University of Montenegro
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Biotehnički fakultet

Agriculture and Forestry
Poljoprivreda i šumarstvo

4

Agriculture and Forestry, Vol. 62. Issue 4: 1-288, Podgorica, 2016

ISSN 0554-5579; E-ISSN 1800-9492; DOI: 10.17707/AgricultForest
COBIS.CG-ID: 3758082 www.agricultforest.ac.me

Agriculture and Forestry - *Poljoprivreda i šumarstvo*
PUBLISHER - *IZDAVAČ*
University of Montenegro – Univerzitet Crne Gore
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Indexed in (by alphabethical order): AGRICOLA, AGRIS, CAB Abstracts, CAB Direct, CABI full text, COBISS, CrossRef, DOI, DOAJ, Directory of Research Journal Indexing (DRJI), Directory Indexing of International Research Journals, EBSCO (currently included in EDS EBSCO Discovery Service), Elektronische Zeitschriftenbibliothek EZB (Electronic Journals Library), Genamics JournalSeek, Global impact factor (GIF), Google Scholar, HathiTrustt, Index Copernicus, International Scientific Indexing (ISI), Israel Union List of Electronic Journals (ULE), J-Gate, JournalRate (Academic journal search tool), Journals Impact factor (JIF) by Global Society for Scientific Research (GSSR), KoBSON, MALMAD, National Agricultural Library – USA, ProQuest, Research bible, RoMEO, Regional Information Center for Science and Technology (RICeST) – Iran, Rubriq database, The Open University Library, The Rudjer Bošković Institute Library, Ulrichsweb, Universal Impact Factor, WorldCat, XML, Zeitschriftendatenbank (ZDB), Zoological Record of the Thomson Reuters, ...

The journal "Agriculture and Forestry" is funded by the Biotechnical faculty, Co-funded by the Architecture Faculty of the University of Montenegro, the Ministry of Science & the Ministry of Agriculture and Rural Development of Montenegro

CIP – Каталогизација у публикацији
Централна народна библиотека Црне Горе, Цетиње
ISSN 0554-5579
COBIS.CG-ID 3758082

CONTENT

DOI: 10.17707/AgricultForest.62.4.01

Vanessa GIL, Luis REY, Marta BARBERÁ, Ignacio R CASTANON, Myriam R VENTURA [1]

TANNIN CONTENT AND CHEMICAL COMPOSITION OF UNCONVENTIONAL AND CONVENTIONAL FEED FOR RUMINANTS

SUMMARY

An interesting challenge for animal nutrition scientists is introduce alternative feedstuffs that would help them overcome the issues of environmental harshness and the forages high production costs in tropical, subtropical and arid areas. The objective of this study was to contribute to the characterization of nutritional potential of unconventional and conventional feed with special attention to the profiles of phenolic compounds and condensed tannin, which could cause effects on meat and milk composition, when consumed in large quantities by animals. The species were: *Opuntia ficus-indica, Agave Americano, Arundo donax*, used for farmers to feed animals from wild populations and *Triticum spp, Avena spp, Hordeum spp* and *Pisum spp*, species natives cultivated as forages for animals, from seeds of species conserved by farmers in the Canary Islands (Spain). Nutrient content and the profiles in secondary compounds would differ between species and that their quantity and qualitative interactions would influence nutritive value. Crude protein (CP) was: 4.2% (*Opuntia ficus-indica*), 6.7% (*Agave Americano*), 10.7% (*Arundo donax*), 11.2% (*Hordeum spp*), 12.6 % (*Triticum spp*), 13.9% (*Avena spp)* and 18.4%, *Pisum spp*). Condensed tannins ranged from 0.0% (Opuntia ficus-indica) to 2% (*Pisum spp*), and total phenols from 0.6% (*Hordeum spp)* to 9.2% (*Pisum spp*). Organic matter digestibility (OMD) ranged from 46% (*Triticum spp*) to 88.6% (*Agave Americano*). Based on IVOMD, digestible energy of the different species was estimated to range from 6.5 to 11.8 MJ DE/kg DM. Current results support the thesis that, some alternative local feedstuffs have potential to be used in ruminant feeding strategies.

Keywords: Opuntia, Agave, Pisum, Hordeum, Arundo

INTRODUCTION

Herbaceous and legume plants have been largely reported to be used for small ruminant feeding as feed resources, but in tropics, subtropics, arid and dry regions, during dry seasons this feedstuff is normally scare and limited. Good

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

economic alternative for livestock feeding is to produce good quality forage with native well adapted species and use as well spontaneous species which grow easily without too much care. Some of them are known to be tolerant to drought having the ability to accumulate green fodder over several seasons, controlling erosion, providing fodder reserves for times of dearth and contributing, as legumes plants, for positive impacts on soil fertility with organisms which fix atmospheric nitrogen (Le Houérou, 2000). Nevertheless, their use has been limited by scant research on their nutritional and chemical properties, in particular on anti-nutritional factors. The presence of tannins in browsing legumes plants is normally associated with limiting factor to the utilization as feedstuffs (Mangan, 1988; Kumar and Vaithiyanathan, 1990); but may be advantageous by protecting dietary protein from digestion in the rumen and thus increasing total supply of protein for absorption (D, Mello, 1992).

In Canary Islands there are some native species (*Triticum spp., Avena spp., Hordeum spp.* and *Pisum spp.*) traditionally cultivated for feeding animals and other which grow spontaneously (*Opuntia ficus-indica, Agave americano, Arundo donax*) which have been used by local goat and sheep keepers to feed animals as forage resources. *Agave spp* and *Opuntia ficus-indica* are usually thrived in semiarid regions such as Mexico, Australia, and Africa used as supplements in small ruminant diets, without compromising animal performance (Vasta *et al., 2008)*. In the case of *Arundo donax* is usually considered a noxious weed, most efforts are dedicated to controlling it and there are few trials dedicated to its management as forage. It is a highly productive species, yielding from 30-40 t/ha/year biomasses in low yield areas such as Greece or Spain, to 171 t/ha/year in the USA (CIPC, 2011). Grazing of the young shoots and leaves, which are the only parts palatable to livestock, may help to control its development during the dry season (USDA, 2012).

However, there is still scarce literature about their particular characteristics and nutritive value which had lead to a poor promotion at local and regional level, even in some species with a risk of losing native seeds and germoplasm of it.

The objective of this study was to assess the nutritive value of some unconventional and conventional feed, species used to feeding animals, specially taking into account their profiles on phenolic compounds and condensed tannins for select properly the species and the variety of the legumes shrubs for animal nutrition.

MATERIAL AND METHODS

The species used in this study were *Opuntia ficus-indica, Agave americano, Arundo donax*, used to feed animals sporadically for farmers from wild populations from different localities in Gran Canary (Latitude 27º 55´ 45´´; Longitude 15º 23´ 20´´). The original plants were randomly selected from spontaneous populations which have been developed without irrigation or fertilization. The other native's species from Canary used were *Triticum spp.*,

Avena spp, Hordeum spp. and *Pisum spp.* which were specifically cultivated on the Cabildo's Experimental Farm in Gran Canary. Fresh samples were weighed, cut and dried at 60 ◦C to about 90 g/kg dry matter (DM). They were then ground to pass a 1mm screen prior to duplicate chemical analyses. Dry matter, ash and crude protein (CP) were determined according to standard methods as described in AOAC (2000) (methods 930.15, 942.05, and 976.05, respectively). Ash-free neutral detergent fibre (NDFom) was determined using sodium sulphite in the ND according to Van Soest et al. (1991). The acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined following the procedure of Van Soest *et al.* (1991).Condensed tannin (TC) analysis and total phenolic content (PC) were performed by the vanillin-HCL method of Burns (1963) and the technique of Makkar, H. and Al. (1993) respectively; *In vitro* dry matter digestibility (DMD) and organic matter digestibility (OMD) was determined according to the two stage pepsin-cellulase method (Pepcel) (Aufrere, 1982). Based on digestible OM, digestible energy (DE, MJ/kg DM) content of the species was estimated as 0.0185 × digestible OM (NRC, 1988). To assess the content of nutrients according to the type of plant, an analysis model of variance with a factor of variation (ANOVA-1) was used (SAS, 2000).

RESULTS AND DISCUSSION

In Mediterranean countries, livestock nutrition is based on the natural grazing and supplementary feeding consisting mainly of fodder, by-products and concentrates. The feed used is usually cereals, hay, consisting mainly of annual legumes and seeded small grains, such as oats and vetch. But in arid and semi-arid areas are characterized by limited food resources and the production of green fodder is scare, which is reflected in weight loss, poor reproductive performance and low overall production. The future of arid and semiarid regions depends on the development of sustainable agricultural systems and on the cultivation of appropriate crops. Such crops must successfully withstand water shortage, high temperature, and poor soil fertility (Nefzaoui, and Ben Salem. 2002).

Table 1. Chemical composition on conventional and unconventional feed (g/kg DM)

Species	DM	OM	CP	NDF	ADF	ADL	ASH	PT	TC
Triticum	295	757	126	62	367	35	121	0.8	1.0
Avena	206	714	139	559	326	11	144	0.9	1.3
Hordeum	250	792	112	419	210	12	95	0.6	1.3
Pisum	156	750	185	282	218	38	111	9.2	1.4
Opuntia	91	558	42	317	77	10	330	1.5	0.0
Agave	120	722	67	200	152	40	125	2.0	0.1
Arundo	298	751	107	568	313	40	193	1.1	0.1

(Dm) dry matter; (om) organic matter; (cp) crude protein ;(ndf) neutral detergent fibre; (adf) acid detergent fibre; (adl) acid detergent lignin; (ash) ash in g/kgdm. (pt) total phenols (% of tannins as tannic acid equivalent), (tc)condensed tannins content (%of tannins as catechin equivalent),

However, there are resources, unconventional feed, that can be produced in this semi-arid conditions, which provides the necessary nutrients to improve the nutritional status of the animals, some of them have been selected and analyzed in this study to compare nutritive value among them; the chemical composition, are seen in table 1.

The DM content of the species was 91–298 g/kg fresh forage, and OM content of the different species analyzed was 558–792 g/kg DM (Table 1). Both DM and OM content were lower (P<0.05) in the *Opuntia ficus* specie and *Agave americano,* with differences significantly among the other species, high water content could affect nutritive value of this species.

CP content ranged from 42 to 185 g/kg DM, and it was lower (P<0.05) in the *Opuntia ficus* specie and higher (P<0.05) in the *Pisum* specie. These values obtained in the *Opuntia* on DM, OM, CP and Digestibility is similar to results described by Flachowsky *et al*., 1985. Bouaziz *et al., (*2014) found lower CP content (3.5%) on *Agave Americano* collected on Tunisia.NDFom content of varieties ranged from 200 to 620 g/kg DM, and it was lower (P<0.05) in the *Agave Americano* and higher (P<0.05) in the *Triticum* specie.

Condensed tannins contents ranged from 0.0% in the *Opuntia ficus* to 2 % of tannins as catechin equivalent, in the *Pisum* specie, and total phenols contents ranged from 0.6% in the *Hordeum* specie to 9% of tannins as tannic acid equivalent in the *Pisum* specie.

Both TC and PC contents were higher in the *Pisum* specie, similar results found in legume shrub called *Tagasaste by Ventura et al.,* (2002). Moreover no differences occurred for cereal species and in the *Agave americano* and *Arundo donax* in the condensed tannins contents. Nevertheless just in case of concentrations greater than 50 g/kg DM, tannins can decrease food intake (Barry and Forss, 1983) and this should limits the use of feedstuffs even though they may have high protein content.

Table 2. Dry matter digestibility (DMD),organic matter digestibility (DMD), digestible organic matter (DOM) in g/kg DM and estimate digestible energy (DE) in MJ/kg DM

	DMD	OMD	DOM	DE
Triticum	0.47	0.46	351	6.5
Avena	0.60	0.58	415	7.7
Hordeum	0.68	0.69	540	10.0
Pisum	0.82	0.80	605	11.2
Opuntia	0.73	0.80	448	8.2
Agave	0.89	0.88	639	11.8
Arundo	0.45	0.47	359	6.6

The *In vitro* organic matter digestibility (IVOMD) (Table 2) ranged from 0.464 in the *Triticum* specie to 0.886 in the *Agave Americano*, were it was the higher (P<0.05) value. Moreover IVOMD was quite high and similar than in the *Pisum specie* and in the *Opuntia ficus*. Digestibility is similar to results described by Flachowsky *et al*., 1985 in the *Opuntia spp*. Digestible OM content of the

species was 351–639 g/kg DM, with differences among species. Finally, based on IVOMD, digestible energy of the different species was estimated to range from 6.5 to 11.8 MJ DE/kg DM. It was lower in the *Arundo donax* and in the *Triticum* specie and higher ($P<0.05$) in the *Agave Americano, Pisum specie* and *Opuntia ficus.* ED was similar among *Pisum specie* and *Agave americano,* due to the higher IVOMD on both of them. An exception in the *Arundo donax* and in the *Triticum* specie, estimated DE of the species analyzed was similar and higher to *Medicago arborea* and to other forage legumes shrubs such as *Bituminaria* varieties or tagasaste reported by Ventura et al. (2002; 2004; 2009). Similar results of Chemical composition and digestibility were described by Ahmed et al., (2009) for *Arundo donax.*

Moreover DE content of all species analyzed was higher and in the range (6.9-9.2 MJ DE/kg DM) estimated by INRA (1988) for cereals, legumes straws and medium quality alfalfa hay.Current results support the thesis that, some alternative local feedstuffs have potential to be used in ruminant feeding systems strategies.

CONCLUSIONS

The value of forages as supplements is mainly depending on their capacity to provide essential nutrients to the rumen microbial population and/or critical nutrients (anti-nutritive factors) to meet the host animal requirements, thus increasing or reducing the efficiency of feed utilization (Elliot and McMenimen, 1987). In this study we can see that there are many differences in chemical composition, in the condensed tannins and in the total phenols contents within the different species analyzed. Condensed tannins and total phenols contents were low in the different species analyzed a exception of total phenols contents in the Pisum specie were it was quite high. Although, more research should be done on the relation between the quantity of total phenols and tannins and the possible toxic effects on animals or the reduction on voluntary dry matter intake and palatability. Based on CP, IVOMD and digestible energy results, all the species analyzed are within the range estimated by INRA (1988) for cereals, legumes straws and medium quality alfalfa hay. Current results support the thesis that, these alternative local feedstuffs have potential to be used in ruminant feeding systems strategies, although more research is needed to see productive responses on animals feeding these plants.

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DOI: 10.17707/AgricultForest.62.4.02

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MICROBIAL COMMUNITY PROFILES IN RESPONSE TO DIFFERENT SOIL MANAGEMENTS IN SANDY SOIL

SUMMARY

The growing human population has higher and higher food demand, which requires an increase in efficiency of agricultural production. Healthy and fertile soils are essential to satisfy this demand. The microbial community structure is an excellent indicator of the soil fertility and the diversity of bacteria and fungi. In our work we compared the effect of organic and conventional cultivation methods on the microbial community profiles of sandy soils in the Nyírség region, Hungary. These fields have topographical heterogeneity therefore the sampling sites were divided into top of hill and bottom of hill. Sampling was done in autumn 2013, from the 0-30 cm and the 30-60 cm soil depths. The phospholipid fatty acids (PLFA) were used for the monitoring of microbial community. PLFAs break down when the cell dies therefore these molecules show the community structure in a real time. In the 0-30 cm soil layer of organically managed field the PLFA structure was significantly different between top and bottom of hill, but the difference were low. In conventionally managed field, high differences were found between the PLFA groups measured in the top and bottom of hill. The PLFA values were higher in the top of hill in organic field, while in case of bottom of hills sites higher PLFA values were measured in conventional farming system. In the deeper soil layers the tendencies were found similar to the upper soil layer, but the measured values were lower.

Keywords: cultivation system, microbial community structure, PLFA, topography, soil depth

INTRODUCTION

The soil microbial community largely determines the soil fertility by influencing the dynamics of organic matter and nutrient cycles (Liu et al., 2006; Bowles et al., 2014). These microbes are responsible for breakdown of dead plant residues and turning those to the carbon cycle. Higher microbial activity and biodegradation were observed in the ecological or reduced tillage cultivation than in conventional management system (Ge et al., 2013; Mangalaserry et al., 2015).

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Therefore it is important to apply properly selected cultivation method, which results in favourable conditions for the soil microbes (Prasad et al., 2016).

Before 1989 intensive farming was conducted in large fields with large volume of the used cheap pesticides and fertilizers. The harmful effects of agricultural chemicals to the biodiversity and human health have already known (Snedeker, 2001; Colborn et al., 1993). Nowadays, in Hungary the use of sustainable agricultural methods is preferred (Birkás et al., 2004).

The objective of this study was to compare the effects of ecological and conventional farming systems on microbiological properties of soil. We investigated the effects of studied management systems and the modification effects of relief of the studied area, and we observed the stronger effect of management compared to the relief.

MATERIALS AND METHODS

The sampling sites are located in the Nyírség region, in the north-eastern part of Hungary, where the main soil type is acidic sand (Arenosols). In this region the climate is moderately cold-dry. In the investigated year the main annual temperature was 12.5 °C and the annual precipitation was 486 mm in sampling areas. Because of the topographical heterogeneity of this region, samples were collected from top of hills and bottom hills.

Sampling sites were the followings:

1. Ecological, top of hill (ET) (214036°82'E, 475849°17'N, 156 m above sea level);
2. Ecological, bottom of hill (EB) (214031°48'E, 475848°81'N, 151 m above sea level);
3. Conventional, top of hill (CT) (214051°10'E, 475841°35'N, 158 m above sea level);
4. Conventional, bottom of hill (CB) (214054°64'E, 475842° 43'N, 153 m above sea level).

The studied areas located at the Research Institute of Nyíregyháza, Debrecen University, where the ecological crop production has been carrying since 1997. In the studied sampling period, rye was cultivated in both management systems. In both cultivation methods ploughing was applied up to 30 cm depth and deep loosening up to 60 cm depth in every 5th year. In conventional area the bottom of hill was liming in autumn 2012 because of the low pH (3.89±0.03) of soil.

Samples were collected in autumn 2013 (22 and 24 October), from two depths, 0-30 cm and the 30-60 cm, in four repetitions (one repetition was a composite sample of four point samples). Samples were stored frozen before analysis. The soil temperature was varied in the range between 10.9 – 17.5 °C in the sampling days.

