et al. 2014). Planning of green spaces gives as a result better quality

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of life in the cities and higher value of space in general (Vukotić-Lazar et al. 2016)

The greenery of kindergartens and schools is an important part of the system of a city's greenery. Besides that, the green areas of these objects have a great significance regarding sports and recreation of children. The green areas of kindergartens, schools and other educational institutions have multiple functions like ecological, sanitary, educational and recreational (Gačić and Stavretović 2008). Numerous authors (Fjørtoft 2004, White 2004, Bell and Dyment 2008, Dyment et al. 2009, Lucas et al. 2010) indicate the positive influence of green areas on social, psychological and motor development of children.

The composition and characteristic of the existing woody species on the green areas of kindergartens affect their aesthetics and functionality. Besides this, these areas may have some woody species which are of an invasive or allergenic character. Allergenic woody species may negatively influence the children's health, or provoke certain allergic reactions. Pollen allergies are seasonal diseases that are related to the time of flowering allergenic plants (Nestorović et al. 2011). Pollen various grasses, trees and weeds cause in sensitive people allergic reactions. The most common allergic disease is allergic rhinitis, bronchial asthma, allergic conjunctivitis, eczema, hives (Urticaria) (Nestorović et al. 2011). Pre-school and school age children are especially vulnerable and increasing the number of those who suffer from allergies caused by pollen of plants. The most common allergic disease in the world is allergic rhinitis by whom in various parts of the world affects 10-25% of the population, with a high representation of preschool and school-age children and adolescents (Nestorović et al. 2011).

Invasive woody species could threaten biodiversity and disturb aesthetics of the green kindergarten's area by sudden and quick spreading.

The aim of this research is the insight into the composition and characteristics of the existing woody species on the areas of chosen kindergartens in Belgrade. The research has included the survey of the main elements of tree growth, as well as the assessment of vitality and decorativeness of each tree on the chosen areas. The number of invasive and allergenic plant species has been established on every study area.

MATERIAL AND METHODS

The research has been conducted on the green areas of four public kindergartens in Belgrade. Those areas where the study surfaces are located were chosen randomly in different parts of the city. The kindergarten „Suncokreti" is located in the eastern part of Belgrade. In the southern part of Belgrade, the kindergarten „Gorica"is located. The kindergarten „Nevena" is situated in the western part of Belgrade. The research has also been conducted on the green area of the kindergarten „Ježurko", in the northern part of the city.

Determination of the woody species has been conducted according to Vukićević (1996). The height of a tree (h) has been determined by Blume-Leis' altimeter according to Banković and Pantić (2006). The diameter of a tree (d) has

been measured by millimetre cross traverse crisscross in two directions under the right angle at the chest height of 1.30m. The height of a trunk (b) has been determined by a meter or Blume-Leis' altimeter. The survey of the width of a treetop (ld) has been conducted by a ribbon in two directions under the right angle (Stavretović et al. 2010).

Besides the aforementioned parameters, the vitality and decorativeness of each tree have been assessed according to the method Stavretović et al. (2010). The assessment of vitality of every single tree has been conducted according to the following principle: the mark 1 is given to the dry tree or the tree with obviously present signs of illness and damage to a large extent. The mark 2 is given to the tree which possesses visible signs of illness and damage or decay. The vital tree with a tendency of survival is given the mark 3. The mark 4 is given to the tree that keeps good vitality and possesses signs of illness or damage to a slight extent. Healthy, exceptionally vital tree without any visible signs of illness and damage is given the mark 5.

The marks for decorativeness have been formed according to the following principle. The tree without any decorative characteristics has been given the mark 1. The mark 2 has been given to the tree which possesses very few decorative characteristics; whereas the mark 3 is given to the tree which appearance has certain flaws. The mark 4 is given to the tree with very good decorative characteristics which possesses certain flaws to a slight extent. The tree with exceptional decorative values, which habitus does not possess visible flaws is given the mark 5.

The obtained data have been processed in the statistical program SPSS Statistics 17.0. Descriptive statistical analysis has been given for every researched area: mean value ($\bar{x}$); middle error of the arithmetic mean ($S_{\bar{x}}$), coefficient of variation (cv %), the relation between mean values of species (%).

Allergenic species have been determined according to Igić et al. (2008). Recorded allergenic woody species have been divided into species which allergenic characteristic of pollen are as follow: 1. very slightly conspicuous, 2. slightly conspicuous, 3. moderate, 4. moderate to strongly conspicuous, 5. very strongly conspicuous (D'amato et al. 2007, Nestorović et al. 2011). Invasive plant species have been defined according to Vrbničanin et al. (2004) and Borišić et al. (2007).

RESULTS AND DISCUSSION

The total number of 41 woody species with 359 trees has been recorded on those four study areas. One invasive species with the total number of 38 trees (10.6%) is present. 12 allergenic species with 133 trees have been ascertained, which is 37.0% of the total number.

The total number of 11 species with 36 trees has been recorded on the green area of the kindergarten "Ježurko". The approximate area of green space of this kindergarten is about 600 m². The majority of trees on this area are relatively young, which is showed by the values of diameters and heights in the Table 1.

Table 1. Average values of the analysed characters of trees in Kindergarten „Ježurko“

		d (cm)				h (m)				b (m)				ld (m)			
Tree Species	N (%)	x̄	Sx	cv (%)	%	x̄	Sx	cv (%)	%	x̄	Sx	cv (%)	%	x̄	Sx	cv (%)	%
Fraxinus ornus L.	3	32,4	/	/	203,8	10,4	/	/	123,8	1,8	/	/	100,0	5,3	/	/	151,4
Platanus orientalis L.	3	7	/	/	44,0	6,3	/	/	75,0	1,7	/	/	94,4	2,3	/	/	65,7
Platanus acerifolia (Ait.) Wild.	31	7,8	1,0	43,1	49,1	6,6	0,7	36,7	78,6	1,8	0,1	12,0	100,0	2,8	0,3	41,0	80,0
Pirus communis L.	3	6,7	/	/	42,1	3,5	/	/	41,7	1,3	/	/	72,2	1,8	/	/	51,4
Prunus cerasifera Ehrh.	3	8,0	/	/	50,3	2,5	/	/	29,8	1,3	/	/	72,2	1,2	/	/	34,3
Thuja orientalis L.	37	1,6	0,4	91,1	10,1	1,8	0,3	64,9	21,4	0	0	0	0,0	0,7	0,05	23,6	20,0
Larix decidua Mill.	5,5	17,3	7,4	60,5	108,8	13	0	0	154,8	2,6	0,2	13,3	144,4	4,4	0	0	125,7
Picea abies Karst.	3	5,2	/	/	32,7	5,3	/	/	63,1	0	/	/	0,0	1,6	/	/	45,7
Acer platanoides L.	5,5	21,4	18,3	121,0	134,6	9,8	5,6	81,1	116,7	3	1,1	51,9	166,7	3,8	2,3	85,6	108,6
Quercus borealis Michx.	3	50,1	/	/	315,1	20	/	/	238,1	3,5	/	/	194,4	9	/	/	257,1
Betula pendula Roth.	3	17,4	/	/	109,4	13	/	/	154,8	2,5	/	/	138,9	5,1	/	/	145,7
Average		15,9	6,8	78,9	100	8,4	1,6	45,7	100	1,8	0,3	19,3	100	3,5	0,7	37,6	100

The highest values of growth elements belong to the species *Quercus borealis* Michx. (d = 50.1cm; h = 20m; b = 3.5m; ld = 9m), whereas the lowest recorded values belong to the species *Thuja orientalis* L.(d = 1.6cm; h = 1.8m; ld = 0.7m). There has been noticed that the tree of the species *Pirus commmunis* L. possesses certain flaws, so it has been given the mark 3 for vitality and decorativeness in accordance with that. As all the other trees have been given the high evaluation for vitality and decorativeness (marks 4 and 5) it can be concluded that the species *Pirus communis* L. possesses the lowest evaluation for vitality and decorativeness on this area (table 2).

Table 2. Average values of vitality and decorativeness of trees in Kindergarten „Ježurko“

		VIT				DEK			
Tree Species	N (%)	x̄	s	cv (%)	%	x̄	s	cv (%)	%
Fraxinus ornus L.	3	4	/	/	93,0	5	/	/	113,6
Platanus orientalis L.	3	5	/	/	116,3	5	/	/	113,6
Platanus acerifolia (Ait.)Wild.	31	4,91	0,1	6,1	114,2	4,91	0,1	6,1	111,6
Pirus communis L.	3	3	/	/	69,8	3	/	/	68,2
Prunus cerasifera Ehrh.	3	4	/	/	93,0	4	/	/	90,9
Thuja orientalis L.	37	4,54	0,1	11,4	105,6	4,77	0,1	9,2	108,4
Larix decidua Mill.	5,5	4	0	0	93,0	4	0	0	90,9
Picea abies Karst.	3	5	/	/	116,3	5	/	/	113,6
Acer platanoides L.	5,5	4,5	0,5	15,7	104,7	5	0	0	113,6
Quercus borealis Michx.	3	4	/	/	93,0	4	/	/	90,9
Betula pendula Roth.	3	4	/	/	93,0	4	/	/	90,9
Average		4,3	0,2	8,3	100	4,4	0,05	3,8	100

Out of the 11 recorded species, 5 possess allergenic characteristics of pollen to a certain extent. The species *Betula pendula* Roth. is characterized by very strongly expressed allergenic characteristics of pollen. One tree of this species has been recorded on the researched area. The species *Acer platanoides* L. - 2 trees and *Thuja orientalis* L. - 13 trees have moderately conspicuous allergenic characteristics of pollen. The species *Fraxinus ornus* L. - 1 tree, *Platanus x acerifolia* (Ait.) Willd. - 11 trees have very weakly expressed allergenic characteristics of pollen. The trees of the species *Thuja orientalis* L. and *Platanus x acerifolia* (Ait.) Willd. are relatively young, so their characteristics of pollen are going to achieve its full potential in the years to come.

The invasive woody species are not present on this green area either, but they are present in the surroundings; the species *Ailanthus altissima* (Mill.) Svingle and *Robinia pseudoacacia* L., which are well known for their invasive character, have been noticed on the green surfaces in the nearby residential area.

The kindergarten “Nevena” is situated in the western part of Belgrade. The approximate area of green space of this kindergarten is about 2000 m². 13 species with the total number of 40 trees is present in its garden. The values obtained from the measured parameters are given in the table 3. The highest recorded values belong to the species *Cedrus deodara* (Rohb) G. Don. (d = 36.3cm; h = 15.5m; ld = 6.9m) and *Tilia argentea* DC. (d = 37.4cm; h = 8.5m; ld = 5.1m).

The lowest recorded values (d = 1.2cm; h = 1.4m; ld = 0.6m) belong to the species *Pseudotsuga menziesii* (Mirb.)Franco. which stems from the fact that the trees of this species are very young. The trees on this green area are given the highest assessments for their vitality and decorativeness (table 4). The species of *Thuja orientalis* L., *Picea abies* Karst., *Picea orientalis* Link. are characterized with the lowest assessments for vitality and decorativeness of the trees (mark 3).

Allergenic characteristics of pollen are predominant to a certain extent in 6 of the total amount of 13 recorded species. The species *Tilia argentea* DC is present with the only one tree on this area. Allergenic characteristics of pollen of this species are moderately to strongly conspicuous. There has also been noted the presence of two species with moderately expressed allergenic characteristics of pollen (*Acer platanoides* L. and *Thuja orientalis* L. with one specimen each). The species *Pinus nigra* Arn.- 3 trees, *Cedrus atlantica* (Endl.)Man ex Carr - 3 trees and *Abies concolor* Lindl. et Gord - 3 trees are characterised with weakly conspicuous allergenic effect of pollen.