Before the chemical analysis the larger plant roots were removed in the laboratory, and the air dried samples were sieved (Ø 2 mm). The pH_{KCl} was measured with a Hach-Lange, HQ411D type digital pH meter (Hach-Lange,

Loveland, Colorado, USA) where the soil : KCl ratio was 1 : 2.5. Total carbon (C) and nitrogen (N) content were determined by Dumas method (varioMax CNS, Elementar Analysensysteme GmbH, Hanau, Germany). The organic carbon content was calculated from the humus content, which was measured with an UNICAM UV2 spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA), after the digestion with potassium dichromate and cc. sulphuric acid.

The nitrite-nitrate-N content was measured with a FIA Star 5000 device (Foss, Hilleroed, Denmark), from potassium chloride extracts.

The PLFAs were prepared as described by White et al. (White et al., 1979) with some modifications, in four repetitions. Briefly, 10.00 g (frozen, then incubated and sieved) soil was extracted using a single-phase chloroform-methanol-phosphate buffer system, during 2h with a laboratory shaker.

Then we added chloroform and distilled water to this extract, and filtrated in celite. The filtrate was separated with shaker funnel for the bottom chloroform phase. After the sodium sulphate filtration and vacuum evaporation of the chloroform phase, the phospholipids were separated from neutral lipids and glycolipids using silica acid columns (Chromabond, Macherey Nagel, Germany), followed by methylation of the phospholipids. Samples were stored at -20°C until the analysis. Methyl nonadecanoate was used as internal standard after the methylation. The PLFAs were separated and identified using a gas chromatograph-mass spectrometer system (GC 6890N with MS 5975, Agilent, Santa Clara, CA, USA) with a 100 m Supelco SP-2560 column, in selected ion mode and scan mode as well (50-350 amu).

Gerstel MPS2 autosampler was used for the injection, the injection volume was 3 µl. The PLFAs 14:0, 15:0, 16:0 and 18:0 represented the general bacterial biomass. The branched, saturated PLFAs, as iC15:0, aC15:0, iC16:0, iC17:0, aC17:0 were used as $Gram^+$ as well as the monoenoic and cyclopropane unsaturated C16:1n7c, C16:1n5c, C18:1n9c, cyC19:0 as $Gram^-$ biomarkers. The 10MeC16:0 and 10MeC17:0 represented the Actinobacteria, and C18:2n6 was used as fungi marker. These PLFAs were used to calculate total PLFA concentration (nmol PLFA g^{-1} dry soil). On the bases of measured PLFAs the ratios of these groups were calculated.

All measurements were reported as mean values with standard errors, where statistical analysis was done using internal repetitions respectively (n = 4). IBM SPSS Statistics 22.0 software package (IBM Inc., USA) were used for statistical analyses at P = 0.05 significance level.

One-way analysis of variance (ANOVA) was used for comparing the means of different sampling sites followed by Games-Howell and Tukey-b test. Correlations between investigated parameters were determined using Pearson's correlation.

RESULTS AND DISCUSSION

Generally, the measured values of pH_{KCl} fit in the pH range of most acidic sandy soils (4.02-7.01). Furthermore acidifying effect of fertilizer was also

observed (Pais et al., 1990) in conventional top of hill, but in conventional bottom of hill the liming was resulted in higher pH_{KCl} in autumn 2013. Compared to the two farming systems, favourable chemical values were measured in ecological field on the top of hill, except the inorganic N (Table 1), but at the bottom of hill the investigated parameters were higher in conventional field. The available nitrogen and carbon usually increased with increasing pH (Zhao et al., 2011). Generally, the most investigated parameters were higher in upper (0-30 cm) soil layer and also were higher in the bottom of hill, than in top of hill (except of nitrite-nitrate-N in ecological field). In ecological top of hill significantly higher total and organic carbon were observed and recycled plant residues increased the total nitrogen content and carbon : nitrogen ratio (Chen et al., 2000; Edmeades, 2003).

Table 1. The main chemical parameters of investigated soils

Sampling site	pH_{KCl}	total C *	organic C *	total N *	NO_2^- NO_3^- - N *	C:N ratio
ET 0-30 cm	5.43±0.01c	0.497±0.02b	0.372±0.02b	0.055±0.00b	0.019±0.00b	9.026±0.06b
ET 30-60 cm	5.53±0.17C	0.332±0.06 B	0.232±0.06 B	0.040±0.00 B	0.010±0.00 C	8.241±0.17 C
EB 0-30 cm	4.85±0.06b	0.818±0.01c	0.715±0.02c	0.093±0.00c	0.010±0.00a b	8.839±0.01a b
EB 30-60 cm	5.05±0.35B	0.721±0.01 C	0.589±0.08 C	0.083±0.00 C	0.007±0.00 B	8.658±0.35 B
CT 0-30 cm	4.02±0.04a	0.346±0.00a	0.202±0.00a	0.040±0.00a	0.008±0.00a	8.751±0.06a
CT 30-60 cm	4.15±0.09A	0.222±0.06 A	0.100±0.08 A	0.029±0.00 A	0.004±0.00 A	7.488±0.09 C
CB 0-30 cm	7.01±0.01d	0.943±0.02d	0.729±0.01c	0.096±0.00c	0.020±0.00b	9.778±0.09c
CB 30-60 cm	6.61±0.44D	0.735±0.08 C	0.524±0.06 C	0.081±0.00 C	0.009±0.00 BC	9.116±0.44 D

[a] Data are expressed as mean ± standard errors (n=12).
[b] Within a column followed by different letters represent the differences by one-way ANOVA followed by Games-Howell test ($P < 0.05$).

The management practice affected not only the soil chemical parameters, but also the structure of microbial community. The nutrient supply method has strong effect on the community structure, through the differences of organic matter content, and the value of available substrates (Hartman et al., 2006), and the change of pH affect the structure of microbial community (Rousk et al., 2009). The General bacteria (2.12±0.00 nmol PLFA g^{-1} dry soil), $Gram^+$ (1.59±0.00 nmol PLFA g^{-1} dry soil) and $Gram^-$ (2.05±0.00 nmol PLFA g^{-1} dry soil) bacteria, Actinobacteria (0.48±0.00 nmol PLFA g^{-1} dry soil) and Fungi (0.32±0.00 nmol PLFA g^{-1} dry soil) markers were highest in upper soil layer in conventional bottom of hill, while the lowest values were measured in conventional top of hill (Table 2). In case of these markers and the total PLFA significant differences were observed between two reliefs, but compared to the

two farming systems, the differences were lower in ecological, than the conventional farming system. Furthermore in ecological field the values of measured markers and total PLFA were higher in top of hill, but in conventional plots were higher in bottom of hill. The pH influences of microbial community (Bååth & Anderson, 2003) and liming could cause the increased PLFA values of CB site.

Table 2. Structure of microbial community in autumn 2013

PLFA markers	ET		EB		CT		CB	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
General bacteria [a]	1.30±0.00 c	1.07±0.00 C	1.2±0.00 b	0.69±0.00 A	0.75±0.00 a	0.72±0.00 B	2.12±0.00 d	1.08±0.00 D
Gram $^{+}$ bacteria [a]	1.05±0.00 c	0.98±0.00 D	1.04±0.00 b	0.43±0.00 A	0.53±0.00 a	0.55±0.00 B	1.59±0.00 d	0.73±0.00 C
Gram $^{-}$ bacteria [a]	0.97±0.00 b	0.74±0.00 C	1.08±0.00 c	0.46±0.00 B	0.50±0.00 a	0.45±0.00 A	2.05±0.00 d	0.84±0.00 D
Actinobacteria [a]	0.38±0.00 c	0.16±0.00 A	0.35±0.00 b	0.17±0.00 B	0.17±0.00 a	0.27±0.00 C	0.48±0.00 d	0.42±0.00 D
Fungi (C18:2n6) [a]	0.13±0.00 c	0.08±0.00 C	0.10±0.00 b	0.03±0.00 A	0.05±0.00 a	0.05±0.00 B	0.32±0.00 d	0.11±0.00 D
Gram^{+} : Gram^{-} ratio	0.92±0.00 a	0.76±0.00 A	1.05±0.00 c	1.05±0.00 C	0.95±0.00 b	0.82±0.00 B	1.29±0.00 d	1.16±0.00 D
Fungi : General bacteria ratio	0.10±0.00 c	0.07±0.00 C	0.08±0.00 b	0.04±0.00 A	0.07±0.00 a	0.07±0.00 B	0.15±0.00 d	0.11±0.00 D
Actinobacteria : General bacteria ratio	0.30±0.00 d	0.39±0.00 D	0.29±0.00 c	0.25±0.00 C	0.23±0.00 b	0.22±0.00 A	0.22±0.00 a	0.25±0.00 B
Total PLFA	3.83±0.00 c	3.29±0.00 D	3.77±0.00 b	1.78±0.00 A	2.00±0.00 a	1.92±0.00 B	6.56±0.00 d	3.02±0.00 C

[a] Data are expressed as mean (nmol PLFA g^{-1} dry soil) ± standard errors (n=4).

[b] Within a raw different letters represent the differences of means according to one-way ANOVA followed by Tukey-b test ($P < 0.05$), the lowercases (a-d) are marked the results of 0-30 cm soil layer and capital letters (A-D) are marked the results of 30-60 cm soil layer.

Low proportion of easily available carbon source increases the value of Gram^{+} bacteria (Esperschütz et el., 2007), while values of Gram^{-} bacteria markers was increased when the quantity of easily available, unstable carbon forms were increased in the soil (Peacock et al., 2001). Therefore, the measured lower Gram^{+} : Gram^{-} bacteria ratio in ET and CT indicates higher value of easily available carbon forms in this sites.

The increasing organic matter input results in increase of fungi, thereby the increase of Fungi : Bacteria ratio (Frostegård et al., 1996). We measured higher Fungi values from upper soil layer in conventional bottom of hill and the ecological top of hill, but the organic carbon was higher in the two bottom of hill and the fungi was higher in the CB and ET, there was not significant relationship

between the two parameters. The value of fungi depends also on the pH. When the pH lower, the conditions are unfavourable for bacteria, and the fungi community increases (Rousk et al., 2009). However, we not found negative correlation between the values of Fungi and pH (0.467, $P < 0.01$). The relationship between pH and general bacteria markers was significantly positive (0.455, $P < 0.01$)

Higher ratio of Actinobacteria: General bacteria in ecological field indicated the progressed decomposition process in sampling time, opposite in conventional field (Bastida et al., 2013).

CONCLUSIONS

Ecological management had favourable effect on the chemical parameters and microbial community of sandy soils in Nyírség region. The lower differences between two reliefs in ecological field were resulted by favourable conditions and better buffer capacity of soil, providing protection against extreme environmental events. The positive effect of liming was also observed, like increasing pH and microbial biomass in conventional bottom of hill. However, additional studies are needed to understand changes of Fungi : bacteria ratio with increasing pH. Our results revealed that regular recycling of organic matter after harvesting without artificial fertilizer utilization could maintain the fertility of sandy soils.

ACKNOWLEDGEMENT

This work was supported by the Hungarian Research Institute of Organic Agriculture (ÖMKi). We thank Antal Ferenczy† for his valuable advices in the statistical evaluation.

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DOI: 10.17707/AgricultForest.62.4.03

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ALPHA AND BETA PLANT DIVERSITY IN MULTISPECIES AGROECOSYSTEMS OF CENTRAL GREECE

SUMMARY

Intercropping encourages biodiversity, by providing a habitat for a variety of plants, which benefit the population of insects and soil organisms that would not be present in a single-crop environment. The literature about this issue is scarce. Hence, the study of plant species density, alpha (species richness, Shannon, Simpson and Evenness index) and beta diversity (Jaccard similarity index) were held in the University of Thessaly facilities on May 2014, with the use of sample plot (50 x 50 cm). The experimental plots were constituted of the following types of intercropping: Pea-Oats (P-O), Pea-Barley (P-B), Winter Vetch-Oats (WV-O), Winter Vetch-Barley (WV-B), Grass Pea-Oats (GP-O) and Grass Pea-Barley (GP-B). A total number of eight species of herbaceous plants were recorded in all types of intercropping. The average density of herbaceous plants was found significantly higher in the Pea-Barley (21.80 ± 13.68, $p < 0.05$) than the Pea-Oats (10.00 ± 7.61, $p < 0.05$), Winter Vetch-Oats (10.60 ± 17.79, $p < 0.05$), Winter Vetch-Barley (6.80 ± 9.47, $p < 0.05$), Grass Pea-Oats (13.00 ± 12.20, $p < 0.05$) and Grass Pea-Barley (5.00 ± 5.91, $p < 0.05$). The Shannon diversity index was higher in the Pea-Barley (1.33) and lower in Winter Vetch-Oats (0.00), Winter Vetch-Barley (0.00) and Grass Pea-Barley (0.00) ($p < 0.05$). The results also showed that the type of intercropping Pea-Barley (1.00-0.85) favoured the evenness and similarity of plant species in relation to other types of intercropping ($p < 0.05$). Conclusively, intercropping systems clearly have the potential to increase the long-term sustainability of food production under low inputs. Specifically, the type of intercropping Pea-Barley favours alpha and beta plant diversity making this type of intercropping important, favouring parameters of biodiversity both in Greek and in the wider Mediterranean areas. We conclude, also, on the need to enhance agricultural research on these multispecies systems, combining both agronomic and ecological concepts and tools.

Keywords: sustainability, crops, biodiversity, Mediterranean.

INTRODUCTION

Intercropping systems consist of two or more crops growing together and coexisting for a time. This final criterion distinguishes intercropping from mixed

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

mono-cropping and rotation cropping (Li et al., 2013; Brooker et al. 2015). Intercropping is common in countries with high amounts of subsistence agriculture and low amounts of agricultural mechanization. Intercropping is often undertaken by farmers practising low-input, low-yield farming on small parcels of land (Ngwira et al., 2012; Brooker et al. 2015).

Most studies on intercrops systems reporting legume-cereal intercropping, a productive and sustainable system, a resource facilitation which consist of growing, soil's fertility and yield. Latati et al. (2013) confirmed the advantage of intercropping legumes – cereals over sole cropping system, as sustainable agriculture.

The main advantage of intercropping is the more efficient utilization of the available resources and the increased productivity compared with each sole crop of the mixture (Agegnehu et al. 2008). Reversevely, Ofori et al. (1987) indicated that intercropping system causes a decrease of yield due to the problems of harmful grasses, pests and diseases, in addition to the difficulties of harvesting.

Intercropping can conserve soil water by providing shade, reducing wind speed and increasing infiltration with mulch layers and improved soil structure (Young 1997). Spatial arrangement of intercrops is an important management practice that can improve radiation interception through more complete ground cover (Heitholt et al., 2005).

Intercropping of compatible plants also encourages biodiversity, by providing a habitat for a variety of plants, insects and soil organisms that would not be present in a single-crop environment. This in turn can help limit outbreaks of crop pests by increasing predator biodiversity (Altieri and Nicholls 2004). Plants form the critical base of food chains in nearly all ecosystems. Through photosynthesis, plants harvest the energy of the sun, providing both food and habitat for other organisms. Therefore, the study of plant diversity constitutes the key of the ecological balance in the intercropping ecosystems.

The literature about the above issue is scarce. Few studies have examined the role of plant functional diversity and the concept of overyielding in food production systems. Hence, the aims of this study is the estimation of plant species density and alpha diversity (species richness, Shannon, Simpson and Evenness index) in the following types of intercropping: pea-oats (p-o), pea-barley (p-b), vetch-oats (v-o), vetch-barley (v-b), vetch-oats (v-o) and vetch-barley (v-b) providing environmental services that have impacts beyond the field scale, either spatially, e.g. services to the local or the global community, or temporally, e.g. modifications of the environment for future generations.

MATERIAL AND METHODS

A number of field experiments have been carried out in Thessaly plain (Experimental Farm of the University of Thessaly, Velestino, central Greece, 2014) with coordinates Lat: 390 23', Lon: 22° 45', and altitude 87.5 m (Fig.1). The soil at the site was a deep, moderately fertile, clay loamy soil that was classified as Calcixerollic Xerochrept (USDA, 1975).

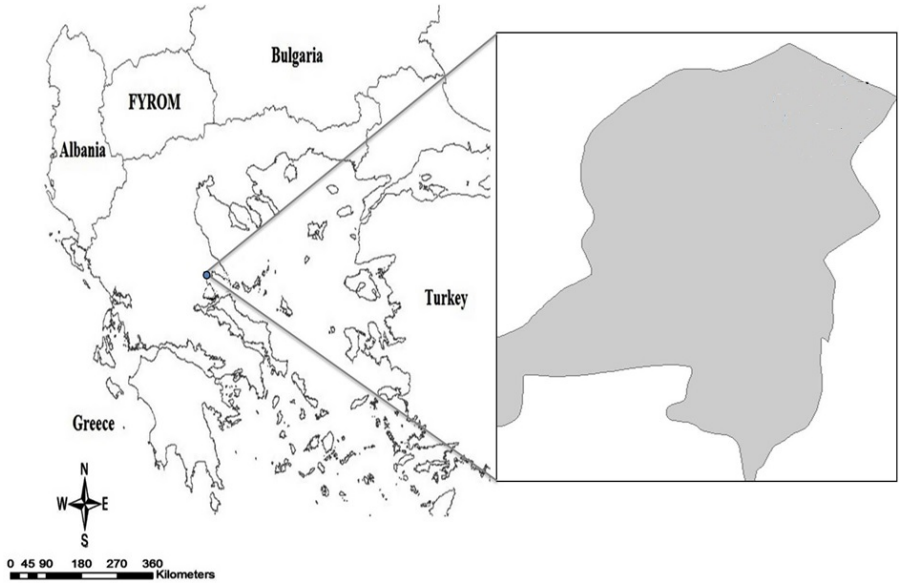

Figure 1. Study area

The study area is part of the *Quercion ilicis* and *Oleo-Ceratonion* subzones of the *Quercetalia ilicis* zone of the Mediterranean-type vegetation at altitudes up to 50 m a.s.l. (above sea level).

The climate in Greece is typical Mediterranean with cool humid winter and very dry and hot summer. Thessaly, the largest Greek lowland and the centre of the country's agricultural production, is characterized by a more continental climatic character with colder winters and hot summers.

Sampling

The sampling of herbaceous vegetation was carried out in May 2014 in randomly selected plots of 0.25 m^2, in order to estimate plant density and diversity (Cook and Stubbendieck 1986, Solomou and Sfougaris 2013) (Fig. 2).