Table 3. Average values of the analysed characters of trees in Kindergarten „Nevena“

		d (cm)				h (m)				b (m)				ld (m)			
Tree Species	N (%)	x̄	Sx	cv (%)	%	x̄	Sx	cv (%)	%	x̄	Sx	cv (%)	%	x̄	Sx	cv (%)	%
Pinus nigra Arn.	7,5	15,0	0,3	3,7	95,5	6,6	1,0	25,5	110,0	1,6	0,06	6,2	114,3	2,4	0,2	12,6	88,9
Cedrus atlantica (Endl.)Man. Ex Carr.	12,5	23,5	10,2	97,2	149,7	7,7	2,7	77,0	128,3	0,9	0,4	91,7	64,3	4,1	1,3	72,2	151,9
Prunus cerasifera Atropurpurea Ehrh.	40	12,1	2,6	86,9	77,1	5,8	0,8	53,9	96,7	2	0,04	8,2	142,9	3,1	0,7	87,9	114,8
Picea orientalis Linkp.	2,5	16,1	/	/	102,5	4,5	/	/	75,0	2	/	/	142,9	1,6	/	/	59,3
Picea abies Karst.	2,5	6,9	/	/	43,9	2	/	/	33,3	0	/	/	0,0	1,5	/	/	55,6
Ulmus effusa Willp.	2,5	11,9	/	/	75,8	7,2	/	/	120,0	1,7	/	/	121,4	3,3	/	/	122,2
Thuja orientalis L.	2,5	18	/	/	114,6	6,5	/	/	108,3	1,5	/	/	107,1	1,6	/	/	59,3
Abies concolor Lindl. et Gord.	7,5	7,3	0,8	17,7	46,5	3,2	0,7	36,5	53,3	0	0	0	0	1,4	0,09	11,2	51,9
Pseudotsuga menziesii (Mirb.)Franco	7,5	1,2	0,1	21,6	7,6	1,4	0,2	29,6	23,3	0	0	0	0	0,6	0,2	50,8	22,2
Acer platanoides L.	2,5	15,4	/	/	98,1	5,5	/	/	91,7	2	/	/	142,9	2,1	/	/	77,8
Tilia argentea DC.	2,5	37,4	/	/	238,2	8,5	/	/	141,7	2,1	/	/	150,0	5,1	/	/	188,9
Celtis australis L.	7,5	2,7	0,2	15,2	17,2	3,8	0,2	9,1	63,3	2,3	0,03	2,5	164,3	1,6	0,07	7,4	59,3
Cedrus deodara (Roxb.)Don	2,5	36,3	/	/	231,2	15,5	/	/	258,3	1,5	/	/	107,1	6,9	/	/	255,6
Average		15,7	2,4	40,4	100	6,0	0,9	38,6	100	1,4	0,09	18,1	100	2,7	0,4	40,3	100

Table 4. Average values of vitality and decorativeness of trees in Kindergarten „Nevena“

		VIT				DEK			
Tree Species	N (%)	x̄	s	cv (%)	%	x̄	s	cv (%)	%
Pinus nigra Arn.	7,5	4	0	0	93,0	4	0	0	90,9
Cedrus atlantica (Endl.)Man. Ex Carr.	12,5	4,6	0,2	11,9	107,0	4,8	0,2	9,3	109,1
Prunus cerasifera Atropurpurea Ehrh.	40	4,1	0,2	22,9	95,3	4,1	0,2	23,2	93,2
Picea orientalis Linkp.	2,5	3	/	/	69,8	3	/	/	68,2
Picea abies Karst.	2,5	3	/	/	69,8	3	/	/	68,2
Ulmus effusa Willp.	2,5	4	/	/	93,0	5	/	/	113,6
Thuja orientalis L.	2,5	3	/	/	69,8	3	/	/	68,2
Abies concolor Lindl. et Gord.	7,5	5	0	0	116,3	5	0	0	113,6
Pseudotsuga menziesii (Mirb.)Franco	7,5	5	0	0	116,3	5	0	0	113,6
Acer platanoides L.	2,5	5	/	/	116,3	5	/	/	113,6
Tilia argentea DC.	2,5	5	/	/	116,3	5	/	/	113,6
Celtis australis L.	7,5	5	0	0	116,3	5	0	0	113,6
Cedrus deodara (Roxb.) Don	2,5	5	/	/	116,3	5	/	/	113,6
Average		4,3	0,07	5,8	100	4,4	0,07	5,4	100

The invasive woody species on this green area have not been noticed. Invasive woody species like *Robinia pseudoacacia* L. are present in the close surroundings of the kindergarten, in the residential area.

The kindergarten “Gorica“ is located in southern part of Belgrade. The approximate area of green space of this kindergarten is about 7500 m².The presence of 17 species with the total number of 52 trees has been recorded on this green area. The table 5. presents the measured parameters for every species: the diameter of a tree (d), the height of a tree (h), the width of a treetop (ld), the height of a trunk (b). Species like *Cedrus atlantica* (Endl.) Man. ex Carr., *Thuja giganthea* Nutt., *Abies concolor* Lindl. et Gord. characterize with the highest values of diameters and heights of the trees. The highest evaluations of vitality and decorativeness (table 6) have been granted to the trees of the species *Acer platanoides* L. (VIT = 5; DEK = 5) and *Cedrus atlantica* (Endl.) Man. ex Carr. (VIT= 4.5; DEK= 4.9). The species *Cedrus atlantica* (Endl.) Man. ex Carr is present with the total number of 8 trees, which is the most numerous on this area. The lowest measured values of growth elements (d = 0.6cm; h = 0.4m; ld = 0.3m) belong to the species of *Thuja orientalis* L. It is necessary to indicate that all the individual trees of the aforementioned species are very young, recently planted, which explains given low values. However, this species has been credited with the high evaluation of vitality and decorativeness (VIT = 4; DEK = 4).

Table 5. Average values of the analysed characters of trees in Kindergarten „Gorica“

		d (cm)				h (m)				b (m)				ld (m)			
Tree Species	N (%)			cv (%)	%			cv (%)	%			cv (%)	%			cv (%)	%
Acer platanoides L.	2	17,9	/	/	210,6	4,5	/	/	121,6	1,9	/	/	82,6	1,9	/	/	135,7
Quercus borealis Ten.	2	3,4	/	/	40,0	4	/	/	108,1	3	/	/	130,4	0,5	/	/	35,7
Cedrus atlantica (Endl.) Man. Ex Carr.	15	18,1	8,0	125,7	212,9	6,8	2,0	85,6	183,8	0,6	0,3	142,7	26,1	2,9	0,8	76,6	207,1
Pseudotsuga menziesii (Mirb.) Franco	7	13,4	6,6	97,7	157,6	5,7	2,4	85,3	154,1	0,9	0,5	115,8	39,1	2,2	0,9	81,8	157,1
Betula pendula Roth.	6	14,2	7,4	89,4	167,1	5,2	1,7	57,0	140,5	1,4	0,4	54,9	60,9	2,6	0,6	42,8	185,7
Koelreuteria paniculata Laxm.	9	3,5	0,08	5,2	41,2	3,2	0,2	13,3	86,5	1,1	0,04	7,7	47,8	1,3	0,05	9,0	92,9
Prunus cerasifera Ehrh.	2	1,9	/	/	22,4	2,5	/	/	67,6	1,9	/	/	82,6	0,6	/	/	42,9
Pinus nigra Arn.	9	4,3	0,4	23,5	50,6	1,9	0,3	32,8	51,4	0	0	0	0,0	1,3	0,2	32,4	92,9
Sorbus scandica Fries.	2	2,4	/	/	28,2	2,5	/	/	67,6	2,1	/	/	91,3	0,6	/	/	42,9
Thuja gigantea Nutt.	2	25,5	/	/	300,0	7,5	/	/	202,7	1,9	/	/	82,6	2,3	/	/	164,3
Thuja orientalis L.	4	0,6	0,1	23,6	7,1	0,4	0	0	10,8	0	0	0	0,0	0,3	0	0	21,4
Prunus avium L.	2	0,8	/	/	9,4	1,5	/	/	40,5	1,3	/	/	56,5	0,2	/	/	14,3
Acer pseudoplatanus L.	2	3,6	/	/	42,4	2,3	/	/	62,2	1,9	/	/	82,6	0,8	/	/	57,1
Abies concolor Lindl. et Gord.	7	18,3	5,7	61,9	215,3	6,4	1,7	52,2	173,0	1,1	0,4	67,1	47,8	2,4	0,3	26,8	171,4
Picea pungens Engelm.	6	1,2	0,2	23,4	14,1	1,5	1,0	115,5	40,5	0	0	0	0,0	0,6	0,3	81,5	42,9
Tilia argentea DC.	17	13,0	2,2	51,1	152,9	5,7	0,6	29,3	154,1	1,8	0,04	5,8	78,3	3,2	0,4	36,2	228,6
Picea abies Karst.	6	2,8	2,1	129,4	32,9	1,5	1,0	115,5	40,5	0	0	0	0,0	0,6	0,3	81,5	42,9
Average		8,5	3,3	63,1	100	3,7	1,1	58,6	100	2,3	0,2	39,4	100	1,4	0,4	46,9	100

Out of the total number of 17 species recorded, 8 species possess allergenic characteristics. The species *Betula pendula* Roth. possesses very conspicuous allergenic characteristics of pollen and it is present on this green area with 3 trees. The species of the genus *Tilia* spp., which allergenic characteristics are moderately to strongly expressed, have the total number of 7 trees. Two species (*Acer platanoides* L. - 1 tree, *Thuja orientalis* L.- 2 trees) have moderately expressed characteristics of pollen. Four species (*Cedrus atlantica*

(Endl.) Man. ex Carr - 8 trees, *Pinus nigra* Arn. - 5 trees, *Abies concolor* Lindl. et Gord. – 4 trees, *Acer pseudoplatanus* L. - 1 tree) have weakly expressed allergenic characteristics of pollen.

Invasive woody species have not been recorded on this area, even though they are present in the surroundings of the kindergarten. The presence of the species *Ailanthus altissima* (Mill.) Svingle, *Robinia pseudoacacia* L.as well as *Acer negundo* L. has also been noticed.

Table 6. Average values of vitality and decorativeness of trees in Kindergarten „Gorica“

		VIT				DEK			
Tree Species	N (%)	x̄	s	cv (%)	%	x̄	s	cv (%)	%
Acer platanoides L.	2	5	/	/	128,2	5	/	/	122,0
Quercus borealis Ten.	2	4	/	/	102,6	3	/	/	73,2
Cedrus atlantica (Endl.) Man. Ex Carr.	15	4,5	0,3	16,8	115,4	4,9	0,1	7,3	119,5
Pseudotsuga menziesii (Mirb.) Franco	7	4,2	0,2	11,8	107,7	4,5	0,3	12,8	109,8
Betula pendula Roth.	6	4	0	0	102,6	4,3	0,3	13,3	104,9
Koelreuteria paniculata Laxm.	9	3,6	0,2	15,2	92,3	3,8	0,2	11,8	92,7
Prunus cerasifera Ehrh.	2	4	/	/	102,6	4	/	/	97,6
Pinus nigra Arn.	9	4	0	0	102,6	4	0	0	97,6
Sorbus scandica Fries.	2	3	/	/	76,9	4	/	/	97,6
Thuja gigantea Nutt.	2	3	/	/	76,9	4	/	/	97,6
Thuja orientalis L.	4	4	0	0	102,6	4	0	0	97,6
Prunus avium L.	2	4	/	/	102,6	4	/	/	97,6
Acer pseudoplatanus L.	2	4	/	/	102,6	4	/	/	97,6
Abies concolor Lindl. et Gord.	7	3,5	0,3	16,5	89,7	4	0	0	97,6
Picea pungens Engelm.	6	4	0,6	25,0	102,6	4	0,6	25,0	97,6
Tilia argentea DC.	17	3,7	0,2	13,6	94,9	4,1	0,1	8,1	100,0
Picea abies Karst.	6	4	0,6	25,0	102,6	4	0,6	25,0	97,6
Average		3,9	0,2	12,4	100	4,1	0,2	10,3	100

The kindergarten “Suncokreti” is located in the eastern part of Belgrade. The approximate area of green space of this kindergarten is about 11000 m². The total number of 26 species with 227 trees has been recorded on the green area which belongs to the kindergarten. The maximum values of the elements of growth (table 7) belong to the species *Quercus virgiliana* Ten. (d = 41.2cm; h = 13.5m; b = 1.5m; ld = 9.2m) and *Liriodendron tulipifera* L. (d = 38.5cm; h = 22.5m; b = 2.8m; ld = 11.1m), whereas the minimum values have been recorded at the species of *Fraxinus angustifolia* Vahl. (d = 2.4cm; h = 3.1m; b = 1.3m; ld = 2.3m) and *Malus domestica* Borkh. (d = 4.4cm; h = 2.5m; b = 1.5m; ld = 1.9m).

Table 7. Average values of the analysed characters of trees in Kindergarten „Suncokreti“

		d (cm)				h (m)				b (m)				ld (m)			
Tree Species	N (%)	x̄	Sx̄	cv (%)	%	x̄	Sx̄	cv (%)	%	x̄	Sx̄	cv (%)	%	x̄	Sx̄	cv (%)	%
Thuja orientalis L.	0,5	7,6	/	/	48,4	6,5	/	/	74,7	0	/	/	0,0	2,4	/	/	52,2
Picea pungens Engelm.	2,2	8,2	1,4	33,0	52,2	6,5	1,1	35,0	74,7	0,4	0,4	200	26,7	2,6	0,5	39,1	56,5
Prunus avium L.	1,6	16,5	6,7	70,7	105,1	4,8	0,5	17,6	55,2	1,5	0,1	13,3	100,0	5,0	1,0	34,0	108,7
Laburnum anagyroides Med.	2,7	6,2	0,6	21,4	39,5	3,6	0,3	2,16	41,4	1,5	0,08	11,8	100,0	2,0	0,2	20,2	43,5
Prunus cerasifera Atropurpurea Ehrh.	12	12,4	1,4	52,3	79,0	7,0	0,3	22,9	80,5	1,5	0,06	17,5	100,0	4,5	0,2	25,5	97,8
Tilia grandifolia DC.	6	28,0	3,3	38,7	178,3	12,7	0,9	23,4	146,0	2	0,1	22,3	133,3	7,5	0,7	30,0	163,0
Betula pendula Roth.	7,1	10,9	2,1	69,6	69,4	7,9	1,3	60,5	90,8	1,9	0,1	25,8	126,7	3,7	0,5	48,9	80,4
Acer platanoides L.	11,4	20,5	1,3	29,4	130,6	12,3	0,4	13,3	141,4	1,9	0,06	13,6	126,7	6,0	0,5	35,9	130,4
Fraxinus angustifolia Vahl.	2,2	2,4	0,4	36,0	15,3	3,1	0,6	36,0	35,6	1,3	0,2	29,5	86,7	2,3	0,4	36,6	50,0
Prunus armeniaca L.	0,5	15,9	/	/	101,3	9	/	/	103,4	1,4	/	/	93,3	4,7	/	/	102,2
Robinia pseudoacacia L.	20,7	20,5	0,8	24,6	130,6	11,9	0,4	20,9	136,8	2,1	0,04	11,0	140,0	5,8	0,3	30,2	126,1
Tilia argentea DC.	1,1	28,5	4,4	22,0	181,5	14,0	0,5	5,1	160,9	1,9	0,5	37,2	126,7	7,2	0,1	2,9	156,5
Quercus virgiliana Ten.	1,1	41,2	2,0	6,9	262,4	13,5	1,0	10,5	155,2	1,5	0,2	22,8	100,0	9,2	0,4	6,9	200,0
Catalpa bignonioides Walt.	3,8	9,9	0,9	22,9	63,1	8,7	0,7	20,6	100,0	1,7	0,08	12,5	113,3	3,9	0,3	22,1	84,8
Cercis siliquastrum L.	2,2	8,9	0,6	14,5	56,7	4,1	0,1	6,1	47,1	1,5	0	0	100,0	2,7	0,07	5,6	58,7
Acer campestre L.	2,2	26,8	2,7	20,3	170,7	14,2	1,5	20,8	163,2	1,7	0,2	19,2	113,3	5,9	0,07	2,4	128,3
Rhus typhina L.	0,5	10,9	/	/	69,4	3,5	/	/	40,2	1,9	/	/	126,7	4,5	/	/	97,8
Crataegus monogyina Jacq.	1,1	6,1	1,9	44,8	38,9	4,1	0,1	5,1	47,1	1,5	0,05	4,6	100,0	2,6	0,4	24,0	56,5
Juglans regia L.	1,1	13,0	9,3	101,3	82,8	7,5	5,0	94,3	86,2	1,7	0,05	4,0	113,3	5,4	3,0	79,1	117,4
Aesculus hypocastanum L.	2,2	12,7	2,6	41,5	80,9	7,2	0,7	19,9	82,8	1,8	0,02	2,7	120,0	3,9	0,7	33,6	84,8
Malus domestica Borkh.	0,5	4,4	/	/	28,0	2,5	/	/	28,7	1,5	/	/	100,0	1,9	/	/	41,3
Cedrus atlantica (Endl.)Man. Ex Carr.	3,8	27,7	1,3	12,3	176,4	18,6	0,7	10,2	213,8	1,8	0,1	17,7	120,0	5,6	0,2	10,4	121,7
Abies concolor Lindl.et Gord.	0,5	7,5	/	/	47,8	6,5	/	/	74,7	0	/	/	0,0	2,2	/	/	47,8
Picea abies Karst.	1,1	16,3	0,1	0,9	103,8	10,4	0	0	119,5	1,4	0	0	93,3	2,1	0	0	45,7
Liriodendron tulipifera L.	0,5	38,5	/	/	245,2	22,5	/	/	258,6	2,8	/	/	186,7	11,1	/	/	241,3
Prunus cerasus L.	11,4	6,5	0,3	20,0	41,4	4,6	0,1	14,5	52,9	1,5	0,02	5,3	100,0	3,7	0,2	18,9	80,4
Average		15,7	2,2	34,2	100	8,7	0,8	21,9	100	1,5	0,1	23,5	100	4,6	0,5	25,3	100

The tree of the species *Liriodendron tulipifera* L. is very vital without any visible signs of illness or damage, so it consequently got the mark 5 for its vitality and decorativeness (table 8). The trees of the species *Prunus cerasus* L., *Laburnum anagiroides* Med., *Rhus typhina* L.and *Crataegus monogina* Jacq. got the lowest marks for their vitality and decorativeness.

Table 8. Average values vitality and decorativeness of trees in Kindergarten „Suncokreti“

		VIT				DEK			
Tree Species	N (%)	x̄	s	cv (%)	%	x̄	s	cv (%)	%
Thuja orientalis L.	0,5	5	/	/	147,1	5	/	/	142,9
Picea pungens Engelm.	2,2	3,2	0,6	38,7	94,1	3,2	0,6	38,7	91,4
Prunus avium L.	1,6	1,7	0,7	69,3	50,0	2	1	86,6	57,1
Laburnum anagyroides Med.	2,7	2	0	0	58,8	1,6	0,2	34,2	45,7
Prunus cerasifera Atropurpurea Ehrh.	12	3,2	0,09	13,3	94,1	3,4	0,1	19,5	97,1
Tilia grandifolia DC.	6	4,5	0,2	11,7	132,4	4,6	0,2	14,5	131,4
Betula pendula Roth.	7,1	3,2	0,3	28,7	94,1	3,5	0,2	21,9	100,0
Acer platanoides L.	11,4	4,1	0,1	13,2	120,6	4,4	0,2	16,9	125,7
Fraxinus angustifolia Vahl.	2,2	4	0	0	117,6	4	0	0	114,3
Prunus armeniaca L.	0,5	2	/	/	58,8	1	/	/	28,6
Robinia pseudoacacia L.	20,7	2,9	0,1	23,0	85,3	2,8	0,1	30,2	80,0
Tilia argentea DC.	1,1	4	0	0	117,6	4,5	0,5	15,7	128,6
Quercus virgiliana Ten.	1,1	4	0	0	117,6	4	0	0	114,3
Catalpa bignonioides Walt.	3,8	3,7	0,3	20,4	108,8	4	0,2	14,4	114,3
Cercis siliquastrum L.	2,2	4	0	0	117,6	4	0	0	114,3
Acer campestre L.	2,2	3,7	0,2	13,3	108,8	3,7	0,2	13,3	105,7
Rhus typhina L.	0,5	2	/	/	58,8	3	/	/	85,7
Crataegus monogyina Jacq.	1,1	2	0	0	58,8	2,5	0,5	28,3	71,4
Juglans regia L.	1,1	3	0	0	88,2	3,5	0,5	20,2	100,0
Aesculus hypocastanum L.	2,2	4	0	0	117,6	4	0	0	114,3
Malus domestica Borkh.	0,5	3	/	/	88,2	3	/	/	85,7
Cedrus atlantica (Endl.)Man. Ex Carr.	3,8	4	0	0	117,6	4	0	0	114,3
Abies concolor Lindl.et Gord.	0,5	4	/	/	117,6	3	/	/	85,7
Picea abies Karst.	1,1	3	0	0	88,2	3	0	0	85,7
Liriodendron tulipifera L.	0,5	5	/	/	147,1	5	/	/	142,9
Prunus cerasus L.	11,4	3,9	0,08	9,3	114,7	3,8	0,09	10,6	108,6
Average		3,4	0,1	12,0	100	3,5	0,2	18,2	100

Out of the total number of 26 species recorded on this area, 9 species possess allergenic characteristics of pollen. The species *Betula pendula* Roth., which pollen has very conspicuous allergenic characteristics, is present on this area with 13 trees. The species of the genus *Tilia* spp., with moderately to strongly conspicuous characteristics of pollen, also have 13 trees. There has been recorded somewhat higher number of trees with moderately conspicuous allergenic characteristics of pollen. The total number of 26 trees, arranged into three species (*Acer platanoides* L. - 21 trees, *Acer campestre* L. - 4 trees, *Thuja orientalis* L. - 1 tree) possess the aforementioned characteristic of pollen. The two species (*Cedrus atlantica* (Endl.) Man. Ex Carr. – 7 trees and *Abies concolor* Lindl. et Gord. – 1 tree) are characterized by weakly conspicuous allergenic characteristics of pollen, while the one species (*Juglans regia* L. - 2 trees) possesses very weakly conspicuous characteristics of pollen. 38 trees of the invasive species *Robinia pseudoacacia* L. have been recorded on this green area, and the same species is present in the nearby surroundings.

Woody species on the green areas in urban environment improve the conditions of the environment and have positive influence on the quality of city life and human health (Stavretović *et al.* 2010, Ninić-Todorović *et al.*, 2015, Vujičić *et al.* 2016). Stavretović *et al.* (2010) measured the elements of growth and estimated the indicators of vitality and decorativeness of trees in Mali park in Obrenovac (part of Belgrade). The results of their research have showed that the trees of the species *Platanus x acerifolia* (Ait.) Willd., *Tilia grandifolia* DC. and *Fraxinus ornus* had the best functional characteristics. Species *Platanus x acerifolia* (Ait.) Willd., *Populus bolleana* Lauche, *Ulmus effusa* Willd. were singled out, as representative species of significant vitality and decorative value in Futoški park in Novi Sad (Ninić-Todorović *et al.* 2015)

All allergenic species are divided into three groups: trees, grass and weed. 24 species are kept track of in Serbia, because their pollen has allergenic characteristics: hazel bush, alder, yew, cypresses, elm, poplars, maple, willow, ash, birch, hornbeam, platanus, walnut tree, oak, pine, hemp, grass, linden, plantagineum, sorrel, nettle, pigweed, wormwood and ambrosia (Nestorović *et al.* 2011). Likewise, there are three maximums of pollen concentration in the air in Serbia: early spring – anemophilic trees and bushes, summer – grass, and summer-autumn herbaceous biannual weed. Pollen of the species *Betula pendula* Roth. is very strong allergen, and it could be found in big concentrations in the air – up to 5000 pollen grains/m³ in air (Puc 2003). As it has already been stated, besides trees, grass and weed are also allergenic species. The numerous authors (Stavretović *et al.* 2006, Stavretović *et al.* 2010a; Stavretović *et al.*, 2010b, Stavretović *et al.* 2011, Petrović *et al.* 2013) have noted a significant presence of allergenic species of grass and weed on different types of urban green areas.

CONCLUSIONS

On the study green areas of 4 kindergartens in the suburbs of Belgrade has been recorded 41 woody species with 359 trees. The largest number of trees has been recorded on the green area which belongs to the kindergarten “Suncokreti” (the total number of 227 trees), while the smallest number of trees belong to the garden of “Ježurko” kindergarten (the total of 36 trees).

The trees in the garden of the kindergarten “Suncokreti” prevail with regards to their height (the average height is 15.7m) and the treetop width (the average value is 8.7) when compared to the trees on the other areas. As opposed to this, the lowest average values of treetop height and width belong to the trees in the garden around “Gorica”. The highest average evaluations of vitality and decorativeness belong to the trees located on the green areas of the kindergartens “Ježurko” and “Nevena”. A significant number of young trees, without any visible signs of illness or damage, has been noticed here, what resulted in high average evaluations of vitality and decorativeness. The trees which belong to the kindergarten “Suncokreti” have been given the lowest average evaluations of vitality and decorativeness.

Allergenic species are present in a significant number on these researched areas (133 trees (37.0%). The largest number of allergenic trees has been recorded in the garden of the kindergarten “Suncokreti” (62 trees), while the smallest number has been noticed in the garden of the kindergarten “Nevena” (12 trees). The maximum recorded number of the species with allergenic characteristics *Betula pendula* Roth. and *Tilia* spp. has been recorded on the green area of the kindergarten “Suncokreti” (13 each). The biggest number of trees which pollen has allergenic characteristics to a certain extent belongs to the category of species with moderately conspicuous allergenic characteristics of pollen. Invasive species have been spotted only on the green area of the kindergarten “Suncokreti” (*Robinia pseudoacacia* L. - 38 trees). With regards to that, monitoring the state of this area is necessary in order to avoid uncontrolled spreading of the species *Robinia pseudoacacia* L.. Woody species on the green areas in urban environment improve the conditions of that environment and have positive influence on the quality of city life. The dynamics of growth and development of woody species, as well as their vitality and decorativeness depend on the conditions in which the species grow.

While planning and designing, it would be desirable not to predict allergenic woody species on the green areas of kindergartens. The trees that are planned for logging on these areas should be replaced with the species which do not have allergenic characteristics, or at least with the species which allergenic characteristics of pollen are very weakly conspicuous. It would be advisable also to conduct regular maintenance, especially mowing, which would reduce and put under the control allergenic herbaceous species. The species with the invasive character are not desirable either because of the possibility of instant and quick spreading and thus endangering the survival of the other species as well as disturbing the aesthetics of the mere green area. The species which possess some poisonous parts, as well as the species with thorns, are also not desirable on the green areas of kindergartens.

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REFERENCES

Banković, S. and Pantić, D. (2006): Dendrometrija. Beograd, Srbija, Šumarski fakultet.

Boršić, I., Milović, M., Dujmović, I., Bogdanović, S., Cigić, P., Rešetnik, I., Nikolić, T. and Mitić, B. (2008): Preliminay check-list of invasive alien plant species in Croatia. Natura Croatica. 17: 55-71.

Bell, A. and Dyment, J. (2008): Grounds for health: the intersection of green school grounds and health promoting schools. Environmental Education Research 14: 77-90.

D'amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi Maesano, I., Behrendt, H. and Van Cauwenberge, P. (2007): Allergenic pollen and pollen allergy in Europe. Allergy 62: 976-990.

Dyment, J., Bell, A. and Lucas, A. (2009): The relationship between school ground design and intensity of physical activity. Children's Geographies 7, Iss. 3.

Fjørtoft, I. (2004): Landscape as Playscape: The Effects of Natural Environments on Children's Play and Motor Development. Children, Youth and Environments 14(2): 21-44.

Gačić, A. and Stavretović, N. (2008): Značaj i uticaj zelenih površina školskih dvorišta na razvoj dece. Zbornik radova „Ekološka istina 08", ured. Radivoje Pantić, Tehnički fakultet, Univerzitet u Boru, Bor, Srbija 305-308.