Data were evaluated for normality and homogeneity with the Kolmogorov–Smirnov and Shapiro–Wilk tests. Data were transformed using log(x + 1) when necessary to meet normality assumptions. For the analysis of plant data, a general linear model (GLM, Type III Sum of Squares) (one-way ANOVA) was used. Tukey's HSD (honestly significantly difference) pairwise comparison tests were used with $p < 0.05$. Statistical analyses were performed using the software package IBM SPSS Statistics ver. 19.0 for Windows (SPSS Inc., IBM Company, Chicago, IL, USA 2010).

Several alpha-diversity indices such as species richness, Shannon–Wiener, Simpson and evenness were calculated using Species Diversity and Richness IV software (comparisons among the type of intercropping systems were made with the randomization test of Solow (1993)) (Seaby and Henderson 2006). For a detailed description of the mathematical background of the above indices, see

Seaby and Henderson (2006). A variety of methods for measuring beta diversity are available, among them similarity measures are the simplest and the most commonly used, being calculated from presence/absence data (Koleff et al. 2003). The similarity of plant communities among the types of intercropping systems was examined using the Jaccard index (Koleff et al. 2003) to express beta diversity, again using Species Diversity and Richness IV software (Seaby and Henderson 2006).

Figure 2. Sampling quadrate of 0.25 m^2 (0.5 m × 0.5 m)

RESULTS AND DISCUSSION

A total number of eight species of herbaceous plants were recorded in all types of intercropping in the study area [three (Pea-Oats), four (Pea-Barley), one (Winter Vetch-Oats), one (Winter Vetch-Barley), three (Grass Pea-Oats) and one (Grass Pea-Barley)] (Table 1). The highest mean density of herbaceous plants (21.80±13.68 individuals/m2, $p<0.05$) (Figure 3) were recorded in the Pea-Barley. Tengberg (Salas et al. 1997) indicates that diversity of agricultural systems, agricultural species and main species are three component of agrobiodiversity. Multiple cropping, especially intercropping, is one way to increase agroecosystems diversity (Marshall et al. 2003, Azizi et al. 2015).

Mohler, and Liebman (Mclaughlin and Minrau 1995) demonstrated that intercropping of barley and pea and barley monoculture were similar in plant species richness approximately. However, plant species richness in pea was the most (Mahn 1984, Mclaughlin and Minrau 1995, Azizi et al. 2015).

The type of intercropping pea-barley showed the highest similarity in plant species composition (table 1). Beta diversity is an important property of ecosystems because it provides information about the partitioning of habitats by species and constitutes an empirical and theoretical link between alpha (the local diversity of a community) and gama diversity (Cornell & Lawton 1992, Medianero et al. 2010). It captures a fundamental aspect of the spatial pattern of diversity, and its study is fundamental to understanding the geographic patterns of species richness (Whittaker 1972, Koleff 2005, Medianero et al. 2010).

Table 1. Herbaceous plant species in the study area**.**

Species	Family	Type of intercropping					
		Pea-Oats	Pea-Barley	Winter Vetch-Oats	Winter Vetch-Barley	Grass Pea-Oats	Grass Pea-Barley
Anthemis arvensis	Asteraceae	+	+				
Chrysanthemum segetum	Asteraceae				+		
Sinapis arvensis	Brassicaceae	+					+
Sinapis alba	Brassicaceae					+	
Convolvulus arvensis	Convolvulaceae	+	+			+	
Papaver rhoeas	Papaveraceae		+				
Avena sterilis	Poaceae		+			+	
Hordeum murinum	Poaceae			+			

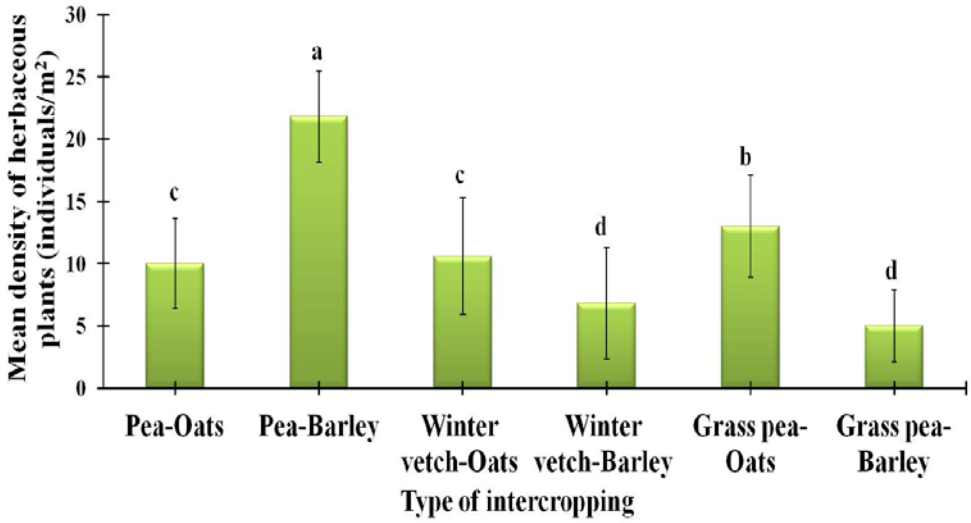

Figure 3. Mean density of herbaceous plants in all types of intercropping

Factors influencing species turnover among local fauna are usually a combination of environmental and geographical variables (i.e. Geographic distance) (Borcard et al. 1992), and determining their relative weighting is crucial for understanding the shaping of biogeographic patterns (Duivenvoorden et al. 2002, Medianero et al. 2010).

Table 1. Alpha and Beta plant diversity in all types of intercropping.

Index	Type of intercropping					
Alpha diversity	Pea-Oats	Pea-Barley	Winter Vetch-Oats	Winter Vetch-Barley	Grass Pea-Oats	Grass Pea-Barley
Species richness	3	4	1	1	3	1
Shannon	0.97	1.33	0	0	1.02	0
Simpson	2.42	3.74	1	1	2.64	1
Evenness	0.46	0.64	0	0	0.49	0
Beta diversity						
Jaccard	0.86	0.91	0	0	0.84	0

CONCLUSIONS

Intercropping systems clearly have the potential to increase the long-term sustainability of animal food production under low inputs. Specifically, the type of intercropping - nitrogen symbiosis fixation by legume was used as important resource for intercropping during growing cycle of cultivated plants – in Pea-Barley cultivation favours alpha and beta plant diversity making this type of intercropping important, favouring parameters of biodiversity both in Greek and in the wider Mediterranean areas.

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DOI: 10.17707/AgricultForest.62.4.04

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INTEGRATING THE FLAME TREATMENT IN THE GROWING CYCLE OF ASPARAGUS FOR A SUSTAINABLE CULTIVATION

SUMMARY

Manual, mechanical and chemical techniques are used to control weeds in asparagus fields. Chemical weed control is permitted by protocols, except for organic production, but prohibited during spear harvest, because shoots continuously emerge. In the harvest phase, the bed must be kept free from weeds to facilitate picking. Inadequate weed control increases infestations over the years. In this context, liquefied petroleum gas (LPG) flaming is effectively used in three phases of the annual cycle: harvest period, fern production period, end of growing cycle. A specific range of flaming machinery has been designed and adjusted for accomplishing treatments in each phase. During the harvest period, a broadcast flaming machine performs the treatment multiple times. Field tests identified suitable operative parameters to control weeds effectively, safeguarding the emerging spears (5km h^{-1} treatment speed, 0.8 bar gas pressure). During the fern production phase, intra-row flaming is applied to eliminate weeds and prevent seed dispersal. A special flaming machine is used to move through the narrow aisles, treating both sides at the same time (4÷5 km h^{-1} speed, 1.2 bar gas pressure). A laminar air flow provides a barrier to protect the fronds from rising heat. At the end of the growing cycle, the fern fronds are shredded mechanically and the residues are burned, eliminating disease inoculum and weed seeds. The flaming machine promotes the ignition of fern residues, improving combustion. A recent innovation is represented by a combined machine for shredding and flaming the residues simultaneously, reducing work time (3÷4 km h^{-1} speed, 1.6 bar gas pressure).

Keywords: Asparagus officinalis, flaming, weeds, organic production, mechanization.

INTRODUCTION

Asparagus is a perennial crop that is produced in several regions of Italy. With a total area harvested of about 5560 ha, Italy ranks 9th in the world, and 3rd

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

in Europe after Germany (19634 ha) and Spain (9900 ha) (Benson, 2012; FAOSTAT, 2016). The major areas of Italian asparagus production are located in Campania (1300 ha), Veneto (1600 ha), Emilia Romagna (1000 ha) and Puglia (900 ha). Each area has specific weed problems and differences in climate and soil types that affect weed management decisions (Falavigna, 2007).

Two distinct periods occur during the multiyear asparagus production cycle: stand establishment requires two years after planting; the following mature planting phase has an economic lifespan of 5 to 20 years. Both periods require specific weed management strategies with respect to plant development and related constraints.

Before planting the asparagus crowns, special care must be given to prepare the site by eliminating perennial weeds (Abell et al., 2006). Timely weed control during stand establishment assures optimal crop development. Mechanical cultivation of the beds, hand removal of weeds or herbicide treatments are needed during the first establishment year until a uniform fern cover is produced (Pedreros et al., 2002).

In established asparagus plantations weed control is only possible during a short window of opportunity, lasting about 4 months. In spring, before the spears emerge, preharvest cultivations and/or pre-emergence herbicides can be used for weed control. The harvest season spans from early spring through early summer, lasting up to eight weeks. In this period the spears develop continuously and the fields are harvested every 1-3 days. To facilitate harvest and increase soil temperature the beds must be kept weed-free. Control of annual weeds is difficult since the spears provide no shade to reduce weed competition. If infestations spread into the field during the harvest period they have to be removed manually or mechanically. Disc cultivation of the bed tops will interrupt harvest to for up to 10 days, reducing yield and profit (Mohler, 2001). Chemical weed control is prohibited in harvest.

Once harvest is finished the spears grow into dense ferns for the rest of the season, to replenish the nutrients for next year's spear production. Adequate weed control during the fern stage is required to prevent seed dispersal and weed seedbank buildup (Brock, 2012). Inadequate control increases infestations over the years, compromising yield and stand duration. Postharvest cultivation is possible until the fern becomes too tall to limit mechanical activity. However a heavy fern cover restricts light, thus providing competition against late emerging annual weeds. The application of herbicides from the end of harvest is an option for summer weed control in conventional production. (Boydston, 1995).

To improve the sustainability of asparagus, both in conventional and organic farming systems, an innovative weed management strategy was tested. LPG flaming was tested to control weeds in the three main phases of the annual asparagus growing cycle: the harvest period, the fern growth period, the end of the growing cycle. In this paper, the range of dedicated flaming machinery developed specifically for use in asparagus fields is described. Flaming may be a sustainable option for weed management, reducing the competition of annual and

perennial weeds, but avoiding the use of chemicals, while safeguarding yield and quality of produce.

MATERIAL AND METHODS

An array of flaming machines was developed by "Officine Mingozzi", an Italian based company specializing in flaming machinery for the farm. Researchers at CREA-ING tested the innovative machines to establish appropriate operative parameters for effective weed control during the different phases of the growing cycle. Field tests were conducted in a 6-year-old commercial asparagus plantation, grown with cultivar "Ercole", on a two hectares farm located in a primary production area of Latium region. The field design consisted in a single line of plants per row, an 80 cm bed width, a 25 cm plant-to-plant spacing, a density of 18000 plants per hectare, and a 2.2 m row-spacing for a total row length of about 4545 m/ha.

The open flame treatments were carried out using three models of flaming machines. These machines share some common features: they are tractor-mounted and connected via three-point linkage; they are limited in size and weight, therefore suitable for use with the compact tractors commonly available on asparagus farms. All models carry two LPG cylinders and the fuel is supplied to the burners in the gaseous phase. This is an important distinctive feature of the tested machines, in contrast with other makes and models that use LPG in the liquid phase. Vapor withdrawal from the LPG cylinders assures safety of use in field work. Accidental damage to the burners or the gas line (field obstacles and uneven terrain) would cause a gas leakage that will quickly dissipate into the air, yet no ignition could occur because the percentage of LPG in the LPG/air mixture would be beyond the limits of flammability.

The following three flaming machines were employed in the field trials: 1) a *broadcast flaming machine* to eliminate weeds in pre-emergence and during harvest; 2) an *intra-row flamer* to selectively remove weeds along the ferns in post-emergence; 3) a *combined machine* with a shredding apparatus coupled to a flaming unit, to treat the residues and clean the field at the end of the season.

Pre-emergence flaming was tested in spring before the spears emerged. Post-emergence selective flaming was tested in the harvest phase, on emerging spears (Figure 1). The tested *broadcast flaming machine* (model PTR-C) has 15 gas burners spaced 9 cm apart, mounted on a pipe manifold perpendicular to the row. The flames are directed under a horizontal hood, that extends rearward for about 60 cm to hold down the heat and increase treatment efficacy. The burners have a 1.2 mm diameter nozzle, providing a nominal LPG flow rate of 1.62 kg/h at 0.8 bar gas pressure (Tomasone et al. 2015a). The work width of the machine is 1.6 meters, therefore the flames reach the bed and the adjacent areas on both sides of the row (Figure 2).

In the fern growth phase, mechanical cultivation is used for inter-row weed control, instead selective intra-row flaming can be applied to handle the weeds developing along the row, to avoid weed maturity and seed dispersal.

Fig. 1: Weeds emerging in the harvest phase

Fig. 2: Broadcast flaming in the harvest phase

The aisles between the rows gradually close as the ferns develop. A compact flaming machine, mounted in front of a compact tractor, was specifically designed to access the narrow aisles (Figure 3). The tested *intra-row flamer* (model PFV-D) is provided with a double boom for treating both sides in one pass (Figure 4).

On each boom, 8 burners are assembled in two groups each with four torches. The inclination of the torches can be adjusted to optimize the coverage of the bed area. A laminar air flow is coupled to the flaming apparatus, providing an air curtain barrier to hold down the heat and protect the fronds. The ventilation openings are aligned above the torches and connected to a fan blower, powered by a hydraulic pump connected to the tractor's PTO. (Tomasone et al. 2009; Tomasone et al. 2010). The diameter of the nozzles on the burner is 1.2 mm, the gas pressure at work is set at 1.2 bar, giving a nominal LPG flow rate of 2.1 kg/h. As the tractor moves in the field along adjacent aisles, the treatment is completed on both sides of the bed.

Fig. 3: Side view of the front-mounted flamer

Fig. 4: Double boom intra-row flamer at work

At the end of the season the fronds turn yellowish and the plants dry out. At this stage the foliage must be cut at ground level. The residues are shredded mechanically and either left on the ground or otherwise burned in the field

To improve the combustion of crop residues, in this time of season when field moisture levels are generally high, the *broadcast flaming machine* (model

PTR-C) can be used to promote the ignition of previously shredded fern residues (Figure 5). The treatment speed is fast and the gas pressure is high (6 km/h speed, 1.4 bar) to produce the powerful flames needed to dry out and burn the debris.

A recent innovation was designed for the end-of-season treatment. It is a tractor-mounted *combined machine* (model PTR-K), having a front shredding unit coupled with a rear broadcast flaming unit (Figure 6). The two operations, shredding and flaming, are carried out in a single-step, thus reducing field passes and work time (3 km h^{-1} speed, 1.0 bar gas pressure). The flail shredder carries standard "Y" shaped flails, total cutting width is 1.4 m across and power requirement is 26-30 kW. The heating unit is directly connected behind the flail shredder. The combustion chamber is closed above by an insulated stainless steel hood. The flames are fired by an array of 8 burners, evenly spaced across on a single row manifold. The temperature under the chamber is constantly held above 650 °C (Tomasone et al., 2015b).

Fig. 5: Flaming to promote fern combustion

Fig. 6: Single-step shredding and flaming ofern

RESULTS AND DISCUSSION

The flaming machines have been tested in the field trials and suitable operative parameters have been identified for applying the heat treatment in the different growing phases. The rate of application of lpg, i.e. The kilograms of gas per hectare, was the main parameter considered. Lpg application rate was obtained by adjusting the tractor's forward speed and the pressure of the gas flow. The appropriate work parameters identified in the tests are shown in table 1 for the different machines and for the treatments made in the different growing phases.

Broadcast flaming can be used in spring before the spears emerge to effectively control early-germinating weeds at an early phase and reduce early-season weed competition. One or more pre-emergence heat treatments can assure a weed-free bed from the beginning (diver, 2002). Flaming can be applied regardless of wet soil conditions, common in spring, and without further soil disturbance that would bring new weed seeds to the surface.

Effective control of weeds is achieved in the harvest phase by applying multiple broadcast flame treatments over the whole surface of the asparagus bed.

The time of exposure to the flames, of the emerging spears, must be kept to a minimum to avoid damage to the partially emerged spears. Appropriate timing is critical for control during the harvest period, subsequent passes are repeated at different time intervals, depending on weed pressure throughout the season. The appropriate work parameters (gas rate, treatment speed, gas pressure, gas consumption) for broadcast flaming (model ptr-c) are shown in table1.

Table 1. Flaming machines used in the different crop phases and appropriate operative parameters

Machine type:	Model	Phase of the crop cycle:	LPG pressure	nozzle diameter	LPG consumption per burner	Number of burners installed	Machine total LPG consumption	Work width	Treatment Speed	Work capacity	Rate of LPG application
			(bar)	mm	(kg/h)	N°	(kg/h)	cm	(km/h)	(h/ha) *	(kg/ha)
Compact broadcast flaming machine	PTR-C	Harvest period	0.8	1.2	1.62	15	24.3	160	5	0.91	22.1
Intra-row flaming on ferns	PFV-D	Fern vegetation	1.2	1.2	2.10	16	33.6	140	4	1.14	38.2
Compact broadcast flaming machine	PTR-C	End of season	1.4	1.2	2.35	15	35.3	160	6	0.76	26.7
Combined shredding and flaming machine	PTR-K	End of season	1.0	1.8	4.22	8	33.8	160	3	1.52	51.2

* Data calculated for a field having a distance between rows of 2.2 m (inter-row spacing)

Intra-row flaming applied in the fern phase has shown to selectively eliminate weeds and prevent seed dispersal. The compact front-mounted flaming machine safely moves through the narrow aisles. The laminar air flow provides a barrier against the heat and protects the fronds. After the ferns have put on sufficient vegetative growth, their stems become resistant to heat and the plants can easily withstand repeated treatments. Optimal speed, LPG pressure and LPG dosage rate for model PFV-D are shown in table 1.