Igić, R., Boža, P., Anačkov, G. and Vukov, D. (2008): Atlas alergijskih biljaka Novog Sada [Atlas of allergic plants in Novi Sad]. Novi Sad, Srbija; Prirodno-matematički fakultet u Novom Sadu, „Simbol".

Jankovska, I., Straupe, I., Brumelis, G., Donis, J., and Kupfere, L. (2014): Urban forests of Riga, Latvia-pressures, naturalness, attitudes and management. Baltic Forestry, 20(2), 342-351.

Lucas, A. and Dyment, J. (2010): Where do children choose to play on the school ground? The influence of green design. Education 38: 3-13.

Ninić-Todorović, J., Mladenović, E., Čukanović, J., Sentić, I., Lakić, a., Todorović, D., Todorović, I. (2015): Bioecological dendroflora characteristics of the District park in Novi sad. Agriculture and Forestry, 61 (2), 51-60.

Nestorović, M., Jovanović, M., Šovljanski, G., Bajić-Bibić, Lj., and Jokić, J. (2011): Priručnik za alergene biljke. Beograd, Srbija; Prirodnjački muzej.

Pretzsch, H., Biber, P., Uhl, E., Dahlhausen, J., Rötzer, T., Caldentey, J., Koike, T., Con, T., Chavanne, A., Seifert, T., Du Toit, B., Farnden, C. and Paulet, S. (2015): Crown size and growing space requirement of common tree species in urban centres, parks, and forests. Urban Forestry & Urban Greening, 14(3), 466-479.

Petrović, J., Stavretović, N., Đuričić, S., Jelić, I. and Mijović, B.(2013): Invazivne biljne vrste i trčci i mravi kao potencijal njihove biološke kontrole na primjeru spomenika prirode „Bojčinska šuma" (Vojvodina, Srbija). Šumarski list 1-2: 61-69.

Puc, M. (2003): Characterisation of pollen allergens. Annals of Agricultural and Environmental Medicine 10: 143–149.

Stavretović, N., Janjić, V. and Paunović, E. (2006): Prisutnost biljne vrste Ambrosia artemisiifolia L. u zelenim površinama Beograda. Zbornik radova naučno-stručnog skupa o prirodnim vrednostima i zaštiti životne sredine, „Ekološka istina 2006", Sokobanja, Srbija: 321-324.

Stavretović, N., Petrović, J. and Đurić, M. (2011): Invazivne biljne vrste u travnim površinama nekih parkova. Acta herbologica 20 (2): 121-131.

Stavretović, N., Stevanović, J., and Mijović, A. (2010a): Invazivne biljne vrste u travnim površinama stambenih naselja Beograda. Acta herbologica 19 (1): 39-47.

Stavretović, N., Vučković, M. and Stajić, B. (2010b): Classification of trees and tree species in Obrenovac "Mali Park" by the elements of growth, vitality and ornamentalness. Archiv of Biological Science 62: 1119-1024.

Vrbničanin, S., Karadžić, B. and Dajić-Stevanović, Z. (2004): Adventivne i invazivne korovske vrste na području Srbije. Acta herbologica 13 (1): 1-12.

Vujičić, M., Tomićević-Dubljević, J., Tomićević-Gavrilović, D. (2016): The socioeconomic and health effect of green infrastructure on the Vračar municipality, city of Belgrade. Agriculture and Forestry 62 (3): 165-174,, DOI 10.17707/agriculturforest.62.3.1.

Vukićević, E. (1996): Dekorativna dendrologija. Beograd, Srbija; Naučna knjiga.

Vukotić-Lazar, J., Popović, S. (2016): Organized proviion of greenery in Belgrade in order to upgrade the quality of life within the city. Agriculture and Forestry 62 (4): 253-266, DOI 10.17707/agriculturforest.62.4.26.

White, R. (2004): Interaction with nature during the middle years: Its importance in children's development and nature's future. Retrieved October 28..

DOI: 10.17707/AgricultForest.63.2.13

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LENGTH-WEIGHT RELATIONSHIP OF NINE FISH SPECIES FROM BOSNIA AND HERZEGOVINA

SUMMARY

This study provides data on the length-weight relationships (LWR) of 9 fish species from Bosnia and Herzegovina. Also, paper provides first comprehensive data of LWRs for endemic *Alburnus neretvae* for which no LWR information was available in Fish Base. Values of b parameter ranged from 2.772 to 3.356, while values of a parameter ranged from 0.001-0.024.

Keywords: Adriatic drainage, linear regression, endemic species, fisheries

INTRODUCTION

Bosnia and Hercegovina inhabited by 118 species of freshwater fish. Within the recorded number of fishes, 17 of them stayed in brackish and salt waters (Sofradžija, 2009). Until now, there were no available data on LWRs of freshwater ichthyofauna representatives except for the *Squalius svalize* (Ivanković *et al*, 2010).

Length-weight (L-W) relationship is one of the most widely used methods in fisheries research and its importance has been well documented (Froese, 2006). The morphometric relationships between length and weight (LWRs) can be useful tool in environmental monitoring program such as in the calculation of fish weight at a certain length and the conversion of an equation of growth in weight and vice versa (Petrakis & Stergiou, 1995).

The aim of the present study was to report for the first-time length-weight parameters for 9 species from Bosnia and Hercegovina, including one endemic for which no estimates were available in Fish Base (Froese and Pauly, 2014).

MATERIALS AND METHODS

Samples were collected using multi mesh size gillnets (EN 14757 standard), from October of 2010th to August of 2014th. In total, it was analyzed 408 individuals from 9 species sampled on several localities and water bodies within Adriatic catchment of Bosnia and Herzegovina (South-eastern part of the country). Species were identified in the field, measured to the nearest 1 mm (total length, TL) and weighed to the nearest 0,1 g (weight, W). The following mathematical function was used for estimation of LWRs (Ricker, 1975):

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

$$W = aL^b ,$$

Where:
W is total body weight (in gr);
L is total length (TL) of body (in cm);
a and *b* are the coefficients of the functional regression between *W* and *L*.

The 95% confidence intervals (Cls) of the parameters and the statistical significance of the regression relationship (r^2) were estimated. The values of function parameters (*a* and *b*) were estimated by linear regression analysis based on log transformed equation log *w* = log *a* + *b* (log *l*) (Ricker, 1975). The determination coefficient (r^2) was used as an indicator of the quality of the linear regressions.

RESULTS AND DISCUSSION

The sample size, the minimum, maximum, and mean lengths and weights, the values of *a* and *b* with their respective 95% confidence limits and the coefficient of determination r^2 for each species are given in Table 1.

Table 1. Descriptive statistics and estimated parameters of LWR for 9 freshwater fishes from Bosnia and Herzegovina; *: indicates difference of *b* value from 3 (t-test; $p<0.05$)

			Length (cm)		Weight (g)		Regression parameters				
Species	Locality	N	Min	Max	Min	Max	*b*	95% Cl of *b*	*a*	95% Cl of *a*	r^2
Alburnus neretvae (Buj, Sanda & Perea,2010)	Trebišnjica river	75	6.9	13.2	2.7	22.4	2.940*	2.7244-3.1556	0.012	0.0061-0.0179	0.906
Carassius auratus (Linnaeus, 1758)	Lake Bilećko	30	10.3	36.4	12.8	812.3	3.356*	3.1012-3.6108	0.006	0.0021-0.0099	0.965
Gymnocephalus cernua (Linnaeus, 1758)	Lake Bilećko	55	3.8	13.2	0.5	25.3	3.052*	2.9285-3.1759	0.009	0.0070-0.0109	0.978
Lepomis gibosus (Linnaeus, 1758)	Lake Billećko	36	3.9	9.6	0.8	12.1	2.772	2.4760-3.0679	0.024	0.0102-0.0377	0.908
Oncorhynchus mykiss (Walbaum, 1792)	Trebišnjica river	25	12.2	43.7	12.7	705.4	3.118*	2.8946-3.3414	0.001	-0.0029-0.0049	0.972
Rutilus rutilus (Linnaeus, 1758)	Lake Bilećko	80	9.2	25.5	8.6	221.4	3.191*	3.1224-3.2596	0.007	0.0050-0.0089	0.991
Salmo obtusirostris (Heckel, 1851)	Buna river	43	11.5	38.1	22.8	354.5	2.953	1.7574-4.1486	0.015	0.0091-0.0209	0.983
Squalius svalize (Heckel & Kner,1858)	Trebišnjica river	44	14.4	44.1	26.6	989.2	2.849*	2.81176-2.8862	0.019	0.0014-0.0366	0.905
Tinca tinca (Linnaeus, 1758)	Trebišnjica river	20	12.7	29.2	29.4	451.2	2.978*	2.8016-3.1544	0.017	0.0072-0.0268	0.986

The *b* values ranged from 2.772 for *Lepomis gibossus* to 3.356 for *Carassius auratus*, while values of *a* parameter ranged from 0.001-0.024. For four species, the *b* values were higher than 3 (t-test; $p<0.05$), for three cases the b values were lower than 3 (t-test; $p<0.05$), while for the one species the *b* values of the L-W relationships were close to 3 and did not differ significantly (Table 1).

Results of this study are in accordance with Froese (2006), who reported that *b* values for teleost fish should fall within the expected range of 2.5 and 3.5. Differences in LWRs of fishes may be attributed to several factors, such as number and length range of the sampled specimens, gonad maturity, sex, diet, stomach fullness, and growth phase (Bagenal & Tesch, 1978; Froese, 2006; Wootton, 1990); these factors, however, were not consider in the present study. Froese (2006) demonstrated through a meta-analysis involving LWR data of 1773 species that 90% of the intercept values ranged between 0.001 and 0.05. In our study, all species showed *a* values within the range presented by Froese (2006). We found negative allometric growth for *Squalius svalize* (*b* - 2.849). These results differ from the available results of Ivanković *et al.* (2010) who reported positive allometric growth (*b* – 3.47). For *Salmo obtusirostris* we found that in river Buna it showed almost isometric growth (*b* - 2,953) while Treer *et al*. (2003) reported negative allometric growth for translocated population from Jadro river (*b* - 2,750). This species is endemic to relatively small region of Eastern Adriatic drainage and data about this species are very scarce. Therefore, reported data are very important for further knowledge acquiring about this rare trout species. It is noteworthy that for endemic *Alburnus neretvae* the data represent the first description of LWRs based on Fish Base (Froese and Pauly, 2014).

CONCLUSIONS

In conclusion, our study provides valuable information for the FishBase database and contributes to fishery research, management and conservation of freshwater fish fauna of Trebišnjica and Buna river which are either under heavy impact of hydro plants (Trebišnjica river) or will be in close future (Buna river). Such dramatic impact on river habitat causing or will cause habitat changing (riverine to artificial lake habitat) which could effect in changing of reported L-W relationship of affected populations.

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REFERENCES

Bagenal, T.B. & Tesch, F.W. (1978): Age nad growth. In: T. B. Bagenal (Ed) Methods for the assessment of fish production in fresh waters. Blackwell Scientific Publication, Oxford, pp. 101-136.

Froese, R. (2006): Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendation. Journal of Applied Ichthyology 22, 241-253.

Froese, R. & Pauly, D. (2014): FishBase. World Wide Web electronic publication. Available at: http://www. Fishbase.org (accessed on 04.2015)

Ivanković, P., Treer, T., Piria, M & Knezović, Z. (2010): Diet and growth of endemic Neretva chub Squalius svallize from the Neretva river area, Bosnia and Herzegovina. Folia Zoologica 59, 51-56.

Petrakis, G. & Stergiou, K.I. (1995). Weight-length relationships for 33 fish species in Greek waters. Fishery Research 21, 465-469.

Ricker, W.E. (1975): Computation and interpretation of biological statistics of fish populations. Bulletin Fisheries Research Board of Canada, 191, 1-382.

Sofradžija, A. (2009). Freshwater fishes of the Bosnia and Herzegovina. Council of Bosnian Intelectuals Congres, (In Bosnian), Sarajevo. 353 p.

Treer, T., Aničić, I., Safner, R., Odak, T. & Piria, M. (2003): Note on the growth of endemic soft-muzzled trout Salmothymus obtusirostris translocated into a Dalmatian river. Biologia, Bratislava, section Zoology 58, 999–1001.

Wooton, R.J. (1990): Ecology of teleost fishes. Chapman & Hall, London, pp.404

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MUTAGEN DEPRESSION AFTER RECURRENT CHEMICAL MUTAGEN ACTION AT FIRST WINTER WHEAT GENERATION

SUMMARY

The strategy of investigation combined the effects of mutation depression evident at first generation on cell and plant level and peculiarities of recurrent mutagen action. The main purposes of investigations in this area were determination genotype-mutagen interaction for modern Ukrainian winter wheat varieties, identify less sensitive for DAB (1,4-bisdiazoatsetilbutan) genotypes and evolution recurrent mutagenesis as a method in difference alteration on first step of mutation breeding program, which limited next stages by quantity of material for selection. Here we report cytogenetic, plant growth and development characteristics of mutation induction variability of the new wheat varieties and some relationships between means of plants grows and developments, morphometrical parameters, cytogenetic characteristics and different concentrations and types of mutagens at first generation after DAB treatment.

Keywords: chemical mutagenesis, winter wheat, chromosomal aberrations, 1,4-bisdiazoatsetilbutan,

INTRODUCTION

DAB (1,4- bisdiazoatsetilbutan) as a mutagen is not so widely used for breeding practice as previous investigated in our experiment mutagens (gamma-rays and nitrosoalkylureas), but it more suitable for reverse genetic approach in modern functional genomics and for programs for genetic improving indigenous cultivars in South-East Asia (FAO-IAEA programs for coordinating investigations in chemical mutagens) (Shu *et al*, 2011). DAB as a mutagen factor is traditionally related to radio-mimetic chemical mutagens substances due to similarity of mutagen effects appearance amid this mutagen and physical mutagens like as gamma- and x-rays.

Mutagen effects on cell and whole plant level are the key factors which limited either winter wheat productivity for agricultural purpose or number of

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families for breeding program in obtaining next generation material for identification and selection of mutants. Consequences of mutagen action on cell level (chromosomal aberrations) are closely connected with future mutation rate. Influence of mutagen factors action is depended on next parameter: physiological parameter of mutagen action object, genotype of object, type of mutagen action (acute, chronicle), nature of mutagen, doses or concentration of mutagen, fractional of dose or concentration, time of exposure, concentration or appearance of free active oxygen, temperature and other environmental conditions (Zhang et al, 2015; Nazarenko, Kharytonov, 2016).

This article is a part of our investigation of recurrent mutagen treatment of winter wheat varieties. In previous parts we developed effects of genotype-mutagen interaction after gamma-ray irradiation and nitrosomethilurea (NMU) nitrosoethilurea (NEU) (Nazarenko, 2017a; Nazarenko, Izhboldin, 2017)

Recurrent mutagenesis includes the exposure to mutagen action of progeny of plants that had been treated in previous generation. The strategy of treating the progeny of previously treated plants is well-known as recurrent action. Investigators studied a wide range of mutagens including different types of physical mutagens (different types of radiation) and the chemical mutagen (EMS); the alternation of EMS with irradiation was also studied. The results of these experiments did not bear out the expected results and were at best mixed. In most cases, radiosensitivity, mutation rate and spectra remained unaffected with repeated irradiation of subsequent generations. In our investigations we used other types of chemical mutation factors (nitrosoalkylureas, DAB) and alteration these mutagens with gamma-rays. We obtained new results according to reduce radiosensetivity, mutagen depression after recurrent mutagenesis and determined some new laws for recurrent mutagen action. In case of mutagen alteration we ran on with trivial, normal reaction on mutagen action (Chaima *et al*, 2012, Nazaenko, 2017b).

DAB are related to special group of mutagens “supermutagens” (as classified by Rapoport). Special ability of this group is induction mutations on level of comparable mutagen without high damages, which influence on survival ability of plant material (Jovtcheva et al, 2002; Özel et al, 2015). Supermutagens induct 50-60 times more mutations than relevant by their consequences for surviving and plant development doses of gamma rays or fast neutrons (Albokari, 2014). But DAB in spite of previous chemical mutagens (nitrosoalkylureas), by its action more similar to physical mutagens (like as gamma-rays) than for other chemical mutagens and don’t so site-specific.

Other feature (general for all chemical mutagens) is induction of gen mutations on peculiar DNA-sequence rather than structural changes. It is depends on chemical nature of specific mutagen. That’s why chemical mutagenesis is one of the important methods for modern genetics investigations (as for example for reverse genetics, for different types of tilling’s methods). We can predict (in certain limits) more probably types of future mutations with higher rates (according to preferable DNA sequences for mutagen action) (Juchimiuk-

Kwasniewska, 2002; Natarajan, 2005).

Mutagenic effects of chemicals have been assessed by both analysis of chromosomal aberrations (Rakhmatullina and Sanamyan, 2007) and investigation plant development and grows at first generation under field conditions.

Parameters traditionally used to estimate the degree of plant injury in the M_1 generation are: 1. Seedling height, determined at a particularly stage soon after germination. 2. Root length, determined soon after germination in controlled environment conditions. 3. Emergence under field conditions or germination. 4. Survival under field or controlled environment conditions. 5. Number of florets, flowers or inflorescences per plant. 6. Number of florets or flower parts per inflorescence. 7. Number of seed set. 8. Number of seeds per plant (Khaled *et al*, 2016).

Mutated plants typically show reduced fertility, mainly caused by chromosomal changes during meiosis. Plant surviving, pollen fertility and yield structure were studied for identification of mutagen depression evident at first generation (Karthika and Subba, 2006; Nazarenko, 2017b).

Most of the observed effects in the M_1 generation are physiological. Plant injuries in the M_1 generation are indicative of the degree of the effects of mutagens on plants and can be determined quantitatively in various ways. Physical injury is commonly measured using such parameters as reductions in germinability of seeds, growth rates of seedlings, vigor, sterility and even lethality of plants.

Chromosomal abnormalities in irradiated mitotic cells range from breaks, through exchanges, laggards and anaphase bridges, dicentric and centric ring formations, terminal fragments with telomeric signal at only one end and interstitial fragments that appear as double minutes without any telomeric signals (Rakhmatullina and Sanamyan, 2007). For crops like wheat, individual tillers (side branches) originate from different cells of the embryo of the treated seeds. If an aberration occurs in one of these cells, it will be carried in the tiller developed from that cell (Bolzarn and Bianchi, 2006; Huaili *et al*, 2005; Shu *et al*, 2011).

The main purposes of investigations in this area were determination genotype-mutagen interaction for modern ukrainian winter wheat varieties, identify less sensitive for DAB genotypes and evolution recurrent mutagenesis as a method in difference alteration on first step of mutation breeding program, which limited next stages by quantity of material for selection.

MATERIALS AND METHODS

Seeds of (in brackets method of obtaining varieties or used mutagens) Favoritka, Lasunya, Hurtovina (irradiation of initial material by gamma rays), line 418, Kolos Mironovschiny (field hybridization), Sonechko (chemical mutagenesis, nitrosodimethilurea (NDMU) 0.005%) and Kalinova (chemical mutagenesis, DAB 0.1%), Voloshkova (termomutagenesis – low plus temperature at plant development stage of vernalizaion has been used as mutagen

factor) of winter wheat (*Triticum aestivum* L.) were subjected to chemical mutagen 1,4- bisdiazoatsetilbutan (DAB) – 0,1 and 0,2 % presoaked. Each treatment was comprised of 1000 wheat seeds. Exposition of chemicals mutagens was 18 hours. These concentrations and exposure are optimal for the breeding process that has been repeatedly established earlier. (Ahloowalia *et al*, 2004; Nazarenko, 2016). Non-treated varieties were used as a check for each variety.

Treated seeds were sown in rows with inter and intra-row spacing of 50 and 15 cm, respectively, to raise the M_1 population. M_1 plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval. Data on seed germination and surviving plants were recorded considering whole plots of M_1 population. Data on yield structure components (plant height, general number of culms, number of productive culms, spike length, spikelets per spike, number of grain per spike, grain weight per spike and plant, 1000 grains weight) were taken from 50 randomly selected plants of each treatment representing more or less all types of morphological plants (Sanamyan *et al*, 2010).

The seeds used in this study were of the M_0 generation. After mutagen treatment dry seeds were germinated in Petri dishes under 24 – 72 hours (depends on presoaking and mutagen action), temperature $+25^0$C. After wards central primary roots were cut and fixed in solution of alcohol and acetic acid (in proportion 3:1) for 24 hours. Fixation material was stored in 70% alcohol solution under temperature 2 ^{0}C (20 – 25 roots per variant). Cytological analysis was carried out by the standard method at temporary press-time preparations of root tips (1 – 1.5 mm) stained with acetocarmine (has been prepared by Remsderh). Tissue maceration (if it needs for analysis) was carried out at 45% solution of acetic acid (during 5 minutes on bane-marie under 60^0C). Anaphase of cell division was observed by light microscope JNAVAL. No less than 900 cells in proper phases of mitosis were observed in each variant, number of samples were about 20 – 25 per variant (Lifang *et al*, 2001; Rank *et al*, 2002; Natarajan, 2005; Nikolova *et al*, 2015).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by ANOVA. Used the standard tools of the program Statistica 8.0 for factor analysis (ANOVA module).

RESULTS AND DISCUSSION

Analysis of grows and development of plants

In M_1 population, observations were recorded seed germination and plant surviving, pollen fertility, plant height, spikes/plant, spike length, kernels/spike, 1000-grain weight, yield/plant (table 1 – 2). Standard error (±SE) values of the treated populations are at tables too.

The results on germination of seeds, survival rate of plants derived from treated and untreated seeds are tabulated (Table 1). Germination and survival

abilities of seeds reduce compared to untreated seeds of the initial variety in all cases.

Germination and survival abilities of seeds reduce compared to untreated seeds of the initial variety in all cases except one (Hurtovina, DAB, 0.1 %). Plant survival ability ranges from 85 (Favoritka) to 80% (Kolos Mironivschini, Hurtovina) at 0.2% DAB, while it ranged from 98 to 92% under untreated control. Some varieties have been shown this parameter without significant difference from difference concentration (between 0.1 and 0.2 – Kalinova, Sonechko, Favoritka, Lasunya).

In general, the correlation between the concetration value and survival abilities of plants is on least level from other mutagens (-0.5).

Table 1. Main parameters of grown of winter wheat plants at M_1 generation

Trial	Germination, %	Survival after winter, %	Germination, %	Survival after winter, %
Variety	Kolos Mironivschini		Kalinova	
Check	98±0.57	91±0.93	94±0.94	88±0.98
DAB, 0.1%	85±1.03*	84±0.96*	84±0.51*	83±0.68*
DAB, 0.2%	82±0.63*	80±1.01*	81±0.79	81±0.78
Variety	Voloshkova		Sonechko	
Check	92±0.57	87±0.93	94±0.94	89±0.98
DAB, 0.1%	84±0.96*	84±1.10*	84±1.02*	84±1.10*
DAB, 0.2%	80±0.74*	79±0.90*	84±1.06	83±1.10
Variety	Favoritka		Hurtovina	
Check	98±0.57	91±0.93	92±0.94	84±0.98
DAB, 0.1%	84±0.92*	84±0.98*	85±0.71*	84±1.10
DAB, 0.2%	86±0.98	85±1.11	81±0.86*	80±1.10*
Variety	Lasunya		Line 418	
Check	98±0.57	94±0.93	93±0.94	92±0.98
DAB, 0.1%	86±1.11*	86±0.90*	89±1.14*	88±1.10*
DAB, 0.2%	84±1.70	83±1.16	85±0.61*	84±1.09*

* - difference is statistically significance from check at $P_{0.05}$

Correlation between the concentration of mutagens and pollen fertility was -0.68 (table 2). It was significantly lower in comparison to gamma-ray and nitrosoalkylureas. For some varieties such as Favoritka, Hurtovina, Lasunya there were not any statistically difference between DAB 0.01 and untreated check. As we can see from these tables parameters of surviving and pollen fertility are not so responsible on mutagen action (in spite of previous mutagens) and, as a consecutive, only partly suitable for evolution mutagen depression in case DAB.

Table 2. Pollen fertility after mutagen action, %

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Check	95.0	93.1	89.7	96.7	95.7	98.6	96.8	93.0
DAB, 0.1%	95.4	90.0*	73.4*	90.5*	93.1	95.9	94,2	89.7*
DAB, 0.2%	88.3*	88.7*	70.0*	88.8*	89.4*	90.8*	91.5*	88.1*

* - difference is statistically significance from check at $P_{0.05}$

All parameters of the crop yield structure have been studied. Components such as plant height, 1000 grain weight, grain weight per plant, number of grains per spike, grain weight per spike, general number of culms, number of productive culms, spike lengths have been developed. Only three (plant height, grain weight per spike, and 1000 grain weight) showed statistically difference level of mutagen depression under any concentration action. But for this type of mutagen sometimes we observed luck of mutagen depression under DAB 0,1 and (for one variety) DAB 0.2, in spite of other mutagens (Nazarenko and Kharytonov, 2016; Nazarenko, 2017b).

Regarding the plant height, correlation between the concentration and the indicator constituted -0.68, (high invert correlation). This parameter decreases if the concentration increases. Gradual decrease in height is a tendency, but we ran on with substantial differences between the varieties. Variety Kalinova has not been shown depression effect under any concentration of DAB.

The indicator of grain weight per spike was not informative in case of DAB, 0.1 %. The correlation coefficient was -0.52. It was reliable only under DAB 0.02 % concentration.

The thousand grain weight is the most reliable for mutagen depression evalution (similar as other mutagens). We observed depression in all variants except Kalinova, DAB 0.1 The correlation coefficient was -0.71.

Variety Kalinova are less sensitive to DAB as mutagen compared with other genotypes for all variants.

Table 3. Correlation between DAB concentration and some components of yield structure of M_1 varieties.

Parameter	Plant height	Number of culms	Spike lengths	Number of spikelets	Number of grains per spike	Grain weight per spike	Grain weight per plant	1000 grain weight
Concentr.	-0.68	-0.20	0.06	-0.08	-0.42	-0.52	-0.41	-0.71

Chromosomal aberrations analysis

At table 4 we represent dates of the results of next parameters analyzed: general number of observing mitosis in primary roots tips, number of cells in appreciate phase with visible chromosomal aberrations rearrangements, total rate of chromosomal aberrations. As we can see from table 1 frequencies of aberrations were changed from 3,3 % (Kalinova, DAB 0,1 %) to 14,2 % (Voloshkova, DAB 0,2 %) percent from total number of cells in division in experimental microscope samples. All the variants are statistically substantially dissimilar from each other and from the check.