At the end of the growing cycle, the use of the *broadcast flaming machine* promotes the ignition of the shredded residues, improving the complete combustion of crop debris. Optimal parameters for model PTR-C are also shown in table 1. Furthermore the combined machine, model PTR-K, can be conveniently used at the end of the season. The combination, in a single pass, of fern shredding and residue burning improves the combustion of residues because

they are delivered directly into the flames. The residues flow through the heat chamber and ignite before they drop to the ground. The combined treatment streamlines the organization of field work, allowing a timely clean-up of the field while reducing inoculum pressure. Flaming of residues has shown some efficacy towards phytosanitary issues (Tomasone et al. 2015b). Leaving the residues in the field increases disease inoculum, affecting in particular foliar diseases of asparagus, such as rust and purple spot (Hausbeck et al. 2008; Johnson 1990). Plant debris can also harbor insect pests such as eggs of the asparagus aphid (Folwell et al. 1990). Optimal treatment parameters for model PTR-K are indicated in table 1.

CONCLUSIONS

The broadcast flaming machine is conveniently used for selective weeding of the asparagus bed throughout the harvest period. No treatment residues are produced by the flame treatment and therefore no interruption of harvest operations is required. In the fern phase the heat treatment can be effectively applied for selective in-row flaming. The flames blow through the relatively heat-tolerant asparagus stems, selectively killing the weeds.

This physical method is a significant tool for in-row weed control, available for organic farmers as a non-chemical option. Broadcast flaming used at the end of the crop cycle improves the ignition and combustion of fern residues, but it requires a two-step process (shredding first). The combined machine is a further innovation that reduces work time and field traffic. In both cases the intense heat provided by flaming, in addition to the heat developed by the burning biomass, drastically improves the destruction of field inoculum and weed seeds laying on the soil's surface. To date, many specialized asparagus farms, in Italy and abroad, are currently using the machines and the methods described. In general 'flaming' is considered a low environmental impact technology, since it avoids the use of chemical herbicides in agriculture. Flaming is a technology that may be used both in conventional and organic farming because LPG gas is allowed by the specific regulations of these production systems.

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DOI: 10.17707/AgricultForest.62.4.05

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EFFECTS OF NON-GENETIC FACTORS ON DAILY MILK PRODUCTION IN AWASSI BREED OF SHEEP IN MACEDONIA

SUMMARY

Several basic – fixed factors on daily milk yield have been examined in total of 132 Awassi breed sheep, during two – year production period (2011 and 2012). The examination includes the following factors: lactation number, lactation type, date of milk recording, number of newborn lambs, month or year of milk recording and length of suckling period. Their influence on individual milk yield measured in morning, afternoon and evening milking, total daily milk yield and percentage of milk fat has been examined. A total of 904 lactation tests in sheep, aged from first to eight lactation were included in the research. All data were analyzed using a multi-factorial fixed model.

The influence of certain factors was studied using the F-test. Analyzes were made using the SSPS set of programs. Most of the factors (number of lactation, date of milk recording, month or year of milk recording and length of suckling period), except the number of newborn lambs or fertility, had a highly significant influence ($P<0.01$) on daily milk production (milk from the morning, afternoon and evening and total amount of milk, as well as % of milk fat) in examined breed of sheep. Highest daily milk production was determined among sheep in sixth lactation and lowest among those in eighth lactation. On the other hand, the highest percentage of milk fat was determined among sheep in seventh lactation and the lowest among those in second lactation.

Three types of lactation curve were identified in examined sheep of which most abundant is the one with one peak. These data suggest that the Awassi breed, as breed with high milk production, retains the level of high and standard milk production to an older age, which is a prerequisite for profitable and sustainable livestock production.

Keywords: Awassi, daily milk yield, influence of factors, lactation curve

INTRODUCTION

Awassi breed of sheep as local fat-tailed sheep from Middle East is dairy breed with unique physiological characteristics. Due to its high production of

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

milk, Awassi breed is used as parent breed during advancing of milk production in many local breeds of sheep and breeds with low milk production from Asian and African continent (Gursoy et al., 1988).

The original non-selective type of this breed had extremely low production traits with low milk yield, which makes it equal with indigenous breeds of sheep in Macedonia (ovchepolian and sharplanina sheep). However, through systematic genealogic selection for high milk yield and infusion of blood from East-Friesian sheep with intensive diet, it was created a type of this breed, with high milk production. This breed of sheep is imported in Macedonia in 1969 from Israel. Its existence of many years in this region demonstrates successful acclimatization, with high capabilities for production of sheep milk. It is imported in order to increase the milk yield in domestic breed of sheep, with low milk production, through the method of crossbreeding.

According to data from Food and Veterinary Agency, Department for identification and registration of livestock, of the total number of sheep in Macedonia (around 700.000 sheep), the percentage of sheep with blood of Awassi breed (purebred and crossbreeds) is about 5% (35.000 sheep).

Having regard to the importance of regular and accurate individual milk recording, during selection of dairy sheep population, this work is an attempt to study the basic or fixed factors that affect the daily milk yield in Awassi breed of sheep. In other words, the purpose of this research was to establish the extent and manner in which basic (fixed) factors affect the amount of milk measured during the test day in Awassi breed of sheep.

MATERIAL AND METHODS

Tests were performed in total of 132 Awassi sheep, located on a farm in the village Gradishte, in Kumanovo surrounding. The sheep ranged in age from second to eighth lactation. Individual milk yield in sheep is tested once a month using A4 method, during a period of two lactation years (2011 and 2012). During the test day, milk yield was tested for all milking (morning, afternoon, evening) whose number depends on the period of lactation.

Test of milk production was performed from the moment of weaned the lambs until drying of sheep. A total of 8 milk tests were realized. The basic variable factors during the test were: individual milk yield in sheep measured in the morning, afternoon and evening milking, total daily milk yield and percentage of milk fat.

Each of these factors was examined on total of 904 test days or samples for total sheep i.e. lactations. The influence of several basic or fixed factors was examined on the following so called variable factors: date of birth of lambs, lactation number, month of milk test, percentage of milk fat, number of newborn lambs.

The statistical analysis of data is performed according to the following fixed LS - model:

$$Y_{ijklmn} = \mu + L_i + T_j + TD_k + NL_l + MY_m + SP_n + e_{ijklmn}$$

Where:

Y – determination of one of five described variable factors or traits,
μ – average for that trait,
L_i – effect of i-th lactation (i = 1, 2, 3, 5, 6, 7, 8),
T_j – effect of j-th type of lactation curve (j = 1, 2, 3),
TD_k – effect of k-th test day (k = 1, 2, 3, 4, 5, 6, 7, 8, 9),
NL_l – effect of l-th number of newborn lambs (l = 1, 2),
MY_m – effect of m–th month of milk yield measuring (m = 1,….16),
SP_n – effect of prolongation of suckling period on n-th sheep for lactation l measured as linear regression and
e_{ijklmn} – represents residual for record of effects that are not observed.

The statistical significance of effects was determined using F-test.

RESULTS AND DISCUSSION

Average production

Average value of the five examined traits i.e. factors, are presented in Table 1. The milk yield is reduced from 0.59 l in the morning, to 0.40 l in the afternoon and 0.39 in the evening. The average total daily milk yield was 1.36 l with fat content of 7.59%.

These average indicators for productivity characterize Awassi breed of sheep, as a typical representative of specialized breeds of sheep for milk.

Table 1. Estimates of LS-analyses of the daily milk yield of the Awassi ewes

Trait	Mean	SE	95% Confidence interval	
			Lower Bound	Upper Bound
Milk yield – morning, l	0.594	0.027	0.541	0.646
Milk yield – mid day, l	0.406	0.017	0.371	0.440
Milk yield – evening, l	0.393	0.020	0.353	0.433
Milk yield – total, l	1.359	0.053	1.255	1.463
Fat, %	7.587	0.120	7.352	7.822

Influence of different factors on milk production

The following factors have significant influence during the examination:

– Number of lactation, which represents the influence of age,

– Number of test day, which represent the stage of lactation,

– Month and year of measurement, that represent the average or moderate influence on diet, climate and breeding conditions and

– Suckling period, which represents the stage of lactation, after the start of milk test (Table 2).

Table 2. Influence of the effects on the daily milk yield traits, F-statistics

Source	Df	Milk morning	Milk midday	Milk evening	Milk daily	Fat, %
Lactation	6	3.151^{**}	3.056^{***}	1.858^{ns}	3.929^{***}	0.602^{ns}
Type	2	5.517^{**}	2.537^{ns}	3.343^{*}	5.276^{**}	0.182^{ns}
Test day	8	2.945^{**}	3.868^{***}	2.758^{***}	5.180^{***}	1.100^{ns}
No of lambs	1	1.836^{ns}	2.891^{ns}	0.608^{ns}	0.002^{ns}	0.015^{ns}
Month/year	15	10.088^{***}	5.519^{***}	6.412^{***}	4.617^{***}	35.563^{**} *
Suckling period	1	11.708^{***}	13.507^{***}	7.061^{***}	15.412^{***}	0.308^{ns}
R Squared		0.682	0.683	0.508	0.705	0.673

*ns – non significant, * – P<0.05; ** – P<0.01; *** – P<0.001

It seems that the number of newborn lambs does not affect the variation of daily milk production, whereas the type of lactation curve only affects some of the measurements of daily milk yield. The influence of all these effects is explained in detail below.

When the selected factors determine 50-70% of the variation of analyzed traits, then the same can be used for future studies for construction of models for genetic evaluations.

Influence of number of lactation

With the increase of age, milk yield slightly increases from first (0,556 l) to third lactation (0,618 l) during morning milking, decreases in fifth lactation (starting from the end of fourth lactation) and reaches a maximum during sixth lactation, after which slightly reduces to 0,45 l, during eighth lactation.

Similar dynamics was determined for afternoon and evening milk yield and for total milk yield in test day (Table 3).

Table 3. Effect of lactation number on the daily milk yield traits, LS-means ± SE

Lact	Milk morning	Milk midday	Milk evening	Milk daily	Fat, %
1	0.556±0.037	0.378±0.024	0.389±0.028	1.274±0.073	7.551±0.166
2	0.593±0.029	0.377±0.019	0.389±0.022	1.325±0.058	7.455±0.131
3	0.618±0.035	0.425±0.023	0.418±0.027	1.426±0.070	7.564±0.160
5	0.588±0.041	0.389±0.027	0.400±0.032	1.344±0.083	7.599±0.187
6	0.707±0.035	0.458±0.023	0.418±0.027	1.549±0.070	7.596±0.160
7	0.646±0.040	0.448±0.026	0.449±0.031	1.533±0.080	7.765±0.182
8	0.448±0.074	0.364±0.048	0.286±0.055	1.063±0.142	7.578±0.321

These results show that Awassi sheep can be successfully used as heads for production, continuously, in order to preserve sufficient milk productivity. Considering that these examinations are preliminary, we suggest such examinations to be realized in greater number in future, for multiple age groups and classes, in order to achieve more accurate and more reliable statistical analysis. The dynamics of the percentage of milk fat has the same tendency as milk yield i.e. an increase from 7.5% during first to 7.8% during seventh lactation, but it seems that differences between age groups are not significant for this trait. This is confirmed by insignificant effect of the factor, number of lactation in ANOVA (Table 2).

Simultaneous increase of milk production and content of fat with the increase of age, although contradictory, may be a result of parallel development of mammary gland, giving the ability to maintain both high productivity and quality of milk.

The results show that the influence of age is important while setting appropriate models for genetic evaluation. The limited number of sheep in flock would require inclusion of as many heads and similar data for all lactations, in order to achieve higher accuracy in the process of evaluation.

Pacinovski et al. (2014) also found significant influence of lactation on above mentioned traits (morning, afternoon and evening milk) in Awassi breed of sheep.

Influence of the type of lactation curve

The obtained results of analyzed data show the existence of three types of lactation curves in examined Awassi sheep: first type (11) with one pik value, second type (21) also with one pik value and third type (22) with two pik values, during lactation. However, in tested flock, in most cases (around 350 measurements) are sheep of type 21 (Table 4).

Highest milk yield is determined in the second type, with two pik values (22), whereas the most common type (21) shows average productivity.

Such scheme of influence was observed at all traits of milk production: morning, afternoon, evening and total amount of milk for the test day (Table 5).

When determining the statistical influence, significant influence of this factor (type of lactation curve) was determined during morning and evening milking.

Insignificant influence of the type of lactation was determined on the percentage of fat. Obtaining and providing more data will probably be necessary to extract more reliable and more categorical data. Overall, it seems that the effect of the lactation type is not very important as a factor that should be included in the model, but it is important as an indication or criteria for future observation and possible eventual selection.

Table 4. Number of milk yield tests per trait

Factor	Code	No	Morning	Midday	Evening	Daily	Fat%
Total		399	382	372	394	395	394
Lactation	1	80	76	74	79	80	80
	2	131	126	123	128	128	127
	3	73	69	66	73	73	73
	5	35	33	33	34	34	34
	6	38	37	36	38	38	38
	7	34	34	33	34	34	34
	8	8	7	7	8	8	8
Type	11	25	21	21	22	22	22
	21	359	346	337	357	358	357
	22	15	15	14	15	15	15
Test-day	1	55	55	55	55	55	55
	2	55	55	55	55	55	55
	3	55	55	55	55	55	55
	4	55	55	55	54	55	55
	5	51	51	49	51	51	51
	6	47	47	44	47	47	47
	7	41	31	30	40	40	40
	8	25	20	16	24	24	24
	9	15	13	13	13	13	12
No lambs	1	332	318	310	327	328	327
	2	67	64	62	67	67	67
Month and year of test	12012	10	10	10	10	10	10
	22011	15	15	15	15	15	15
	22012	23	23	23	23	23	23
	32011	15	15	15	15	15	15
	32012	26	26	26	26	26	26
	42011	18	18	18	18	18	18
	42012	29	29	29	29	29	29
	52011	21	21	21	21	21	21
	52012	33	33	33	32	33	33
	62011	22	22	22	22	22	22
	62012	33	33	33	33	33	33
	72011	22	22	22	22	22	22
	72012	33	33	33	33	33	33
	82011	22	21	21	21	21	21
	92012	33	20	10	33	33	33
	92011	22	21	21	21	21	21
	102011	22	20	20	20	20	19

Table 5. Effect of type of lactation on the daily milk yield traits, LS-means ± SE

Type	Milk morning	Milk midday	Milk evening	Milk daily	Fat, %
11	0.484±0.046	0.367±0.030	0.323±0.035	1.145 ± 0091	7.596±0.206
21	0.584±0.022	0.387±0.014	0.396±0.017	1.338±0.043	7.650±0.098
22	0.713±0.053	0.463±0.035	0.459±0.041	1.595±0.106	7.515±0.241

According to Dimov (1986) there are three types of lactation curves in dairy breed of sheep: first type, when maximal daily milk yield is measured after the first test, so called. "Cow type", second type, when maximal daily milk yield is measured during first milk test and third type, when during lactation appears second pik value.

Influence of number of milk test

The effect of number of milk test actually shows the stage of lactation. According to most previous examinations in dairy breed of sheep, the average daily milk yield reduces from the beginning to the end of lactation. The decrease was clear and significant for each of the four traits of milk yield (Table 6).

Table 6**.** Effect of consecutive test day on the daily milk yield traits, LS-means ± SE

TD	Milk morning	Milk midday	Milk evening	Milk daily	Fat, %
1	0.781±0.048	0.520±0.031	0.540±0.037	1.848±0.097	7.511±0.219
2	0.740±0.043	0.519±0.028	0.495±0.033	1.755±0.086	7.507±0.194
3	0.686±0.039	0.490± 0.026	0.444±0.031	1.613±0.079	7.388±0.179
4	0.604±0.037	0.435±0.024	0.433±0.029	1.483±0.076	7.365±0.171
5	0.570±0.039	0.397± 0.026	0.374±0.030	1.328±0.078	7.642±0.178
6	0.534±0.042	0.348±0.029	0.330±0.033	1.176±0.085	7.802±0.193
7	0.494±0.052	0.344± 0.034	0.336±0.038	1.071±0.097	7.912±0.220
8	0.519±0.068	0.300±0.050	0.308±0.049	1.043±0.127	7.642±0.289
9	0.415±0.091	0.297±0.059	0.274±0.071	0.915±0.184	7.515±0.423

Having in mind that daily milk yield continuously decreases, at the end of lactation keeps a level of around one liter. This trait is very important and characterizes these sheep as typical dairy type, with strong genetic potential for milk production in an extended period. In most examinations of local breed of dairy sheep, the period of lactation is significantly shorter and the milk yield reaches 0.2–0.3 l at the end of lactation (Astruc et al., 2000, Astruc et al., 2002).

The percentage of fat content also varies throughout the lactation period, but no particular dependence was determined because the influence was insignificant (Table 2). Certain change in reducing the percentage of milk fat from 7.5% in the first to 7.4 in third and fourth test, with an increase in the

following controls to 7.8 or 7.9% and re-reduction at last tests is difficult to explain and predict.

In other examinations, the expected tendency of this trait is increase of the percentage of milk fat by reducing the milk yield (Baldi et al., 1999).

The maintenance of constant and stable rate of milk fat during lactation characterizes these sheep as quite suitable for production of dairy products with a constant quality. Pacinovski et al. (2014) also found significant influence of number of milk test on above mentioned traits (morning, afternoon and evening milk) in Awassi breed of sheep.

Influence of number of newborn lambs

Most of the examined sheep in the flock had one lamb. The results of the analyses showed that during morning milking, sheep with two lambs had higher milk yield in comparison with sheep with one lamb (Table 7). This tendency was opposite during afternoon and evening milking.

Table 7. Effect of number of lambs born on the daily milk yield traits, LS-means ± SE

No	Milk morning	Milk midday	Milk evening	Milk daily	Fat, %
1	0.573±0.027	0.423±0.018	0.402±0.021	1.358±0054	7.596±0.122
2	0.615± 0.034	0.388±0.022	0.383±0.026	1.361±0068	7.578±0.154

As an effect, the number of newborn lambs per sheep was insignificant for all five traits (Table 2). Therefore it is natural to expect that the milk production will not depend on this trait and we consider that this factor is not necessary to be included in further genetic studies.

Unlike these examinations, Pacinovski et al. (2014) determined significant influence of number of newborn lambs on some of above mentioned traits (evening and total amount of milk, total amount of fat), but on other traits (morning, afternoon milk), this factor had no influence at all.

Influence of month of milk test

In complex models of the factor of milk test, data for average differences in various stages of lactation is expected to be provided, having in mind that the last influence is seen from another factor during examination. At the same time, despite this, certain influence of these two factors may be possible.

The significant influence of month in the year shows that this factor should be included in all studies in order to obtain accurate evaluation for other effects which also represent an interest to be studied (Table 2). The evaluation of certain months show that certain increase of daily milk yield can be expected during June and July for morning and evening milking, due to better pasture conditions, but the results for total daily milk yield do not confirm this hypothesis.

Generally, when considering milk production from individual milking (morning, afternoon and evening), during individual years, some changes between individual months may be observed, but summarizing total daily milk yield

seems to limit that influence (Table 8). Therefore, these analyses derive two conclusions. The first is, if the selection is bases on better record and correction of average effects then measurements of milk yield for certain periods – periods of test day, should represent individual indications or traits.

Table 8. Effect of month of test on the daily milk yield traits, LS-means ± SE

MY Test	Milk morning	Milk midday	Milk evening	Milk daily	Fat, %
22011	0.580±0.072	0.489±0.046	0.365±0.056	1.397±0.145	7.427±0.328
32011	0.603±0.066	0.475±0.043	0.416±0.052	1.462±0.134	6.598±0.303
42011	0.525±0.059	0.404±0.038	0.464±0.046	1.366±0.119	7.037±0.270
52011	0.509±0.053	0.387±0.035	0.385±0.042	1.242±0.107	8.838±0.244
62011	0.473±0.051	0.366±0.033	0.420±0.040	1.239±0.103	7.897±0.234
72011	0.575±0.051	0.301±0.033	0.312±0.040	1.182±0.103	8.185±0.234
82011	0.454±0.055	0.286±0.036	0.274±0.042	1.054±0.108	8.106±0.244
102011	0.337±0.073	0.229±0.047	0.218±0.057	0.822±0.146	8.843±0.331
12012	0.872±0.080	0.518±0.052	0.666±0.062	2.015±0.161	8.134±0.365
22012	0.799±0.057	0.584±0.037	0.455±0.044	1.804±0.114	5.767±0.258
32012	0.790±0.050	0.583±0.032	0.345±0.039	1.690±0.100	7.785±0.227
42012	0.861±0.045	0.409±0.029	0.378±0.035	1.618±0.091	6.948±0.207
52012	0.833±0.042	0.399±0.028	0.360±0.033	1.548±0.085	6.512±0.193
62012	0.570±0.042	0.378±0.028	0.450±0.033	1.383±0.085	8.519±0.194
72012	0.492±0.044	0.384±0.029	0.401±0.034	1.293±0.088	8.578±0.200
92012	0.492±0.053	0.428±0.044	0.497±0.037	1.083±0.097	5.279±0.219

The second one is that the type – model for genetic evaluations, which is based on milk yield of separate milking during the test day, has not yet been discussed in literature. Pacinovski et al. (2014) determined significant influence of month of milk test on above mentioned traits (morning, afternoon and evening milk) in Awassi sheep and similar results obtained Alkass et al., (2008) in Awassi sheep in Iraq. More detailed studies and gathering more information will be necessary in future to compare with the model of daily milk yield, as correlations and genetic relations between individual measurements of milk production during the test day.

CONCLUSIONS

The milk yield of sheep from recognized organizations of Awassi breeders or so called Breeding organization for Awassi, during individual test days depends significantly on the number of lactation or age, number of test and month i.e. year.

Milk yield is at certain high level to seventh lactation i.e. to an age of 9. Sheep are with long milking period from 8-9 tests or months (240-270 days), with suckling period of around 60 days.

Changes in daily milk yield, affected by environmental factors, are more noticeable during individual measurements of the test day (morning, afternoon and evening milk), compared to total daily milk yield in the same day.

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DOI: 10.17707/AgricultForest.62.4.06

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SOLANACEAE DIVERSITY IN VIETNAM: A PRELIMINARY TAXONOMIC INVENTORY FOR CONSERVATION AND UTILIZATION

SUMMARY

Solanaceae plays an important role in providing food, vegetables, spices, medicine, and ornamentals in Vietnam. However, solanaceous genetic resources are facing serious threats of erosion and eradication. Our objective is to give a survey on the diversity as well as an evaluation of the role of solanaceous indigenous plants in the local communities to identify the current status and its importance for conservation and sustainable utilization in the future. The data comes from a survey and a collection in central Vietnam as well as herbarium specimens from the collections of Solanaceae in Vietnam. An analysis of these specimens and the result from the survey show that Solanaceae in Vietnam includes 15 genera with 63 species. Besides, there are 6 species belonging to 5 genera being in question regarding the taxonomy. Three genera, *Solanum* (31 species), *Lycianthes* (7 species) and *Physalis* (5 species) represent 68% of the total number of species. Among the 63 recorded species, there are 29 wild species, 22 cultivated species and 12 species being wild and cultivated. 6 species are used as fruits, 41 species as medicine, 16 species as ornamentals, 4 species as spices and 22 species as vegetables. The indigenous solanaceous plants include 24 species belonging to three genera: *Lycianthes* (7 species), *Solanum* (13 species) and *Tubocapsicum* (1 species). The indigenous solanaceous species in Vietnam are mostly wild species, among them there are three endemic species as *Lycianthes baviensis* V. V. Hop, *Solanum robinsonii* Bonati and *Solanum thorelii* Bonati.

Keywords: Solanaceae, diversity, genetic resource, species richness, Vietnam

INTRODUCTION

Solanaceae is one of the most important families of flowering plants to human beings with considerable value in economical, agricultural, and medicinal respects (Bennett, 2010; Knapp et al., 2004; Wiart, 2006), especially valuable they are as vegetable crops (Hawkes, 1999; Mueller et al., 2005; Samuels, 2015).

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

In Vietnam, solanaceous plants play an important role in providing food, vegetables, spices, medicine and ornamentals as well as improving income for the local people (Chi, 1997; Ho, 1999; Hop and Phuong, 2003). In recent years, the solanaceous genetic resources in Vietnam are still facing serious threats of erosion and eradication. The main reasons are the fast replacement by new, high-yielding crop varieties and the intensive farming process, human activities as destruction and non-rational exploitation of forests and lands, urbanization and modernization, the pressure of population with growing food demands, natural disasters along with negligence and insufficient management of humans (Sen and Trinh, 2010; Trinh, 1996).

The Solanaceae are ranked the eighth of 37 families in valuable and rare species that are needed to conserve in Vietnam including 107 rare genetic resources belonging to 26 species and 3 genera (MARD, 2005a), however, conservation of these species received very little attention so far (Tuong et al., 2010; Tuong et al., 2013). One of the biggest obstacles for the conservation of indigenous species including solanaceous species are incomplete statistics, classification and evaluation of them. This would be necessary especially for wild species having close relationships with crops in different ecological regions in Vietnam to provide information for ex-situ or in-situ conservation (Catacutan et al., 2014; Hue et al., 2012). Besides, determining and selection of important indigenous solanaceous genetic resources for conservation have not been done. Therefore, the utilization of germplasm collections, particularly in the wild species, is still very limited (PGRV, 2012; PGRV, 2014; Tuong, 2014; Tuong et al., 2010). The objective of this study is to give a survey on the diversity as well as the evaluation of the role of solanaceous indigenous plants in the local communities to identify the status as well as importance of them for conservation and sustainable utilization in the future.

MATERIALS AND METHODS

The study was designed as an investigation based on the results of a survey and collection in 5 provinces in central Vietnam (Figure 1) from September 2013 to February 2014 and the data come from 308 herbarium specimens from the collections of Solanaceae in Plant Genetic Resource in Vietnam.

The provinces were selected for the survey based on eco-geographic data (Guarino et al., 2005). Identification of Solanaceous species used the method of comparing morphological characteristics with using the identification keys by Ho (1999), Hop (2006) and Zhang (1994). Useful solanaceous plants were determined by surveys and interviews with 155 randomly-selected households base on questionnaire. Additionally, special collecting trips were organized with indigenous experts: traditional doctors, village elders and other local people who knew about useful plants. The information was also checked and compared with documents on medicinal plants by Loi (1995), Chi (1997), Ho (1999) and Batugal (Batugal et al., 2004).

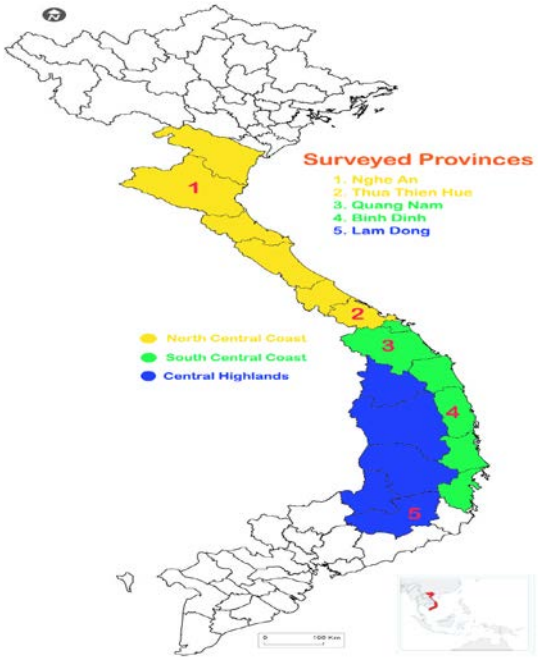

Figure 1. Surveyed provinces in central Vietnam

Classification is based on system by Hunziker (2001). Solanaceous species are re-identified base on detail descriptions and they also updated scientific name according to The Plant List (2016) (version 1.1).

RESULTS AND DISCUSSION

According to solanaceous system by Hunziker (2001), Solanaceae in Vietnam can be classified into 2 subfamilies (*Cestroideae*, *Solanoideae*) with 7 tribes (*Cestreae*, *Nicotianeae*, Francisceae, Browallieae, Datureae, Lycieae, Solaneae) and 5 subtribes (*Nicotianinae*, *Physalinae*, *Iochrominae*, *Capsicinae*, *Solaninae*) with 15 genera (*Cestrum*, *Nicotiana*, *Petunia*, *Brunfelsia*, *Browallia*, *Datura*, *Brugmansia*, *Lycium*, *Physalis*, *Tubocapsicum*, *Capsicum*, *Solanum*, *Cyphomandra*, *Lycopersicon*, *Lycianthes*). In addition, there is one genus (*Atrichodendron*) thats affinity with Solanaceae is debated.

The table 1 shows that the Solanaceae in Vietnam are represented by 15 genera with 63 species. Besides, there are 6 species belonging to 5 genera being doubtful (Table 2). *Solanum* and *Lycianthes* are the most diverse genera. Three genera, Solanum (31 species), Lycianthes (7 species) and Physalis (5 species) represent 68% of the total number of species. *Capsicum* (3), *Cestrum* (2), *Datura* (3), *Lycium* (3) and *Nicotiana* (2) group only few species. *Browallia*, *Brugmansia*, *Brunfelsia*, *Cyphomandra*, *Lycopersicon*, *Petunia* and *Tubocapsicum* are represented by only 1 species each (Table 1 and Figure 2).

Comparing with some countries in Asia the number of genera and species of solanaceous plants in Vietnam is diversity of species (Figure 3). Some genera belonging to Solanaceae are not found in Vietnam as *Anisodus, Atropa, Atropanthe, Hyoscyamus, Mandragora, Nicandra, Nierembergia, Przewalskia, Physaliastrum, Physochlaina, Salpiglossis, Schizanthus, Scopolia, Solandra* and *Withania*. However, the number of species in *Solanum* is quite large with 31 species (only smaller than China and Taiwan).

Table 1. Genera and species of Solanaceae in Vietnam

Sub-family	Tribe	Subtribe	Genus[a]	The number of species [b]
Cestroideae	*Cestreae*		*Cestrum*	2
	Nicotianeae	*Nicotianinae*	*Nicotiana*	2
			Petunia	1
	Francisceae		*Brunfelsia*	1
	Browallieae		*Browallia*	1
Solanoideae	*Datureae*		*Datura*	3
			Brugmansia	1
	Lycieae		*Lycium*	3
	Solaneae	*Physalinae*	*Physalis*	5
		Iochrominae	*Tubocapsicum*	1
		Capsicinae	*Capsicum*	3
		Solaninae	*Solanum*	31
			Cyphomandra	1
			Lycopersicon	1
			Lycianthes	7

[a] Genus *Atrichodendron* is not mention in this table.
[b] Not including the species which being doubtful

Table 2. List of doubtful taxon belong to Solanaceae in Vietnam

Genus	Scientific name	Reasons
Atrichodendron	*Atrichodendron tonkinense* Gagnep.	no herbarium specimen, can belong to Boraginaceae (Ho, 1993)
Cyphomandra	*Cyphomandra godefroyi* Bonati	leaves and interpetiolar stipules characteristic of Rubiaceae (Bohs, 1994)
Lycianthes	*Lycianthes denticulata* (Blume) Bitter.	no herbarium specimen, unknown distribution
Lycium	*Lycium cochinchinensis* Lour.	no herbarium specimen, excluded from this genus (Zhang et al., 1994)
Solanum	*Solanum mauritianum* Scop.	no herbarium specimen, unknown distribution
	Solanum virginianum L.	doubt about existence

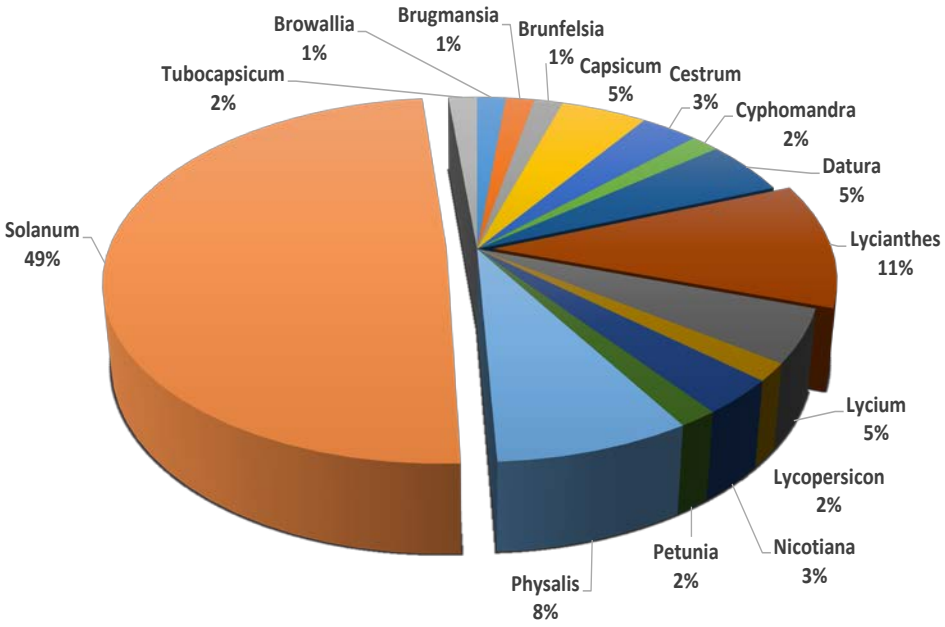

Figure 2. Percentages of genera of Solanaceae family in Vietnam

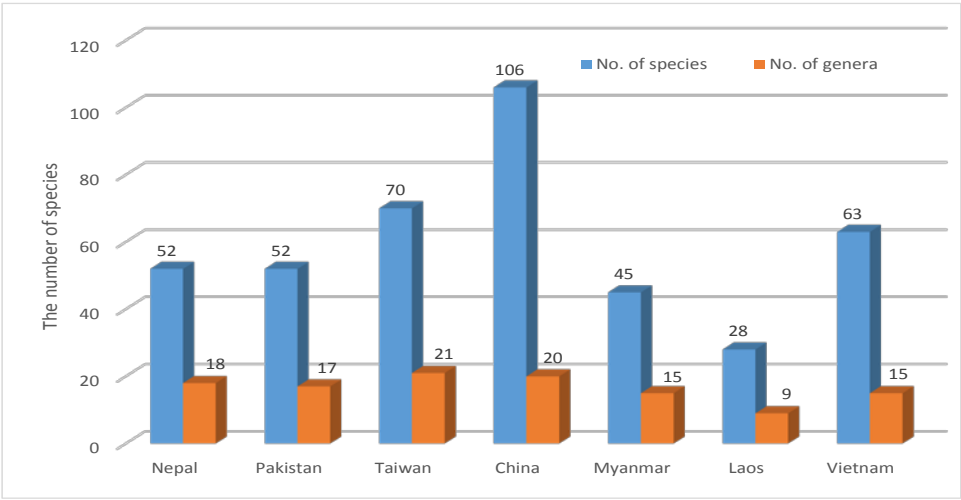

Figure 3. Number of genera and species of Solanaceae family in some countries in Asia

There are 29 wild species, 22 cultivated species and 12 species being wild and cultivated (Table 3 and Figure 4). Genus *Solanum* is highly diverse regarding morphological characteristics as well as its distribution in nature and species of *Solanum* are found everywhere from mountains areas to delta areas. Some wild species are distributed everywhere as *Solanum nigrum* L. and *Solanum torvum*

Sw. while some species are only distributed in the mountains areas as *Solanum erianthum* D. Don.

This genus has many species, which are very similar so it is easy to have confusion as *Solanum capsicoides* or *Solanum viarum* (Hop, 2006). Genus *Lycianthes* has 20 native species in South East Asia (Hunziker, 2001) in which 7 wild species in North Vietnam. This shows that Vietnam is also one of the central areas of this genus in Asia (Hop, 2006; Zhang et al., 1994).