Lower frequency of aberrations in any cases peculiar to varieties obtained by chemical mutation breeding (Sonechko, Kalinova).The higher frequency of aberrations has been obtained by used DAB 0,2 as usual.

Rates of chromosomal aberrations was statistically lower when we used DAB for varieties obtained with chemical mutagenesis. The same situation we observed in case of varieties Kalinova and Sonechko, when NMU and NEU have been used in our previous investigations. Therefore, we can affirm DAB as mutagen by the nature not so depends on object genotypes as nitrosoalkylureas and less damaging mutagens with lower rates. Varieties Sonechko and Kalinova are less sensitive to DAB action. DAB as a mutagen initiated least rates of chromosome damages than nitrosoalkylureas or gamma-rays (Nazarenko, 2016). We can range mutagens in next sequence by its genetic activity (from least to pick) DAB → NEU → NMU → gamma-rays.

From the Table 1 we can see that the higher rates of chromosomal changes in any cases characteristic for variety obtained by mutation breeding with using of thermomutagenesis (Volochkova). All other varieties (field hybridization and after irradiated) were similar by its reaction on DAB – rate of chromosomes rebuildings on 9 – 10 percent. According to this fact recurrent mutagenesis is acceptable method for exploit DAB as a mutagen if we exploit physical mutagens or classical breeding methods for obtaining objects of action (for example DAB after gamma-rays in our pattern or vice versa).

We developed next types of aberrations of chromosomes after investigation of spectra in our samples: chromosomal bridges and double-bridges, fragments of chromosomes and double-fragments, micronucleus, lagging chromosomes. Cases with complicated aberrations (two or more kinds of changes in one mitosis) and ratio fragments till bridges were counted up singly (Table 5). Number of any type of chromosomal changes was leaped with dose ascended (correlation coefficients is on 0,6 – significantly lower than for other mutagens). Previously we observed this evident in our investigations, when more bridges than fragments have been induced with gamma-rays (fragments-bridges ratio lower than 1) too (Nazarenko, 2017a). In this case, like as nitrosoalkylureas, more fragments and double-fragments were caused by DAB (fragments-bridges ratio more than 1) (Nazarenko and Izhboldin, 2017)). We will be able to use this parameter for identify difference between gamma-rays action and chemical mutagenesis in case of unknown mutagen factor.

Table 4. Frequency of chromosomal aberrations in M_1 generation of winter wheat varieties

Variable	Mitosis, number	Chromosomal aberrations		Mitosis, number	Chromosomal aberrations	
		n.	%		n.	%
	Favoritka			Line 418		
Check	984	19	1.93±0.31	962	11	1.14±0.11
DAB, 0.1%	1048	139	5.92±0.69*	906	106	4.01±0.64*
DAB, 0.2%	934	179	10.13±1.03*	983	188	8.99±0.88*
	Lasunya			Hurtovina		
Check	1056	15	1.42±0.19	1034	12	1.16±0.11
DAB, 0.1%	1019	121	5.52±0.69*	1005	143	6.00±0.74*
DAB, 0.2%	844	161	10.19±1.06*	1022	223	11.17±1.05*
	Sonechko			Voloshkova		
Check	1026	8	0.78±0.04	1003	31	3.09±0.34
DAB, 0.1%	1027	56	5.78±0.33*	1002	142	7.99±0.80*
DAB, 0.2%	981	108	8.64±0.51*	912	207	14.20±1.11*
	Kalinova			Kolos Mironivschini		
Check	1047	9	0.86±0.11	909	10	1.10±0.13
DAB, 0.1%	1009	106	3.30±0.14*	1016	129	5.78±0.73*
DAB, 0.2%	851	133	7.60±0.43*	917	190	10.26±1.02*

* – difference statistically significant on $P_{0,01}$

Number of complicated (or combined) aberrations was significantly lower as well as micronucleus and lagging chromosomes then for previous mutagens. Generally, when concentration of DAB was increased the rate of fragments and bridges also has increased. But other situation was for other types. We couldn't observe these types for variety Kalinova at all, only fragments and bridges. For line 418 complicated aberrations are also absent, but present other types. For varieties Favoritka, Lasunya, Hurtovina (all varieties are radiomutants) in some variants under DAB action complicated aberrations are absent too. On the other hand micronucleus, lagging chromosomes are absent for varieties Sonechko and Hurtovina.

The results of three-factor analysis ("genotype", "dose (concentration)" and "mutagen"; in general scheme of analyze we include our date from previous investigation of chemical mutagens and gamma-rays action (Nazarenko and Kharytonov, 2016) shown us that, prevalently, on the rate of chromosome aberrations factor "dose" influenced, then "genotype", then the "mutagen". The part of second and third factors are increased as compared with previous investigations. Thus, we developed that repeated exposure to the similar mutagen

(for example, DAB on the variety obtained by the action of this mutagen) leads to a substantially lower rate of chromosomal aberrations, absence some types of rarely aberrations in spectra.

CONCLUSIONS

The most informative parameters to determine the degree of mutagenic depression in the first generation for plant growth and development were germination and survival rates, pollen sterility, 1000 grain weight. But these parameters are partially suitable for mutagen depression evolution in case of DAB. The least level of mutagen depression by morphometrical indicators we observed in case of Kalinova. Therefore, chemical mutation varieties are less sensible for to same chemical mutagens both either for DAB or for nitrothoalkylureas

DAB as a mutagen substantially lower in chromosomal aberration induction in comparison with previous mutagens (gamma-rays and nitrosoalkylureas). We ranged mutagens in next sequence (from least to pick) DAB → NEU → NMU → gamma-rays. We can predict less quantity of mutations if we use DAB for mutation breeding purpose.

Chemical mutations varieties Kalinova and Sonechko are less sensitive to recurrent mutagenesis with this mutagen. In spite of other chemical mutagens DAB was less peculiar in genotype-mutagen interaction by the level of chromosomal rebuildings, but more peculiar in spectra of aberrations. Complicated (or combined) aberrations haven't been observed for Kalinova and we have not developed micronucleus, lagging chromosomes after DAB action for Kalinova and Sonechko.

Comparing between bridges and fragments after DAB action confirmed reliability of fragments-bridges ratio (prevalence of fragments under bridges for chemical mutagens and opposite situation for gamma-rays) for mutagen nature identification. In general, the rate of any kind of chromosomal aberrations is linearly increased with increase dose of the mutagen.

Recurrent mutagenesis is acceptable method for exploit DAB as a mutagen if we exploit physical mutagens or classical breeding methods for obtaining objects of action.

REFERENCES

Albokari M., 2014. Induction of mutants in durum wheat using gamma irradiation. In: Pakistan Journal of Botany, 46, 317–324.

Bolzarn, A. D., Bianchi, M. S., 2006. Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations. In: Mutation Research, 612, 189 – 214.

Chaima, S., Girard, L., Ezzeddine, F., Pascale, G., 2012. Exposure of Vicia faba to sulcotrione pesticide induced genotoxicity. In: Pesticide Biochemistry and Physiology, 103, 9–14.

Jovtcheva, G., Stergiosa, M., Schubert, I., 2002. A comparison of N-methyl-N-nitrosourea-induced chromatid aberrations and micronuclei in barley meristems using FISH techniques . In: Mutation Research, 517, 47–51.

Juchimiuk-Kwasniewska, J., Brodziak, L., Maluszynska, J., 2011. FISH in analysis of gamma ray-induced micronuclei formation in barley. In: Journal of Applied Genetics,

Huaili Q., Lanming X., Fei H., 2005. Biological effect of the seeds of Arabidopsis thaliana irradiated by MeV protons. In: Radiation Effects & Defects in Solids, 160, 131–136.

Karthika I R., Subba B., 2006. Effect of Gamma Rays and EMS on Two varieties of Soybean. In: Asian Journal of Biological Sciences, 5, 721–724.

Khaled, H., Anderson, D., Brinkworth, M., 2016. Detection of phase specificity of in vivo germ cell mutagens in an in vitro germ cell system. In: Toxicology, 16, 1–10.

Lifang Y., Zengliang W., 2001. Radiobiological effects of a low-energy ion beam on wheat. In: Radiat Environ Biophys, 40, 53–57.

Natarajan, A.T., 2005. Chromosome aberrations: Plants to human and feulgen to FISH. In: Current Science 89, 335-340.

Nazarenko, M., Kharytonov, M., 2016. Characterization of wheat mutagen depression after gamma-rays irradiated. In: Agriculture and Forestry, 62, 4, 267–276.

Nazarenko, M., 2017. Specific Features in the Negative Consequences of a Mutagenic Action. In:Russian Journal of Genetics: Applied Research, 7, 2, 195–196.

Nazarenko, M., Izhboldin, O., 2017. Chromosomal rearrangements caused by gamma-irradiation in winter wheat cells. In: Biosystems Diversity. 25, 1, 25 –28.

Nazarenko, M., 2017. Influence of nitrosoalkylureas on winter wheat plants at first generation after mutagen action. In: Agriculture and Forestry, 63, 1, 319–328.

Nikolova, I., Georgieva, M., Kruppa, K., Molnor-Long, M., Liu, L., Manova, V., Stoilov, L., 2015. Cytogenetic effects in barley root apical meristem after exposure of dry seeds to lithium ion beams. In: Genetics and Plant Physiology, 5, 3 – 9.

Özel, H.B., Kirdar, E., Bilir, N. 2015. The effects of magnetic field on germination of the seeds of oriental beech (Fagus orientalis Lipsky.) and growth of seedlings. Agriculture and Forestry, 61 (3): 195-206.

Rakhmatullina, E.M., Sanamyan, M.F., 2007. Estimation of efficiency of seed irradiation by thermal neutrons for inducing chromosomal aberration in M_2 of cotton Gossypium hirsutum L. In: Russian Journal of Genetics 43(5), 518-524.

Rank, J., Lopez, L.C., Nielsen M.H., 2002. Genotoxicity of maleic hydrazide, acridine and DEHP in Allium cepa root cells performed by two different laboratories. In: Hereditas 136, 13–18.

Sanamyan, M. F., Petlyakoval, J. E., Sharipoval, E. A., Abdurakhmonov, I. Y., 2010. Morphological characteristics and identification of new monosomic stocks for cotton (Gossypium hirsutum L.) In: Advances in Bioscience and Biotechnology 1, 372-383.

Shu, Q.Y., Forster B.P., Nakagava H., 2011 Plant Mutation breeding and Biotechnology, Vienna, CABI publishing.

Zhang, J., Jiang, Y., Guo, Y., Li, G., Yang, Z., Xu, D., 2015. Identification of Novel Chromosomal Aberrations Induced by 60Co-γ-Irradiation in Wheat-Dasypyrum villosum Lines. In: International Journal of Molecular Sciences, 16, 29787–29796.

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PRESOWING SEED TREATMENT METHODS TO OVERCOME DORMANCY IN SEEDS OF *VACHELLIA REHMANNIANA* SCHINZ

SUMMARY

A germination experiment of *Vachellia rehmanniana* seeds was conducted in the laboratory of the Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources from November to December 2016. Mature *Vachellia rehmanniana* seeds were collected from healthy erect trees at Butale Village, Botswana to investigate the effect of different pre-sowing treatment methods on seed germination. The experiment was laid out in a completely randomized design (CRD) with five treatments (control, mechanical scarification, boiling water, hot water and concentrated sulphuric acid (98.8%)). Boiling water had three different levels of time exposure (1, 3 and 5 min) whereas, concentrated sulphuric acid had four different levels of time exposure (15, 30, 45 and 60 min). The results revealed that seed germination percentage, germination mean time and germination index were significantly ($P < 0.01$) affected by pre-treatment methods. The highest significant cumulative germination percentages were recorded in seeds subjected to boiling water for 3 and 5 min, sulphuric acid for 45 and 60 min, and mechanical scarification. Based on the findings mechanical scarification and boiling water techniques are recommended for use in nurseries and by farmers because sulphuric acid is expensive and need to be handled by trained individuals. It is recommended that future research should target increasing the exposure time over five and 60 minutes for boiling water and sulphuric acid treatments, respectively for this species to increase the cumulative germination percentage.

Keywords: *Vachellia rehmanniana*, pre-treatment, seed dormancy, germination percentage.

INTRODUCTION

The *Vachellia*, formerly known as *Acacia* (Kyalangalilwa *et al*., 2013) is a genus of shrubs and trees that belong to the subfamily Mimosoideae of the family Fabaceae (Palgreaves, 1983). They are widely distributed in arid and semi-arid

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regions where they evolved to withstand harsh climatic and environmental conditions (Timberlake, 1980). The genus *Vachellia* is one of the most important tree components of the flora of southern Africa (Timberlake, 1980). *Vachellia rehmanniana* Schinz is very important source of construction wood, firewood (Timberlake, 1980; Hanaoka *et al.*, 2014; Missanjo *et al.*, 2014) and non-wood products (Kassa *et al.*, 2010), such as medicines (Timberlake, 1980; Ali *et al.*, 2012; Missanjo *et al.*, 2014), edible gum and fodder (Hanaoka *et al.*, 2014; Missanjo *et al.*, 2014). In addition, some are nitrogen fixers (Missanjo *et al.*, 2014) and have been used in agroforestry systems (Azad *et al.*, 2011) and soil conservation projects.

Vachellia rehmanniana Schinz, commonly known as silky thorn (Timberlake, 1980; Palgrave, 1983), is a small thorny tree or shrub that grows to 5 m high (Timberlake, 1980), occasionally reaching 10 m (Timberlake, 1980; Palgrave, 1983). It is deciduous with a flat spreading crown (Palmer and Pitman, 1972; Timberlake, 1980). Leaves are yellow-green, neat slender, composed of 15-40 pairs of slender pinnae, each with many small leaflets (Palmer and Pitman, 1972; Palgrave, 1983). Flowers are in round greenish-white, fragrant balls grouped at the ends of young branches (Palmer and Pitman, 1972; Timberlake, 1980; Palgrave, 1983) appearing before or at the same time with leaves between October and December (Timberlake, 1980). Fruits are straight, dehiscent, aligned and twisted hairless flat pods (Timberlake, 1980; Palgrave, 1983), greyish to olivine in colour (Palgrave, 1983). The species occurs in southern Zambia, Zimbabwe, north eastern Botswana and South Africa, particularly in the Limpopo province (Timberlake, 1980). It grows in wooded grasslands, along river banks and on termite mounds (Timberlake 1980; Palgrave, 1983). It is a drought tolerant multipurpose tree species which provides firewood, construction wood, many non-timber products that include fodder and several ecosystem services. The leaves, young shoots, flowers and pods are eaten by both livestock and wildlife (Tsumele *et al.*, 2007).

The most common and cheap method of raising a large number of plant seedlings is through seeds (Kim *et al.*, 2008). Seeds of many leguminous tree species have hard seed coat impervious to water (Teketay, 1996, 1998; Walters *et al.*, 2004), which exerts a physical exogenous dormancy (Falemara *et al.*, 2014). The hard seed coats hinder germination and limit their propagation (Karthika *et al.*, 2016) as well as their use in afforestation programmes (Botsheleng *et al.*, 2014). Seed germination is an important stage in the life cycle of plant species (Evren and Kucukoduk, 2012) because it determines their survival (Yang *et al.*, 2008). The hard seed coat of many forest species have evolved to withstand unfavourable conditions, such as intense heat from sunlight, dispersing animals, severe drought and physical damage (Tadros *et al.*, 2011). Seed dormancy is an adaptation strategy that ensures seeds germinate only when environmental conditions are suitable for the survival of germinates (Falemara *et al.*, 2014).

Vachellia species are characterized by a hard, seed coat which hinders uptake of water and air by the seed (Teketay, 2005; Aref *et al.*, 2011) to trigger

germination. For germination to take place, it requires the seed coat to rupture and allow the absorption of water by the seed. Seeds with a hard seed coat require some pre-treatments before sowing to break dormancy and obtain rapid and synchronous germination. Seed dormancy is referred to as a temporary failure of a mature viable seed to germinate when subjected to favourable germination conditions (Ibiang *et al.,* 2012). Numerous techniques have been used to break dormancy in many species with hard coats including species of *Vachellia* (Teketay, 1998, 2005; Botsheleng *et al.,* 2014; Rasebeka *et al.,* 2014). Different pre-sowing techniques, such as stratification, mechanical, acid and hot water scarification as well as tap water have been widely used (Teketay, 1996, 1998, 2005; Aref *et al.,* 2011; Tadros *et al.,* 2011; Missanjo *et al.,* 2014; Rasebeka *et al.,* 2014; Fredrick *et al.,* 2016) because they can improve germination within a relatively short period of time (Tadros *et al.,* 2011). As with the other leguminous species, seeds of *V. rehmanniana* have hard seed coats, which require pre-sowing treatment to overcome dormancy. However, there is complete lack of information about the pre-sowing methods for overcoming the seed coat-imposed dormancy in *V. rehmanniana*. The objective of this study was, therefore, to investigate the effects of different pre-sowing treatment methods on the germination of seeds of *V. rehmanniana.*

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the laboratory at the Botswana University of Agriculture and Natural Resources (BUAN) from November to December 2016. BUAN is located at Sebele, 23°34' S and 25°57' E with an altitude of 994 m above sea level, 10 km from the centre of Gaborone City, the Capital of Botswana along the A1 North-South highway.

Seed collection and processing

The pods *of V. rehmanniana* were collected directly from different healthy, erect and mature mother trees at Butale Village (North East District), eastern Botswana, in September 2016. Subsequently, the pods were placed in a paper bags and transported to the laboratory in BUAN where the seeds were extracted and mixed together. In the laboratory, seeds were screened to remove those showing some signs of damage, and about 4% of the seeds showed some signs of insect damage. The seeds were kept in a tightly sealed bottle and stored in a cool place awaiting commencement of the study. Prior to the experiment, seeds were immersed in distilled water, and only those that sank and settled at the bottom were used for the experiment. The seeds that floated, which represent unfilled and dead seeds, were discarded.

Seed characteristics

To assess the seed characteristics of *V. rehmanniana,* the three dimensions of the seeds, namely length, width and breadth of seeds were measured using an electronic digital caliper (0-150 mm), and their thousand seed weight were determined by weighing the seeds using an electronic analytical balance (Model:

PW 124). Five replications of 10 seeds and ten replications of 100 seeds were used to determine the mean dimensions and thousand seed weights of the seeds, respectively. The thousand seed weight of the seeds was, then, computed from the mean seed weight of the 100 seeds.

Experimental design and germination experiment

The experiment was laid out in a completely randomized design (CRD) with five treatments, i.e. mechanical scarification, boiling water, hot water, concentrated sulphuric acid (98.8%) and control. The boiling water treatment had three different levels of exposure time (1, 3 and 5 minutes) whereas the concentrated sulphuric acid treatment had four different levels of exposure time (15, 30, 45 and 60 minutes). Each treatment had 100 seeds replicated four times with 25 seeds in each replication. The seeds were germinated in petri dishes lined with cotton wool, which was kept continuously moist with distilled water.

Pre-sowing seed treatment procedures

In the mechanical scarification treatment, the seeds were scarified by carefully nicking the seed coat at the distal end by hand with a nail cutter. In the boiling water treatment, first seeds were enclosed in coffee filter papers and clipped tightly to avoiding any of them falling out and, then, immersed in boiling water for 1, 3 and 5 minutes. They were then removed from the boiling water and left to cool down on a table. Before the hot water treatment, water was boiled to up to approximately 100°C. Then, the simmering hot water (about 98.5 °C) was poured in a 100 ml heat resistant glass beaker containing the seeds, which was left to gradually cool for 24 hours at room temperature. For the sulphuric acid treatment, the method described by Botsheleng *et al.* (2014) was followed. Four hundred seeds were counted from the seed lot and then divided into batches of 100 seeds. The seeds were, then, put into four 100 ml heat resistant non-corrosive glass beakers and concentrated sulphuric acid (98.8%) was added slowly on the side of the beakers to a level where all seeds were covered (about 50 ml). Seeds in the four beakers were left in the sulphuric acid for different time periods (15, 30, 45 and 60 minutes), and they were stirred regularly to ensure equal exposure of the seeds to the acid. After each soaking period, the sulphuric acid was drained off, and the seeds were repeatedly rinsed in running tap water until they were considered safe to handle. The control consisted of seeds that did not receive any treatment.

Germination assessment

The counting of germinated seeds was done daily, and a seed was considered germinated when the radicle reached about 2 mm. The experiment was terminated after 21 days.

Data analyses

Data collected on the germination of seeds were used to calculate germination percentage, germination mean time and germination index for each treatment using the equations below.

Germination percentage (GP), which represents the number of germinated seeds as a percentage of the total number of tested seeds, was computed using the following formula:

GP (%) = (germinated seeds/total tested seeds) × 100

Germination mean time (GMT) and germination index (GI) were calculated as follows (Botsheleng *et al.*, 2014):

GMT (days) = $\Sigma T_i N_i/S$

where, T_i = number of days from the beginning of the experiment, N_i = number of seeds germinated per day and S = total number of seeds germinated.

GI = $(G_1/1) + (G_2/2) +...+ (G_x/x)$

where, G = germination day 1, 2…, and x = the corresponding day of germination.

To test if there were significant differences among treatment means, the data were further subjected to one-way analyses of variance (ANOVA) using Analytical Software (2003), following arcsine transformation of all percentage data. Significant differences of means were tested using Tukey's Studentized Range (HSD) at the significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Table 1. Effect of seed pre-treatment on mean germination parameters of *V. rehmanniana*.

Treatments	Cumulative mean germination (%)	GMT (days)	GI
Control	3.00^{d}	1.50^{c}	0.01^{c}
Mechanical scarification	69.00^{ab}	4.53^{abc}	0.82^{ab}
Boiling water			
1 min	24.00^{c}	6.88^{a}	0.22^{c}
3 min	73.00^{a}	4.56^{abc}	0.87^{a}
5 min	68.00^{ab}	4.54^{abc}	0.81^{ab}
Hot water-24 hrs	4.00^{d}	1.00^{c}	0.03^{c}
Sulphuric acid (98%)			
15 min	4.00^{d}	1.63^{bc}	0.03^{c}
30 min	24.00^{c}	6.42^{a}	0.21^{c}
45 min	52.00^{b}	6.27^{ab}	0.62^{b}
60 min	71.00^{ab}	4.32^{abc}	0.85^{ab}
Significance	**	**	**
HSD	19.99	4.76	0.23
CV (%)	21.13	47.33	21.28

** Highly significant at $P < 0.01$. Means separated using Tukey's Studentized Range (HSD) Test at $P \leq 0.05$; means within columns followed by the same letters are not significantly different. GMT = germination mean time and GRI = germination index.

The mean length, width and breadth of seeds were 7.1 ± 0.08, 6.3 ± 0.08 and 3 ± 0.06 mm, respectively, with minimum and maximum ranges of 6.7-7.5, 5.8 - 6.6 and 2.7-3.2 mm, respectively. The mean thousand seed weight of the seeds was 93.5 ± 0.95 g, ranging between 88.3 and 97 g.

The results indicated that seed germination percentage, germination mean time and germination index were significantly ($F_{(9, 30)} = 51.4$, $P < 0.01$) affected by pre-sowing treatment methods (Table 1). Overall, the highest cumulative germination percentages at termination of the experiment were recorded in seeds subjected to boiling water for 3 minutes (73%), followed by seeds subjected to sulphuric acid for 60 minutes (71%), nicking (69%) and seeds subjected to boiling water for 5 minutes (68%) (Figure 1). No statistical differences in germination percentages were observed among the above treatments. Results also show no significant differences ($P > 0.05$) in cumulative germination percentages between seeds soaked in boiling water (1 minute) and those soaked in sulphuric acid (30 minutes). The lowest cumulative germination percentages were recorded in the control (3%) followed by hot water (24 hours) and sulphuric acid (15 minutes).

Different pre-treatment methods have been used to break dormancy to stimulate prompt and uniform germination of seeds (Teketay, 2005; Sahoo *et al.*, 2007; Aref *et al.*, 2011; Botsheleng *et al.*, 2014; Rasebeka *et al.*, 2014; Frederick *et al.*, 2016). No single pre-treatment method has been reported to be effective across plant species (Amusa, 2011). Mechanical scarification has been shown to enhance germination in seeds of many species (Teketay, 1996, 1998, 2005; Likoswe *et al.*, 2008; Aref *et al.*, 2011; Olatunji *et al.*, 2013; Missanjo *et al.*, 2014). Present results show that mechanical scarification significantly ($P < 0.01$) improved germination compared with the control. These results demonstrated the effectiveness of mechanical scarification in overcoming physical dormancy, which is imposed by a hard seed coat that prevent the seed from taking up water, the first critical step in germination (Teketay, 2005; Chisha-Kasumu *et al.*, 2007). Chisha-Kasumu *et al.* (2007) recorded the highest rate of germination in mechanically scarified *Pterocarpus angolensis* DC. seeds within 5 days of sowing. Mechanical scarification cracks part of the hard seed coat which is a barrier to water uptake and gaseous exchange (Teketay, 2005; Azad *et al.*, 2011) and allow germination to proceed (Olatunji *et al.*, 2013). The results of the present experiment indicate that the seed dormancy found in *V.* rehmanniana is caused by physical rather than embryo dormancy.

Soaking seeds in boiling water (1, 3 and 5 minutes) was effective in improving germination compared with the control. However, seed germination percentages of seeds soaked in boiling water for 3 and 5 minutes were significantly higher ($P < 0.01$) than those soaked for 1 minute. Results indicated that soaking seeds in boiling water for a few minutes break their physical dormancy and allow their subsequent germination. The effectiveness of boiling water observed in this work could probably be attributed to the softening of the hard seed coat, which allowed entrance of water and air into the seed to induce

physiological changes (Teketay, 2005; Pahla *et al.*, 2014, Rasebeka *et al.*, 2014) that subsequently triggered germination (Rasebeka *et al.*, 2014). Present results are consistent with McDonald and Omoruyi (2003) who reported the highest seed germination (70%) in *Dialium guineense* Wild. seeds soaked in boiling water (100 °C) for 5 minutes. The fact that soaking seeds in boiling water for 1 minute was not as effective as the 3 and 5 minutes soaking durations could be attributed to the degree of the seed coat thickness. These results suggest that the seed coat thickness of V**.** rehmanniana requires soaking in boiling water for longer duration.