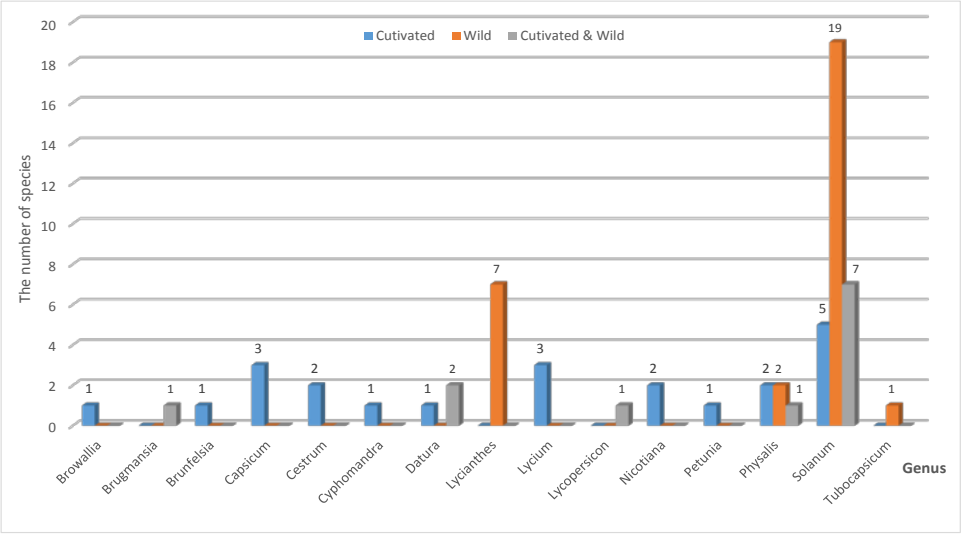

Figure 4. Status of solanaceous species in Vietnam

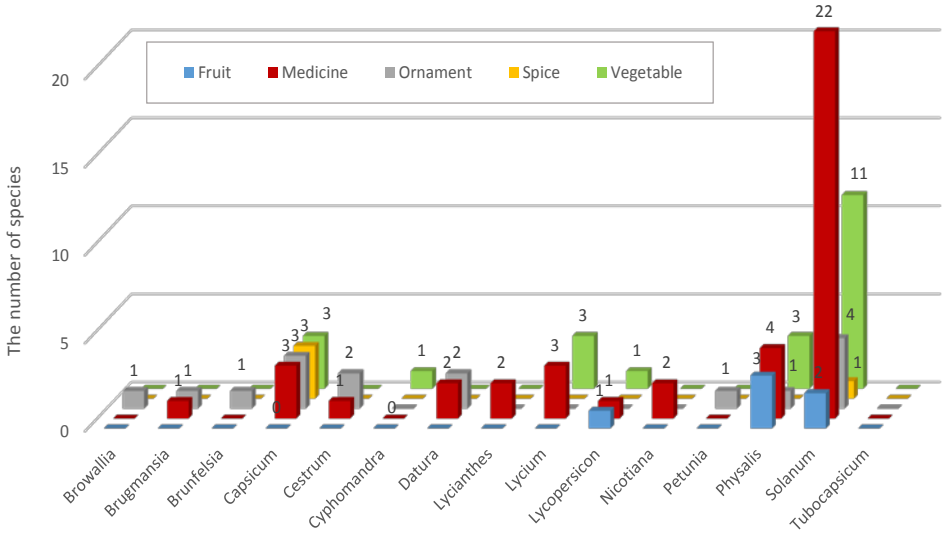

Figure 5. Value of solanaceous plants in Vietnam

Table 3. Species of Solanaceae in Vietnam

Species	Voucher collection	Bio. status[a]	Type[b]	Used[c]
Browallia americana L.	Lâm Đồng, V. V. Hợp 19 (HN)	C	I	O
Brugmansia suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl	Lâm Đồng, N. Đ. Khôi 20 (HN)	C, W	I	M, O
Brunfeldsia pauciflora (Cham. & Schlecht) Benth.	Phú Thọ, V. X. Phương 7861 (HN)	C	I	O
Capsicum annuum L.	Nghệ An, N. N. Thìn 550 (HNU)	C	I	M, O, S, V
C. baccatum	None, recorded in this study	C	I	M, O, S, V
C. frutescens L.	V. X. Phương 3705 (HN)	C	I	M, O, S, V
Cestrum elegans (Brongn.) Schlechter	None, recorded by Ho (1993) and Nhan (1996)	C	I	O
C. nocturnum L.	Hà Nội, T. Đ. Lý 1 (HN)	C	I	O, M
Cyphomandra betacea (Cav.) Sendtn.	None, recorded by G. Bonati (1927) and Nhan (1996)	C	I	V
Datura innoxia Mill.	Hà Nội, N. Tập 2610A-B-C (HNPM)	C, W	I	M, O
D. metel L.	Tuyên Quang, V. X. Phương 6991 (HN)	C, W	I	M, O
D. stramonium L.	Hòa Bình, V. X. Phương 1938 (HN)	C	I	M, O
Lycianthes baviensis V. V. Hop	Hà Tây, Lý-Nhan-Vệ 237 (HN)	W	E	
Lycianthes biflora (Lour.) Bitter	Sơn La, V. X. Phương 16150 (HN)	W	N	M
Lycianthes bigeminata (Nees) Bitter	Hà Giang, sine nom. coll. (HN)	W	N	M
Lycianthes laevis (Dunal) Bitter	Sơn La, V. X. Phương 16142 (HN)	W	N	
Lycianthes lysimachioides	Hà Tây, Đoàn Trung Quốc 3802 (HN)	W	N	
Lycianthes macrodon (Wal. ex Nees) Bitter	Cao Bằng, sine nom. coll. 8295 (HN)	W	N	
Lycianthes neesiana (Wall. Ex Nees) D'Acry & Z. Y. Zhang	Hà Tây, Dư-Croat 77996 (HN)	W	N	
Lycium barbarum	None, recorded by Ho (1993)	C	I	M, V
Lycium chinense Mill.	Hà Giang, N. K. Đào 73 (HN)	C	I	M, V
Lycium ruthenicum Murray	Hà Nội, sin nom. coll. & sine num. (HNIP)	C	I	M, V
Lycopersicon esculentum Mill.	Hà Nội, 72HN Bách-Tâm 283 (HN)	C, W	I	F, M, V
Nicotiana rustica L.	Thanh Hoa, V. X. Phương 10902 (HN)	C	I	M
Nicotiana tabacum L.	Lạng Sơn, N. Đ. Khôi 333 (HN)	C	I	M
Petunia hybrida Vilm.	Lâm Đồng, V. V. Hợp 20 (HN)	C	I	O
Physalis alkekengi L.	Hà nội, 72 HN4 sine nom. coll. 233 (HN)	C	I	M, V
Physalis angulata L.	Lạng Sơn, N. Đ. Khôi 327 (HN)	W	I	F, M, V
Physalis minima L.	Kon Tum, N. H. Hiến 275 (HN)	W	I	M, V
Physalis peruviana L.	Lâm Đồng, N. H. Hiến 676 (HN)	C, W	I	F, M
Physalis pubescens	None, recorded by Ho (1993)	C	I	F, O
Solanum album Lour.	Kon Tum, V. V. Hợp 06 (HN)	C	N	M, V
Solanum aethiopicum	None, recorded in this study	C	I	M, V
Solanum americanum	None, recorded by Ho (1993)	W	I	F, M, V

Solanum capsicoides All.	Lạng Sơn, N. V. Phú 7266 (HN)	C, W	I	M
Solanum cyanocarphium Blume	Kon Tum, N. V. Hiến 240 (HN)	W	N	
Solanum diphyllum L.	None, recorded in this study	W	I	M
Solanum dulcamara L.	Ninh Bình, Đoàn KSVT 4805 (HN)	W	I	M
Solanum erianthum D. Don	Hà Giang, DKH 6208 (HN)	C, W	I	M
Solanum ferox L.	Khánh Hòa, Poilane 3245 (HM)	W	N	M
Solanum incanum L.	Lạng Sơn, Hoàng Vệ 15538 (HN)	W	I	M
Solanum involucratum	None, recorded by Ho (1993)	W	N	M, V
Solanum laciniatum Ait.	Hà Nội, N. V. Phú 16300 (HN)	C	I	M
Solanum lasiocarpum Dunal	Nghệ An, Đoàn KSVT 4201 (HN)	W	N	M, V
Solanum lyratum Thunb.	Lạng Sơn, V. X. Phương 3692 (HN)	W	N	M
Solanum mammosum L.	Lào Cai, N. V. Phú 7651 & 7652 (HN)	C, W	I	M, O
Solanum melongena L.	Lạng Sơn, N. V. Phú 16306 & 16307 (HN)	C	N	M, V
Solanum nienkui Merr. & Chun	Đắk Lắk, N. T. Nhan 695 (HN)	W	N	
Solanum nigrum L.	Lào Cai, Đội KS Việt Trung 2565 (HN)	W	I	F, M, V
Solanum pittosporifolium Hemsl.	Cao Bằng, CBL 552 (HN)	W	N	
Solanum praetermissum Kerr ex Barnett	Hà Tây, HPP 20 (HN)	W	N	
Solanum procumbens Lour.	Phú Thọ, N. V. Phú 7658 (HN)	C, W	N	M
Solanum pseudocapsicum L.	Hà nội, V. V. Hợp 05 (HN)	C, W	I	O
Solanum robinsonii Bonati	Khánh Hòa, N. V. Phú sine num. (HN)	W	E	
Solanum seaforthianum Andr.	Hải Phòng, LX-VN 3229 (HN)	C, W	I	O
Solanum spirale Roxb.	Yên Bái, VN 934 (HN)	W	N	M, V
Solanum thorelii Bonati	Lào Cai, Đoàn KSVT 3344 (HN)	W	E	
Solanum torvum Sw.	Điện Biên, DKH 5875 (HN)	W	I	M, V
Solanum trilobatum L.	None, recorded by G. Bonati (1927), Ho (1993), and Nhan (1996)	W	N	M
Solanum tuberosum L.	Hà nội, sine nom. coll. 7220 (HN)	C	I	M, V
Solanum viarum Dunal	Phú Thọ, V. X. Phương 8047 (HN)	W	I	
Solanum violaceum Ortega	Hà Giang, V. X. Phương 305 (HN)	C, W	N	M, V
Tubocapsicum anomalum (Franch. & Sav.) Makino	Lai Châu, DKH 5915 (HN)	W	N	

[a] Biological status: W = Wild; C = Cultivated
[b] Type: ecological state of a species in Vietnam: N = Native/Indigenous; I = Introduced; E = Endemism;
[c] Used: F = Fruit; M = Medicine; O = Ornament/decoration; S = Spice ; V = Vegetable
Abbreviations: HN = Institute of Ecology and Biological Resources, HNU = College of Natural Sciences - Vietnam National University in Hanoi, HNIP = Hanoi University of Pharmacy, HNPM = National Institute of Medicinal Materials.

The wild species belonging to Solanaceae in Vietnam have common features that grow near forests, wastelands, roadsides and thickets so it is quite convenient for humans to exploit and use them, but also easy to be negatively affected by human activities. The valuable species of solanaceous plants include

51 species (80,95 percentage of total species), in which 6 species for fruits, 41 species for medicine, 16 species for ornamentals, 4 species for spices and 22 species for vegetables (Table 3 and Figure 5). The important solanaceous species in Vietnam are *Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Nicotiana rustica* L., *Solanum tuberosum* L. and *Solanum melongena* L.. The most important medicinal species is *Solanum procumbens* Lour.

Table 4 List of indigenous solanaceous species in Vietnam

Botanical name	Common name	Biological status[a]	Distribution[b]	Type[c]	Used[d]
Lycianthes baviensis	Cà ngủ ba vì	W	N	E	
Lycianthes biflora	Cà hai hoa	W	N/M/S	N	M
Lycianthes bigeminata	Cà ngủ cặp đôi	W	N	N	M
Lycianthes laevis	Cà ngủ nhẵn	W	N	N	
Lycianthes lysimachioides	Cà ngủ dạng trân châu	W	N	N	
Lycianthes macrodon	Cà ngủ cuống to	W	N/M/S	N	
Lycianthes neesiana	Cà ngủ ness	W	N	N	
Solanum album	Cà pháo	C, W	N/M/S	N	M, V
*Solanum cyanocarphium**	Cà trái lam	W	M/S	N	
*Solanum ferox**	Cà lông	W	M/S	N	M
*Solanum involucratum**	Cà tổng bao	W	N	N	M, V
*Solanum lasiocarpum**	Cà trái lông	W	N/M/S	N	M, V
*Solanum lyratum**	Cà đờn	W	N	N	M
*Solanum melongena**	Cà	C	N/M/S	N	M, V
Solanum nienkui	Cà cụm hoa dài	W	M	N	
*Solanum pittosporifolium**	Cà lá cườm thảo	W	N	N	
Solanum praetermissum	Cà đài bao quả	W	N	N	
*Solanum procumbens**	Cà gai leo	C, W	N/M	N	M
*Solanum robinsonii**	Cà robinson	W	M	E	
*Solanum spirale**	Cà xoắn	W	N/M	N	M, V
*Solanum thorelii**	Cà thorel	W	N/M/S	E	
*Solanum trilobatum**	Cà ba thùy	W	M/S	N	M
*Solanum violaceum**	Cà dại hoa tím	C, W	N/M/S	N	M, V
Tubocapsicum anomalum	Ớt ống	W	N/M	N	

[a] Biological status: W = Wild; C = Cultivated

[b] Ditribution: Abbreviations: letters for three areas (N = North, M = Middle, S = South) in Vietnam

[c] Type: ecological state of a species: N = Native; E = Endemism

[d] Used: M = Medicine; V = Vegetable;

* Belong to rare genetic resources exchange international in case special (MARD, 2005b)

The indigenous solanaceous plants include 24 species belonging to three genera: *Lycianthes* (7 species), *Solanum* (13 species) and *Tubocapsicum* (1 species) (Table 4).

The indigenous solanaceous species in Vietnam are mostly wild species, in which there are three endemic species including *Lycianthes baviensis* V. V. Hop, *Solanum robinsonii* Bonati and *Solanum thorelii* Bonati.

CONCLUSIONS

The results of this analysis show that the Solanaceae flora of Vietnam is very diverse with 15 genera and 63 species. Solanum and Lycianthes are the most diverse species in Vietnam. Solanaceous species are mostly wild species, but the most cultivated species are introduced to Vietnam and there are 51 valuable species including 6 species for fruit, 41 species for medicine, 16 species for ornament, 4 species for spice and 22 species for vegetable. Economic value is mainly in vegetables and medicines. The indigenous solanaceous plants include 24 species belonging to three genera with three endemic species. Solanum album, Solanum melongena and Solanum procumbens are indigenous species with high value.

ACKNOWLEDGEMENT

We would like to thank to the Vietnamese government (VIED) and German Academic Exchange Service (DAAD) for financial support in this study

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DOI: 10.17707/AgricultForest.62.4.07

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EFFECT OF NITROGEN FERTILIZATION TYPES ON THE SOIL MICROBIAL BIOMASS AND GREENHOUSE GASES EMISSION

SUMMARY

Global climate models shows patterns of temperature and precipitation changes worldwide. Soil moisture and type of fertilization are key determinants of the microbial processes that determine the fluxes of gases from soil. There are not many research activities including the assessment how land conversion to the grassland can influence the greenhouse effect. The aim of this study was to determine the biomass content of microorganisms in soil and CO_2 and CH_4 emissions in conditions of diversified nitrogen fertilization and soil moisture in the cultivation of pot grass mixtures. The results of the study were treated by two-factor analysis of variance. The linear correlation between analysed gases and between microbial biomass and CO_2 or CH_4 emissions was performed. The volume of soil microbial biomass in the cultivation of grass mixtures was affected by the type of nitrogen fertilization and the level of soil moisture. Approximately 1.5 times bigger microbial biomass was found after fertilization than under control conditions. The same relationship occurred in the comparison between the microbial biomass during wet and dry conditions. Only the volume of CO_2 emission in this pot experiment was affected by the type of nitrogen fertilization. Higher emission of CO_2 was accompanied by increased emission of CH_4. In humid conditions, both mineral and organic fertilization affected positively on soil microbial biomass and the volume of CO_2 emission. From the viewpoint of reducing greenhouse gases emission, inorganic fertilizers used in dry conditions during the land conversion to the grassland, would be the best grassland cultivation method.

Keywords: Grassland, microbial biomass, carbon dioxide, methane

INTRODUCTION

Effect of agriculture on the environment involves a number of factors such as the release of chemicals from soil to water and air (Gałczyńska and Kot, 2010;

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Liu *et al.*, 2014). Gaseous carbon compounds such as: dioxide carbon (CO_2) and methane (CH_4) are two the most important greenhouse gases (GHG), present in the atmosphere, which are produced in part by natural sources.

Because GHC prevent heat emitted by the Earth from escaping to space, changes in their atmospheric concentrations can alter the energy balance of the climate system.

According to FAO, in 2010, agriculture was the third largest contributor to global emissions by sector, with CH_4 accounting for just under half of total agricultural emissions, nitrous oxide (N_2O) for 36%, and CO_2 for some 14% (Reynolds, 2013). In 2012 agricultural activities in the EU-28 generated 470.6 million tonnes of CO_2 equivalent (evaluated for CH_4 and N_2O), corresponding to about 9.6% of total greenhouse gas emissions (information on land use, land use change and forestry – LULUCF is excluded).

GHC emissions from agriculture have increased by approximately 23.8% since 1990 (Eurostat, 2016). LULUCF covers GHG emissions into the atmosphere and removal of carbon from the atmosphere resulting from our use of soils, trees, plants, biomass and timber.

Forests and agricultural lands naturally hold large stocks of carbon, preventing its escape into the atmosphere. For example ploughing up grassland generates emissions but conversion of arable land into grassland can result in protection of carbon stocks or even carbon sequestration (Doblas-Miranda *et al*., 2013).

Many papers suggested, that soil moisture and type of fertilization are key determinants of the microbial processes (Natywa *et al*., 2014), that determine the fluxes of GHG from soil (Nannipieri *et al.*, 2003; Skiba *et al*., 2013).

The aim of this study was to determine the biomass content of microorganisms in soil and two gases emissions (carbon dioxide and methane) in conditions of nitrogen fertilization and soil moisture in the cultivation of pot grass mixtures.

MATERIAL AND METHODS

The pot experiment was conducted in 2014 in the greenhouse on West Pomeranian University of Technology in Szczecin. A mix of grasses was grown in the conditions of mineral and organic fertilization and from two humidity levels (dry and wet) at the same time (30 and 60 % of field water capacity).

In the experiment, the soil material collected from a 30 cm deep layer of soil not used for agricultural production for 15 years (sand: 2-0.05 mm - 60.9%, silt: 0.05-0.002 mm - 35.1%, colloidal clay: 0.002 mm - 4.0%) granular metric composition was sandy clay.

Soil material dedicated to the research characterized, by the criteria of IUNG (Obojski and Strączyński, 1995), slightly acidity and low content of available phosphorus, potassium and magnesium (Table 1).

Table 1. Characteristic of soil material

Reaction pH_{KCl}	Salinity g $NaCl \cdot dm^{-3}$	TOC $g \cdot kg^{-1}$	Humus $g \cdot kg^{-1}$	Organic substance $g \cdot kg^{-1}$	N_{tot} $g \cdot kg^{-1}$	Content of bioavailable forms $mg \cdot kg^{-1}$		
						P	K	Mg
5.9	0.37	9.6	16.6	37.6	0.75	28	83	24

*Source: Own study

Vases of the soil material (11 kg) were fed in the middle of May a mineral fertilizer (ammonium nitrate) and organic (slurry) at a dose of 0.355 g N per vase, which corresponds to 50 kg $N \cdot ha^{-1}$. After a few days grass mixtures were sown to vases. Measurements of soil microbial biomass and emissions of carbon dioxide and methane were carried out at the beginning of June and mid-July in the next day after grass cutting. The second dose of nitrogen fertilization was applied (at the same amount) after the first grass cutting.

In the soil samples collected from the vases (in triplicate) the biomass of living micro-organisms in the soil was determined. The measurements were performed with the use of a physiological method defined in the literature as the SIR method (Substrate Induced Respiration), developed by Anderson and Domsch (1978).

The SIR method characterizes the current presence of the microorganisms in the soil. This method is often used in combination with measurements of CO_2 emission (Liu *et al.*, 2014). For this purpose, the soil samples were analysed with a mass of 10 g, which is enriched with extra carbon source in the form of a mixture of glucose and talc (weight ratio 1:5). The amount of glucose was determined by taking into account the initial deviation values for the matrix used. The prepared samples were then transferred to the columns of the analyser Ultragas U4S and measured the amount of CO_2 evolved after three hours. Microbial biomass was calculated using the equation authors methods:

$$x = 40{,}4y + 0{,}37$$

were:

x – the amount of C contained in the biomass of microorganisms per 100 g d.m. soil, mg;

y – maximum initial production CO_2, $cm^3 \cdot (100\ g\ soil \cdot h)^{-1}$.

Measurements of carbon dioxide and methane were carried out using photoacoustic field gas monitor INNOVA 1412 (Burczyk *et al.*, 2008).

The results of the study were obtained with by a two-factor analysis of variance (1^{st} factor – type of fertilization, 2^{nd} factor – level of soil moisture). The linear correlation between analysed gases and between microbial biomass and carbon dioxide or methane emissions was performer.

The significance of the differences between means (Tukey test) and the value of Pearson correlation coefficient at the confidence level of $p = 0.05$ was calculated using *Statistica 12*.

RESULTS AND DISCUSSION

Soil microorganisms constitute less than 0.5% (w/w) of the soil mass, but they play a key role in soil properties and processes. Microorganisms participate in oxidation, nitrification, ammonification, nitrogen fixation, and other processes which lead to decomposition of soil organic matter and transformation of nutrients. Natywa *et al.* (2014) reported, that soil moisture and type of fertilization are very important determinants of the microbial processes. In pot experiment, the volume of soil microbial biomass in the cultivation of grass mixtures was affected by the type of nitrogen fertilization and the level of soil moisture, too (Table 2).

Table 2. Statistical parameters of two-way of the variance analysis for microorganism biomass (A) and carbon dioxide (B) or methane (C) emissions.

Parameters	A or B or C	F	p	$LSD_{0,05}$
Level of soil moisture (I)	A	14.2	0.000	2950
	B	0.26	0.611	-
	C	0.29	0.594	-
Type fertilization (II)	A	5.10	0.009	3106
	B	3.81	0.027	$1.6 \cdot 10^8$
	C	0.44	0.647	-

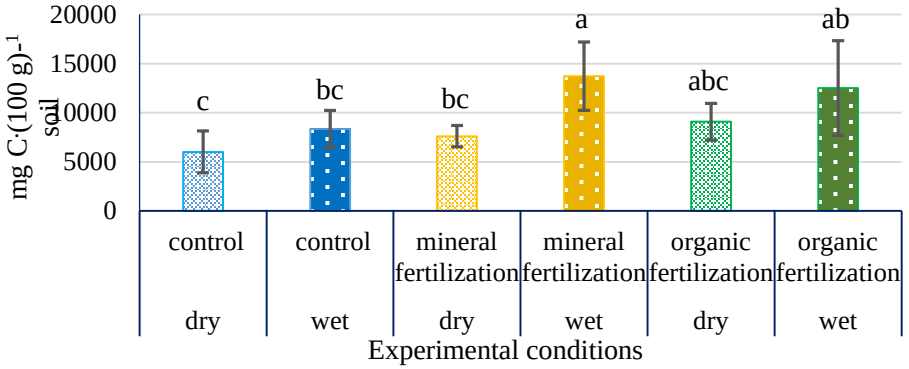
*Source: Own study

Approximately 1.5 times bigger microbial biomass (Fig. 1) was found after fertilization (10731 mg $C \cdot (100\ g)^{-1}$) than under control conditions (7178 mg $C \cdot (100\ g)^{-1}$). It was found that the applied dose of nitrogen fertilisation in the form of ammonium nitrate or slurry (50 kg $N \cdot ha^{-1}$), generally stimulated bacterial growth (Fig. 1).

The influence of mineral fertilisation on the formation of microbial biomass is confirmed by other researchers (Kozanecka *et al*., 1996; Barabasz and Vořišek, 2002). The inhibitory effect of NH_4NO_3 on the total number of bacteria in soil was observed by Kozanecka *et al*. (1996) at a high dose of such fertilization – 240 $kgN \cdot ha^{-1}$.

Generally, it is assumed that slurry is a fertilizer which is comparable to mineral fertilizers in terms of effectiveness and period of activity. It decomposes intensely immediately after application to soil, therefore the long-term effects of slurry on properties of organic matter in soil are rather non-existent.

Yet, contrary to existing opinions, Dębska (2004) found that organic matter in slurry is relatively resistant to decomposition in soil, and research by Mazur and Mazur (2015) indicate that average increase of organic carbon as a result of fertilization in relation to the control group was 1.94 $g \cdot kg^{-1}$ in lessive soil.



Source: Own study

Figure 1. Biomass soil microorganisms depending on the type of experimental conditions (Explanations: error bars are mean ± standard deviation; a, b, ab are homogeneous groups)

Soil water content controls microbial activity and is a major factor that determines the rates of mineralization (Paul *et al.*, 2003, Yan *et al.*, 2015). In analysed experiment average 1.5 times bigger microbial biomass (Fig. 1) was found during wet (11526 C·(100g)$^{-1}$) than during dry conditions (7567 C·(100 g)$^{-1}$). Due to the role of microorganisms in soil processes, their presence and biomass significantly affect the level of CO_2 emission from soils (Nannipieri *et al.*, 2003), which is the result of root respiration and physiological processes of the microorganisms involved in the decomposition of organic material. Emissions of CO_2 from soils appear to be highly variable in heterogeneous soil micro-sites, and they are influenced by the activity of roots, microbial processes, crop residue and litter content, microclimate and catalytic properties of clay colloids (Matteucci *et al.*, 2000).

In this pot experiment, besides two gaseous carbon compounds, only the volume of carbon dioxide emission was affected by the type of nitrogen fertilization (Table 2., Fig. 2 and Fig. 3).

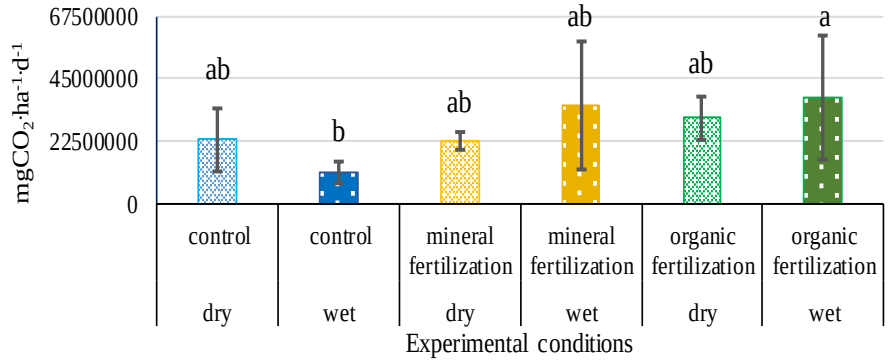


Figure 2. Carbon dioxide depending on the experimental conditions

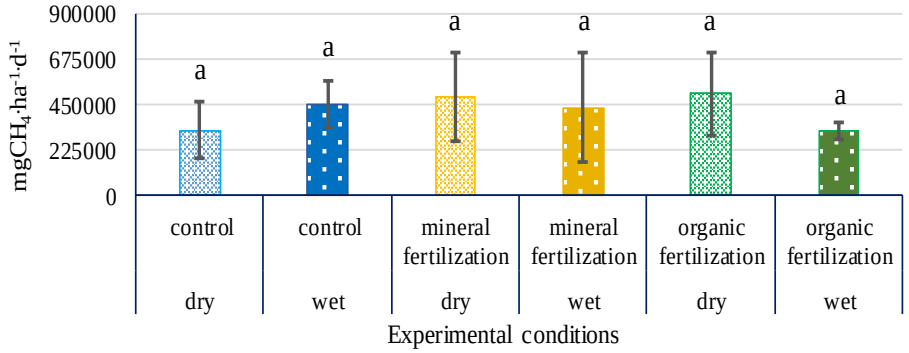


Figure 3. Methane emissions depending on the experimental conditions.

However, generally in dry conditions, higher emission of carbon dioxide was accompanied by increased emission of methane (Table 3).

Table 3. Parameters of statistically significant linear correlation between emissions analysed gases and between biomass soil microorganisms and CO_2 or CH_4 emissions were performed.

Relation, parameters		Experimental conditions					
		c/d	c/w	mf/d	mf/w	of/d	of/w
eCH_4 = $f(eCO_2)$	r	0.8251	-	-	-	0.8727	-
	p	0.0010				0.0002	
$eCO_2 = f(b)$	r	-0.7346	-	-	0.9571	-	0.6342
	p	0.0065			0.0000		0.0268
$eCH_4 = f(b)$	r	-	0.6317	-0.7013	-	-	-
	p		0.0276	0.0110			

Source: Own study

Explanations: e-emission; b-biomass soil microorganisms; c-control; mf-mineral fertilization; of-organic fertilization; d-dry; w-wet

The complex gas cycle of carbon compounds in mineral soil depending on the abiotic and biotic factors should be further researched.

Land cover change in agriculture shows highly contrasting trends in different areas of Europe. The main trend has been towards a conversion of arable land and permanent crops to pasture, set-aside and fallow land (EEA, 2005). The use of knowledge of the environmental results of changes in agricultural land use will affect the assessment of greenhouse gas emission.

CONCLUSIONS

In humid conditions, both mineral and organic fertilization affected positively on soil microbial biomass and the volume of carbon dioxide emission. From the viewpoint of reducing greenhouse gases emission, inorganic fertilizers used in dry conditions during the land conversion to the grassland, would be the best grassland cultivation method.

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DOI: 10.17707/AgricultForest.62.4.08

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BIOLOGICAL STUDIES ON THE AFRICAN FIG FLY, ZAPRIONUS INDIANUS GUPTA (DIPTERA: DROSOPHILIDAE)

SUMMARY

The African fig fly, *Zaprionus indianus* Gupta, is a widely distributed polyphagous drosophilid fly of tropical origin. Its occurrence in Jordan was first reported on date palms from the Central Jordan Valley in June 2012. Studies on biological aspects of a fly population collected from Northern Jordan Valley were carried out under laboratory conditions at 25±1˚C, 75±10% RH, and 14 h photoperiod. Mashed banana fruits with dry active yeast of Saccharomyces cerevisiae were used for the first time as a diet for larval and adult stages. The data obtained showed that the average mating period was 2.5 days, the pre-oviposition period 2.7 days, the oviposition period 42. 7 days, incubation period 24.5 h, hatching of eggs was 91.7%, duration of larval stage 7.4 days, pupal stage 6.8 days, adult male life span 42.2 days, adult female life span was 37.7 days. The larval stage had the highest mortality followed by the pupal stage and then the egg stage. The life cycle lasted 13.9 to 23.2 days with an average of 17.9 days. Emerged adult flies showed a sex ratio of 1.0. The obtained results provided basic data that may help in the management of this pest in Jordan.

Keywords: *Zaprionus indianus*, biology, life cycle, laboratory rearing.

INTRODUCTION

Zaprionus indianus Gupta is a polyphagous drosophilid fly of tropical origin (Pasini and Link, 2011). It successfully colonized the Indian subcontinent more than four decades ago, and more recently South America and North America (Yassin et al., 2008). It was recorded recently for the first time in Jordan (Al-Jboory and Katbeh-Bader, 2012). This species cannot survive at high latitudes and this explains its restriction to tropical and subtropical climates (Karan et al., 1999). Its optimum temperature ranged from 20 to 30˚C (Amoudi et al., 1993). In Saudi Arabia, the mean generation life cycle was recorded as 22.4

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

days at 25˚C which was shorter than that recorded at 30˚C (Amoudi et al., 1991), while in Brazil it was at an average of 13 to 20 days (Stein et al., 2003).The number of generations/year varies from 12 to 16 (Karan et al., 2000; Setta and Carareto, 2005; Nava et al., 2007). Field and laboratory studies on Indian populations showed that *Z. indianus* overwinters in the egg stage and to a few extents as pupae. Eggs remain in quiescent stage until the weather become warmer.

There are no biological data related to the fly populations from Jordan. Therefore, studies on some of its biological aspects for a Jordanian population collected from Jordan Valley 220 m below sea level were carried out under laboratory conditions. Obtained data will be helpful for the establishment of efficient management strategies against the fly populations.

MATERIAL AND METHODS

A stock colony of *Z. indianus* was started with 200 adults collected from sour orange orchard (*Citrus aurantium* L.), Northern Jordan Valley (32°25'54"N/ 35°35'30"E, 213 m below sea level) in March 2013. The flies were maintained in the laboratory at a temperature of (25±1)˚C, relative humidity of (75±10)% in plastic jars (120 x 160 mm) capped with a fine cloth. Small plastic plates (55 x 25 mm) containing mashed banana fruits seeded with dry and active yeast Saccharomyces cerevisiae were placed inside the jar for adult feeding and oviposition and were renewed every 3-4 days. All biological experiments were conducted in an incubator at 25±1˚C, relative humidity of (75±10)%, and 14 hr photoperiod. One virgin female and one male (one day old) were randomly taken to be the parents of experimental flies, and placed into a plastic jar (80 x 75 mm). The procedure was repeated six times. The plastic plates were inspected daily under a stereomicroscope at 40X. Duration of premating, mating, preoviposition, oviposition and postoviposition periods were recorded. Ten eggs (≤ 2 hr old) were transferred to each plate (30 x 25 mm, containing mashed banana fruits) for a total of 60 eggs. Observations were performed at 6 hr intervals. Duration of incubation, fertility and percentage of hatched larvae in each plate were recorded. The hatched larvae (one day old) were transferred in groups of ten larvae to 6 plastic plates (55 x 25 mm) containing mashed banana fruits (a total of 60 larvae). Each plate was placed in a plastic jar (80 x 75 mm) capped with a fine cloth and placed in the incubator.

Observations were performed daily and the duration of each larval instar and percentage of pupae in each plate were recorded. Pupae (one day old) were transferred in groups of ten to six plastic plates (55 x 25 mm) containing moistened filter paper on the bottom of the plate (a total of 60 pupae). Each plate was placed in a plastic jar (80 x 75 mm) capped with a fine cloth and placed in the incubator. Observations were performed daily and the duration of the pupal stage and percentage of emerged adults in each plate were recorded. Female to male ratio was recorded. Longevity of sexually active flies was studied by placing ten couples of virgin flies (one day old) each pair into a glass vial (25 x

95 mm) capped with cotton, and transferred to new vials with fresh media until their death, at intervals of 2-3 days to keep the quality of the media and to avoid toxic effect of metabolites. The same procedure was followed for longevity of sexually inactive flies by placing them separately. Vials were autoclaved and contained Formula 4-24 Instant Drosophila medium (Carolina Biological Supply Company, USA). The used vials were washed in a 10% chlorine bleach solution each time renewing the media. Vials were inspection daily and the dead flies were removed. Longevity of adult flies were recorded. For all investigated parameters, simple mean values were calculated.

RESULTS AND DISCUSSION

All studied biological parameters are shown in Table 1. Our results represent the first data of biological features of Jordanian populations of *Z. indianus* which showed variations in some biological characteristics when compared to other fly populations from different geographical areas.

The mating duration in Jordanian populations of the fly was close to that obtained by Müller et al. (2012) which ranged between 18 s to 6 min and 56 s, with average of 2 min and 16 s). The obtained premating and preoviposition durations were similar because the mated females laid eggs after mating due to availability of ideal oviposition substrate.

Table 1. Parameters of *Z. indianus*

Parameter	Range	$\bar{x}$
Premating duration (day)	2 - 4	2.67
Mating duration (min.)	2.12 - 3.02	2.48
Preoviposition duration (day)	2 - 4	2.67
Oviposition duration (day)	16 - 63	42.67
Postoviposition duration (day)	0 - 10	6.00
Sex ratio (F/M)	4-6/3-6	4.5-4.3
Incubation period (hr)	22 - 28	24.50
Fertility %	80 - 100	91.67
Larval duration (day)	5 - 10	7.36
Larval viability %	50 - 100	75.00
Pupal duration (day)	6 - 8	6.83
Pupal viability %	70 - 100	88.33
Longevity of sexually active female flies (day)	4 - 79	37.70
Longevity of sexually active male flies (day)	12 - 77	42.20
Longevity of sexually inactive female flies (day)	14 - 35	26.00
Longevity of sexually inactive male flies (day)	5 - 78	37.20

Z. indianus premating, mating, preoviposition, oviposition, postoviposition durations (n=6), egg incubation period, fertility percentage, larval and pupal duration and viability, sex ratio (n=60), and longevity of sexually active and sexually inactive adult flies (n=10).