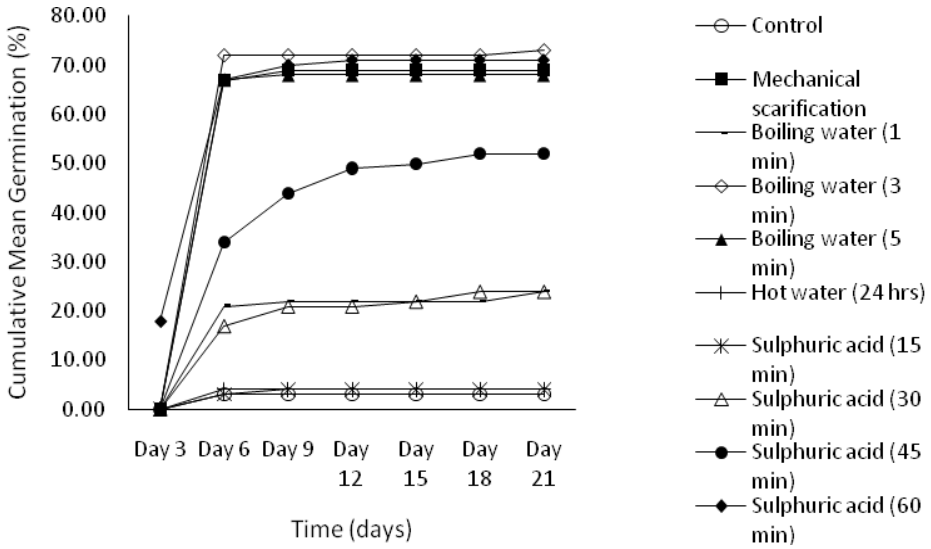

Figure 1. Cumulative mean germination (%) over time as influenced by presowing seed treatments.

Hot water treatment is frequently used to overcome dormancy in seeds with a hard coat. When using this method, seeds are soaked at 40-100 °C for a period of time, depending on the species and seed coat thickness or until the boiling water cools to ambient (Tadros et al., 2011). There was no significant difference in germination percentages between seeds soaked in hot water for 24 hrs and the control. These results are in agreement with studies conducted elsewhere using seeds of different tree species (Teketay, 1996, 1998; Botsheleng et al., 2014; Fredrick et al., 2016). The effectiveness of hot water in breaking seed dormancy varies from species to species (Tigabu and Oden, 2001; Teketay, 2005). For example, Albrecht (1993) reported that soaking seeds for 24 hrs in water boiled to 100 °C was effective in breaking the seed coat dormancy of *Adansonia digitata* L., *Calliandra calothyrsus* Meissner and *Sesbania sesban* (L.) Merr. Tadros et al. (2011) observed that soaking Leucaena *leucocephala* (Lam.) de Wit seeds in 70 °C water for 20 minutes was effective in breaking seed

dormancy and enhancing seed germination. The results indicate that V. rehmanniana has a very tough seed coat, which is in agreement with Aref (2000) who reported hard coats in other *Vachellia* species. The hard seed coat is one of the several strategies that the *Vachellia* species use to survive in the spatially and temporally variable environment (Aref, 2000). It is possible that the hot water in the present study cooled off before the hard seed coat was soft enough to allow entry of water and air.

Sulphuric acid (30, 45, and 60 minutes) improved seed germination compared with the control. This is consistent with results from other studies that reported prompt and uniform germination in hard water impermeable seed coated seeds soaked in sulphuric acid (Muhammad and Amusa, 2003; McDonald and Omoruyi, 2003; Keshtkar *et al.*, 2008; Likoswe *et al.*, 2008; Aref *et al.*, 2011). Seed germination in the present study increased with soaking period in sulphuric acid. Muhammad and Amusa (2003) recorded the highest germination in *Tamarindus indica* L. seeds soaked in sulphuric acid (50%) for 60 minute. Sulphuric acid disrupts the seed coat and expose the lumens of the macrosclereids cells, permitting imbibition of water, which triggers seed germination (Aliero, 2004; Amusa, 2011). The germination of seeds soaked in sulphuric acid for 15 minutes did not significantly differ from the control indicating that *V.* rehmanniana seeds are characterized by hard seed coat, which requires longer soaking periods in sulphuric acid to reduce its thickness.

The lowest mean germination durations (days) were recorded in seeds soaked in hot water for 24 hrs (1) followed by control (1.5) and seeds soaked in sulphuric acid for 15 min (1.63). These together with high coefficient of variation (47.33) revealed indicates that the three treatments had difficulties breaking the hard seed coat exhibited by *V.* rehmanniana recording no germination in almost all the replications. However, the rest of the treatments were evenly spread between 4.32 and 6.88 days with no statically differences. The few seeds which germinated as evidenced by the highest germination percentage (73%) after 21 days across the treatments germinated within a reasonable period. As would be expected, seeds subjected to boiling water for 3 and 5 minutes, sulphuric acid for 45 and 60 minutes and mechanical scarification had the highest germination index ranging from 0.6 to 0.9. As a ratio, this shows that treatments were superior to others.

CONCLUSION

Mechanical scarification, boiling water (3 and 5 minutes) and concentrated sulphuric acid (45 and 60 minutes) proved to be effective in improving seed germination in V. rehmanniana in the present study. However, mechanical scarification and boiling water methods are recommended for use in nurseries and by farmers because sulphuric acid is expensive and needs to be handled by trained individuals. Authors also recommend increased exposure time over 5 and 60 minutes for boiling water and sulphuric acid respectively.

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REFERENCES

Albrecht J. 1993. Tree seed handbook of Kenya. GTZ forestry seed centre, Muguga. Kenya Forest Research Institute, GTZ/KEFRI p 264

Ali A, Akhtar N, Khan BA, Khan MS, Rasul A, Khalid N, Waseem K, Mahmood T and Ali L. 2012. Acacia nilotica: a plant of multipurpose medicinal uses. *Journal of Medicinal Plants Research*, 6(9):1492-6

Aliero BL. 2004. Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa*. *African Journal of Biotechnology*, 3(3):179-181.

Amusa TO. 2011. Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers). *Journal of Horticulture and forestry*, 3(4):96-103.

Analytical Software. 2003. STATISTIX 8 for Windows. Tallahassee, Florida, US.

Aref IM, Atta HAE, Shahrani TA and Mohamed AI. 2011. Effects of seed pre-treatment and seed source on germination of five Acacia spp. *African Journal of Biotechnology*, 10(71):15901-15910.

Aref IM. 2000. Morphological characteristics of seeds and seedling growth of some native acacia trees in Saudi Arabia. *Journal of King Saud University, Agricultural Sciences*, 12(2):31-95.

Azad S, Manik MR, Hasan S and Matin A. 2011. Effect of different pre-sowing treatments on seed germination percentage and growth performance of *Acacia auriculiformis*. *Journal of Forestry Research*, 22: 183. doi: 10.1007/s11676-011-0147-y.

Botsheleng B, Mathowa T and Mojeremane W. 2014. Effects of pre-treatments methods on the germination of Pod mahogany (*Afzelia quanzensis*) and mukusi (*Baikiaea plurijuga*) seeds. *International Journal of Innovative Research in Science Engineering and Technology*, 3(1):8108-8113.

Chisha-Kasumu, E, Woodward, S and Price A. 2007. Comparison of the effects of mechanical scarification and gibberellic acid treatments on seed germination in *Pterocarpus angolensis. Southern Hemisphere Forestry Journal*, 69(1):63-70.

Evren Y and Kucukoduk M. 2012. Dormancy breaking and germination requirements for seeds of *Sphaerophysa kotschyana boiss. Journal of Global Biosciences*, 1: 20-27.

Falemara BC, Chomini MS, Thlama DM and Udenkwere M. 2014. Pre-germination and dormancy response of *Adansonia digitata* L seeds to pre-treatment techniques and growth media. *European Journal of Agriculture and Forestry Research*, 2(1):13-23.

Fredrick C, Muthuri C, Ngamau K and Sinclair F. 2016. Provenance and pre-treatment effect on seed germination of six provenances of *Faidherbia albida* (Delile) A. Chev. *Agroforestry Systems*, doi:10.1007/s10457-016-9974-3.

Hanaoka S, Nakawa N, Okubo N, Omondi SF, Kariuki J. 2014. Seed pre-treatment methods for improving germination of Acacia tortilis. *African Journal of Biotechnology* 13(50):4557-4561.

Holmes RJ, McDonald JNAW and Juritz J. 1987. Effects of clearing treatment on seed bank of the Alinene invasive shrubs *Acacia saligna* and *Acacia cyclops* in the southern and south western Cape, South Africa. *Journal of Applied Ecology*, 24: 1045-1051.

Ibiang YB, Ita EE, Ekanem BE and Edu, NE. 2012. Effect of different pre-treatment protocols on seed germination of Tetrapleura tetraptera (Schum and Thonn). *Journal of Environmental Science, Toxicology and Food Technology*, 2(3):25-29.

Karthika C, Mohamed RK and Manivannan S. 2016. Effect of different pre-treatment on *in vitro* seed germination and seedling development of *Senna Alata* Linn. *International Journal of Pharmaceutical Science and Research*, 7(5):2157-2162.

Kassa A, Alia R, Tadesse W, Pando V and Bravo F. 2010. Seed germination and viability in two African Acacia species growing under different water stress levels. *African Journal of Plant Science*, 4(9):353-359.

Keshtkar AR, Keshtkar HR, Razavi SM and Dalfardi S. 2008. Methods to break seed dormancy of *Astragalus cyclophyllon. African Journal of Biotechnology* 7(21):3874-3877.

Kim RE, Jung HH and Kim KS. 2008. Seed germination of *Carex neurocarpa maxim* is promoted by fluctuating temperatures and seed scarification. *Horticulture Environment and Biotechnology*, 49(3):162-167.

Kyalangalilwa B, Boatwright JS, Daru BH, Maurin O, and van der Bank M. 2013. Phylogenetic position and revised classification of *Acacia s.l.* (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. *Botanical Journal of the Linnean Societ*y, 172: 500-523.

Likoswe MG, Njoloma JP, Mwase WF and Chilima, CZ. 2008. Effect of seed collection times and pre-treatment methods on germination of Terminalia sericea Burch. Ex DC. *African Journal of Biotechnology*, 7(16): 2840-2846.

MacDonald I and Omoruyi, O. 2003. Effect of seed pre-treatment on germination of two surface types of *Dialium guineense*. *Seed Technology*, 25(1):41-44.

Missanjo E, Chioza A and Kulapani C. 2014. Effects of different pre-treatments to the seed on seedling emergence and growth of *Acacia polyacantha*. *International Journal of Forestry Research*, http://dx.doi.org/10.1155/2014/583069.

Muhammad S and Amusa NA. 2003. Effects of sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L). African Journal of Biotechnology, 2(9):276-279.

Olatunji DJ, Maku O and Odumefun OP. 2013. The effect of pre-treatments on the germination and early seedlings growth of *Acacia auriculiformis* Cunn. Ex. Benth. *African Journal of Plant Science*, 7(8):325-330.

Palgrave KC. 1983. Trees of Southern Africa. Struik, Cape Town, pp. 248.

Pahla I, Muziri T, Chinyise T, Muzemu, S and Chitamba J. 2014. Effects of soil type and different pre-sowing treatments on seedling emergence and vigour of *Acacia sieberana*. *International Journal of Plant Research*, 4(2):51-55

Palmer E and Pitman N. 1972. Trees of Southern Africa. A.A.Balkema, Cape Town, pp.783.

Rasebeka L, Mathowa T and Mojeremane W. 2014. Effect of seed pre-sowing treatment on germination of three Acacia species indigenous to Botswana. *International Journal of Plant and Soil Science*, 3(1):62-70.

Tadros MJ, Samarah NH and Alqudah AM. 2011. Effect of different pre-sowing seed treatments on the germination of *Leucaena leucocephala* (Lam.) and *Acacia farnesiana* (L.). *New forests*, 42(3):397-407.

Teketay, D. 1996. Germination Ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. *Forest Ecology and Management* 80: 209-223.

Teketay D. 1998. Germination of *Acacia origena*, *A. pilespina* and *Pterolobium stellatum* in response to different pre-sowing seed treatments, temperature and light. *Journal of Arid Environments*, 38(4): 551-560.

Teketay, D. 2005. Seed and regeneration ecology in dry Afromontane forests of Ethiopia: I. Seed production - population structures. *Tropical Ecology* 46: 29-44.

Timberlake J. 1980. *Botswana acacias*. Ministry of Agriculture. Gaborone, pp. 81.

Sahoo UK, Upadhyaya K and Lalrempuia H. 2007. Effect of pre-treatment and temperature on the germination behaviour of seeds of *Parkia roxburghii* G Don. *Forests, Trees and Livelihoods*, 17(4):345-350.

Tsumele J, Mlambo D and Sebata A. 2007. Response of three Acacia species to simulated herbivory in a semi-arid southern African savanna. *African Journal of Ecology*, 45(3):324-326.

Walters M, Midgley JJ and Somers MJ. 2004. Effects of fire and fire intensity on the germination and establishment of *Acacia karoo*, *Acacia nilotica*, *Acacia luederitzii* and *Dichrostachys cinerea* in the field. BMC. Ecology 4:3; doi: 10.1186/1472-6785-4-3.

Yang QH, Wei X, Zeng XL, Ye WH, Yin XJ, Zhang-Ming W and Jiang YS. 2008. Seed biology and germination ecophysiology of Camellia nitidissima. *Forest Ecology and Management*, 255(1):113-118.

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