The preoviposition duration (2-4 days) was very close to that obtained at 25˚C by Pires *et al*. (2008) and lower than that recorded by Amoudi *et al*., (1991) which ranged from 5 to 8 days in Saudi populations at the same experimental

temperature. The duration of oviposition and postoviposition recorded in the present study were 16–63 and 0–10 days, respectively which were similar to Saudi populations (9-62 and 0–12 day of oviposition and postoviposition, respectively, Amoudi *et al*., 1991). However, in the Brazilian populations oviposition period lasted 14–98 day at 25˚C (Setta and Carareto, 2005). The incubation period was ranged from 22-28 hr and fertility from 80–100%. In comparison, Amoudi *et al.* (1991) reported higher incubation period (28–60 hr) and lower fertility (53–92%). While Stein *et al*. (2003) reported an incubation period of 1–1.5 day and viability of 88.89% which are similar to our results. In Saudi Arabia, Amoudi *et al*. (1991) studied the effect of different temperatures on the life cycle of *Z. indianus*, and recorded duration of 4-11 days for the larval development and 5-9 days for pupa development at 25˚C which was similar to our recoded values. In Brazil, Nava *et al*. (2007) reported a larval duration of 8.2-11.3 day and a pupal 3.4–5.5 day at 25˚C. Matavelli *et al.* (2013) reported a pupal duration of 4-6 days. These results show higher values of larval duration and lower values of pupal duration in comparison with values obtained in our study. The differences in larval duration can be attributed to the composition of the diet provided to the larva which was Formula 4-24 instant *Drosophila* medium in study performed by Amoudi *et al*. (1991), Vilela's diet in Brazilian study performed by Nava *et al*. (2007) and artificial diet based on banana in another Brazilian study conducted by Matavelli *et al.* (2013), while mashed banana fruits with dry and active yeast *Saccharomyces cerevisiae* for larval development in our study.

Brazilian populations of the fly showed mean larval viability of 76% and mean pupal viability of 87.88% (Stein *et al*., 2003) which were very close to our findings. Also Saudi populations of the fly showed similar mean pupal viability to our findings which was 89%, but higher mean larval viability which was 88% (Amoudi *et al*., 1991). On the other hand, the mean mortality in egg, larval, and pupal stages recorded in the present study were 8.33, 25, and 11.67 %, respectively. . Similar results were obtained in Brazil as 11.11, 24, and 12.12 % representing the mean mortality in egg, larval, and pupal stages, respectively (Stein *et al*., 2003).

The evaluation of the number of females and males emerged from 60 pupa was obtained by identifying the sex ratio (F/M) which was 1.04. Similar results were recorded in Brazil as 1.32 and 1.11 which represents higher numbers of females than males (Setta and Carareto, 2005, Matavelli *et al*., 2013), which confirms our findings.

The mean duration of life cycle of *Z. indianus* at 25˚C recorded in our study (17.9 days with a range of 13.9 - 23.2 days) was similar to that reported in Brazil by Setta and Carareto (2005) at the same experimental temperature, which was 18.5 day with a range of 15-25 day, also to that reported in Brazil too by Stein *et al.* (2003) who recorded the mean life cycle as 16.78 day with a range of 13–23.50 day at the same temperature.

The exception was observed when we compared with Saudi populations of the fly, the mean life cycle recorded at 25˚C by Amoudi *et al*. (1991) was 22.4 days and is considered higher than that we obtained, despite being at the same geographical area; they had variable latitude and longitude which may contribute to such differences. The viability data and total development time results agree with findings reported by Nava *et al*. (2007) who found that the temperatures near to 28˚C are the thermal optimum for egg-adult development, allowing shorter development time and high viability. Furthermore, these results indicated that the diet used in this experiment was convenient for rearing this species and achieving successful development under the laboratory conditions.

The mean longevity of sexually active female flies obtained in our study was lower than those recorded by Amoudi *et al.* (1991) for Saudi population of *Z. indianus* which was 50.4 days, while the mean longevity of sexually active male flies obtained in our study has the same value of that recorded by Amoudi *et al.* (1991) which was 42.2 days.

Döge *et al*. (2009) studied the effect of marking virgin adult flies of South American strains of *Z. indianus* with marker pen ink on persistence of marks and longevity at 25˚C and using cornmeal media for rearing adult flies. They obtained mean longevity of non-marked virgin females of 32.9 day which was similar record as in our study, and mean longevity of non-marked virgin males of 18.7 day which was lower than we recorded..

However, studies of longevity of sexually active and inactive flies carried out by Setta and Carareto (2005) for Brazilian population, which were reared on banana-agar media at 25˚C, were found to be higher than that obtained for the Jordanian population of the fly. Also it was observed that sexually active and inactive females lived longer than males, while results from our study showed that females died earlier than males.

Pires *et al*. (2008) evaluated the longevity of sexually active adult flies from two geographic strains of Brazilian population, the Jaboticabal strain and the Valinhos strain at 25±2 ˚C. Our results are in line with the findings of Valinhos strain which had males mean longevity of 53.51 day and females mean longevity of 33.53 day.

Females had a shorter life cycle than males in both cases , due to the oviposition process which was observed in sexually active and inactive female flies and it may cause depletion of protein and energy for egg production. Also we found that the mean longevity of sexually active female flies (37.7 day) is higher than that of sexually inactive female flies (26.0 day), despite they laid fertile eggs at high rate. These differences in longevity values could be due to the experimental conditions such as the culture medium used for feeding adult flies, controlled temperature, photoperiod and uncontrolled relative humidity.

In general, these overall variations in biological parameters from our results could be related to different photoperiod, relative humidity and type of diet provided to parental adult flies for feeding and oviposition. In addition, different fly populations from different geographical areas were studied; flies

were collected in Saudi Arabia from Taif area which is about 1600 m above sea level (Amoudi *et al*., 1991), while flies in the present study were collected from Jordan Valley about 220 m below sea level.

It will be interesting to verify the lower temperature threshold for development and the thermal constant (Degree-Days) values for the egg, larval and pupal stages, which can be used when applying control measures during fruits storage and exportation, especially fig fruits. Finally, there is a need of further research on the ecology of *Z. indianus* to investigate its seasonal dynamics.

CONCLUSIONS

The African fig fly was successfully reared under laboratory conditions at 25±1˚C, 75±10% RH, and 14 h photoperiod on mashed banana fruits with dry active yeast for the first time. Most life cycle parameters were comparable to other populations of the fly in other countries such as Saudi Arabia and Brazil. However, some differences were found which could be due in part to differences in experimental conditions and diet components. The results could be helpful in the management of this pest in Jordan.

ACKNOWLEDGMENTS

We thank institutions that made possible this M.Sc. work: Ministry of Agriculture/Jordan and Mediterranean Agronomic Institute of Bari/Italy (CIHEAM-MAIB). This work was supported by MAIB grants. Also thanks to the National Center for Agricultural Research and Extension (NCARE)/Jordan for the technical support.

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DOI: 10.17707/AgricultForest.62.4.09

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CLONAL MICROPROPAGATION AND SOME PHYSIOLOGY ASPECTS OF ESSENTIAL OIL ROSES VALUABLE CULTIVARS REGENERATION IN VITRO

SUMMARY

Limiting factor for vegetative propagation of essential oil roses is their high rate of viral pathogens damages. Complex system of plants` diagnostics, cleaning up and propagation *in vitro* gives possibility to obtain high quality plant material.

This study aimed to investigate some features of *in vitro* meristem culture in essential oil rose cultivars Festivalnaya, Raduga, Lany, Kooperatorka, Michurinka, Iskra and to evaluate morphological, anatomical and physiological parameters of plants *in vitro*. For induction of microshoot regeneration and leaf formation explants we cultured MS medium with 0.5-1.5 mg l^{-1} BAP, 30 g l^{-1} sucrose, 8 g l^{-1} agar. For *in vitro* chemotherapy, Ribavirin was added to the media. It was found out that February and March were the optimum time for meristem isolation and development. The maximum length of regenerated microshoots was observed in the cultivars Iskra and Kooperatorka at the initial stage of cultivation. Cultivar Festivalnaya was characterized by high multiplication rate (1:5-1:6). Due to the maximum number of leaves per shoot, the minimum leaf shape index, anatomical characteristics of palisade tissue development, the number of chloroplasts in photosynthetic tissue cells, and the rate of vascular bundle sheath development the cultivar Festivalnaya were selected. However, the maximum integral index of photosynthetic activity and viability index were noticed in regenerants of Lany cultivar. Analysis of chlorophyll *a* fluorescence rate indicated that physiological state of leaves was normal in all investigated *in vitro* regenerants.

Keywords: *Rosa damascena Mill*., *Rosa gallica* L., meristem, clonal micropropagation, morpho-anatomy characteristic of leaf, fluorescence.

INTRODUCTION

Rose (genus *Rosa* L., family Rosaceae Juss.) is one of the most valuable essential oil plants cultivated on the south of Russia. Due to its high quality and unique aroma, rose oil is widely used in perfumery and cosmetics,

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

pharmaceutical and food industries. Its antibacterial, antifungal and anti-depressant properties are used in medicine.

In Russia, introduction and breeding of aromatic roses are closely related to Nikita Botanical Gardens. In the first years of its foundation (1815-1816) Kazanlak Rose (*Rosa damascena* var. *trigintipetala*) was introduced. Industrial plantings of essential oil rose were set in the Crimea at the end of the XIX century. Research works on the essential oil roses was launched in 1923 and the first rose collection was created. However, over the last 20 years, most of essential oil rose plantations in the Crimea had been destroyed by anthropogenic factor and plant pathogens. Currently, essential oil rose collection in Nikita Botanical Gardens is being restored and it includes 2 species and 12 cultivars. Traditional methods of propagation and producing high quality healthy planting material of essential oil rose, as well as ornamental roses, are cumbersome and ineffective. In such propagation methods viral, bacterial and fungal diseases are usually transferred from plant to plant. For plants of the genus *Rosa* L. the most common and harmful viruses are *Prunus necrotic ringspot ilarvirus* (PNRSV), *Apple mosaic ilarvirus* (ApMV), *Arabis mosaic nepovirus* (ArMV) (Golino et al., 2007). Thus, method of clonal micropropagation is becoming an increasingly popular as an alternative to vegetative propagation of roses (Mitrofanov et al., 2015).

The aim of this work was to study *in vitro* regeneration characteristics of essential oil rose cultivars and evaluation of morphological, anatomical and physiological parameters of regenerants in the process of clonal micropropagation *in vitro*.

MATERIAL AND METHODS

Investigations were carried out in the Department of Plant Developmental Biology, Biotechnology and Biosafety in Nikita Botanical Gardens from January 2015 to April 2016. Shoots with buds were collected from a pre-tested by ELISA-test and PCR analysis visually symptomless virus-free 5-year-old plants of essential oil rose from the collection of the Nikita Botanical Gardens. The materials for the research were cultivars Festivalnaya (*Rosa damascena* Mill. x *Rosa gallica* L.), Raduga (*R. gallica* subsp. *eriosila* Kell. var. *austriaca* Br. x *R. gallica*), Lany [*R. alba* L. x (*R. damascena* x *R. gallica*)], Kooperatorka (*R. damascena* x *R. gallica*), Michurinka (*R. damascena* x *R. gallica*) and Iskra (Bulgarian cultivar).

Studies on *in vitro* culture were carried out according to the procedures presented by Kyte (2013) and Mitrofanova (2011). Stems were exempted from leaves and spikes, and washed in running tap water for 30 min., and then they were cut into segments with one bud, considering their position on the shoot (top, middle, bottom zones). Explants were sterilized for 30 seconds in 70% ethanol, 7-10 minutes in 1% Thimerosal (Sigma, USA), 15-17 min in 2-3% NaOCl solution with 2-3 drops of Tween-20 detergent. After each reagent explants were washed 3-4 times in sterile distilled water. Meristems 0.2-0.5 mm length were isolated from

vegetative buds under binocular microscope SMZ745T (Nikon, Japan). For morphogenesis induction meristems were placed into the culture vessels with MS medium (Murashige, Skoog, 1962) with 0.1 mg l^{-1} α-naphthylacetic acid (NAA), 0.5 mg l^{-1} gibberellic acid (GA_3), 0; 0.5; 1.0; 1.5 mg l^{-1} 6-benzylaminopurine (BAP), 30 g l^{-1} sucrose and 8 g l^{-1} agar (Agar-agar, Panreac). pH was adjusted to 5.6 with 1N NaOH solution. Ribavirin (virazole, 1-beta-D-ribofuranosyl-1H-1,2,4-triazole-carboxamide, Sigma, USA) was used at 5 mg l^{-1} for elimination of possible viral infection. Culture media were autoclaved at 121°C for 15-20 minutes. GA_3 was added to the medium after autoclaving. The culture vessels were maintained in a climate chamber (Panasonic MLR-352-PE) at the temperature 24°C, 16-hour photoperiod under cool white fluorescent lamps (Philips TL, 40W) with an intensity 37.5 μmol $m^{-2}s^{-1}$. Each treatment was tested three times with ten replications. To induce rooting, microshoots 1.5-2.0 cm length were transferred to 1/2 MS medium for rhizogenesis supplemented with 0.0, 0.5, 0.75, 1.0, 1.5, 2.0 mg l^{-1} indole-3-butyric acid (IBA). Culture medium was injected with nanomaterials - potassium humate or sodium humate. After 4-6 weeks, the percentage of *in vitro* rooted microshoots, number of root/shoot and root length was measured.

To determine microshoot proportions 3-5 plants of each cultivar were taken, leaf morphometry for leaf shape index calculation was performed in 10 replications (Zlobin et al., 2009). Anatomic slides were prepared by the standard technique. Histological and anatomical studies of three essential oil rose cultivars (Raduga, Lany, Festivalnaya) were carried out under the light microscope AxioScope A.1 (Zeiss, Germany) using software Axio Vision Rel. 4.8.2.

Parameters of photosynthetic activity (FA) were measured with portable fluorometer "Florotest" (Institute of Cybernetics. Glushkov National Academy of Sciences of Ukraine, 2010). During the experiments, the following parameters were recorded: the initial level of fluorescence after irradiation (F_0), maximum (F_m) and stationary (F_{st}) fluorescence values after light adaptation. We calculated vitality index and photosynthetic activity – relative quantum efficiency of photosystem 2 (Bajron et al, 2000; Stirbet, Govindjee, 2011).

RESULTS AND DISCUSSION

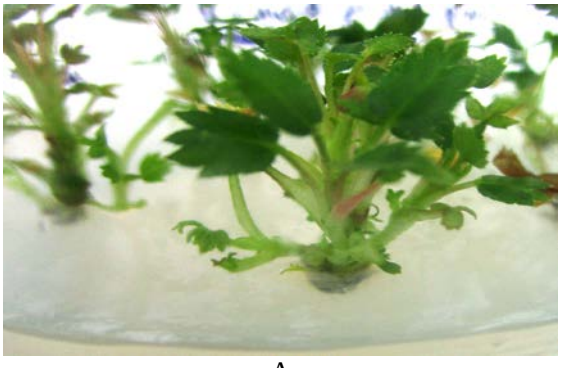
The results of our investigations demonstrated that viability of isolated meristems in six essential oil rose cultivars and their development under *in vitro* conditions depended on the genotype, terms of material collecting, sterilization conditions, type and concentration of plant growth regulators in the culture medium. On the Southern Coast of the Crimea the best terms of explant collecting and introduction of the isolated meristem to *in vitro* conditions were February-April and June. During this period, number of developing meristems was high in all the studied cultivars and reached 94.6% in 'Festivalnaya', 85.6% in 'Iskra', 84.4% in 'Raduga', 83.7% in 'Lany', 81.2% in 'Kooperatorka' and 80.9 % in 'Michurinka'. In September and October number of *in vitro* developing meristems was significantly lower (34-37%). Sterilization yielded 87.5% of

explants free of contamination.

Initiation of meristem development in the studied cultivars was noticed after 7 days culture on MS medium supplemented with 0.5, 1.0, 1.25, 1.5 mg l^{-1} BAP, 0.1 mg l^{-1} NAA, 0.5 mg l^{-1} GA_3. Meristems of the cultivars Lany, Michurinka and Kooperatorka developed more actively on the medium with 1.0 mg l^{-1} BAP. BAP concentration 1.5 mg l^{-1} was optimal for the cultivars Iskra, Raduga and Festivalnaya. In the control (medium without cytokinins) explants developed poorly and died after 21 days of culture. Meristems isolated from vegetative buds of the upper and middle zones of the shoots had high regenerative capacity. GA_3 supplemented to the medium containing BAP intensified regeneration that is corresponds to the data presented by P.K. Pati et al. (2006), A. Ginova et al. (2012). After 21 days of culture on the media supplemented with BAP regeneration of one microshoot with 1-4 compound leaves was noticed in all the studied cultivars. Those shoots were transferred to the fresh medium.

Induction of multiple shoot formation and microshoot length in essential oil rose were largely dependent on BAP and NAA concentrations in the culture medium. Active microshoot elongation was observed in the cultivars Iskra and Kooperatorka at BAP concentration 1.25 and 1.0 mg l^{-1}, respectively.

It is known that BAP, as an exogenous cytokinin added to the medium, reduces apical dominance and induces axillary bud development in roses (Kapchina et al., 2000). The effect of BAP concentrations 0.5, 1.0, 1.25 and 1.5 mg l^{-1} on axillary microshoots induction has been studied. Initiation of multiple shoot formation in essential oil rose cultivars was observed after the third subculture. Axillary buds and microshoots formation was observed on the medium with 1.0 mg l^{-1} BAP in the cultivars Lany, Michurinka, Kooperatorka after the fourth subculture and for the cultivars Iskra, Raduga and Festivalnaya the best concentrations were 1.25 and 1.5 mg l^{-1}. In this condition the multiplication index was maximum. In the cultivar Festivalnaya multiplication index was 1:6-1:10 (Fig. 1C). In the cultivars Iskra, Michurinka and Kooperatorka multiplication index did not exceed 1:8 (Fig. 1A), in Raduga and Lany – 1:7 (Fig. 1B). However, under increasing BAP concentration from 2.0 to 4.0 mg l^{-1} and further subcultures we noticed reduction of regenerative capacity that is corresponds to the data presented by N. Hameed et al. (2006).



A

B C

D

Figure 1. Active shoot formation in essential oil rose cultivars: A - Iskra, B - Raduga, C - Festivalnaya; D - regenerant of the cultivar Lany (bar 1 cm)

One of the most problems in essential oil roses *in vitro* propagation is rooting (Pati et al, 2006; Ginova et al, 2012.). In the process of micropropagation we observed spontaneous microshoot rooting in the cultivars Raduga and Festivalnaya, which is treated as a "hormonal autonomy" of plant cells *in vitro* (Hamburg et al., 1974). To get complete regenerants microshoots were placed on the rooting medium. We used 1/2 MS medium 0.75-2.0 mg l^{-1} IBA. High IBA concentrations resulted in callus formation at the base of microshoots and single roots development. Induction of normal roots 1.5-2.0 cm length was observed on 1/2 MS medium with 0.75 mg l^{-1} IBA. In the cultivars Iskra and Kooperatorka number of microshoots rooted *in vitro* was 68% and 70%, respectively. High rooting percentage was obtained on ½ MS medium with 0.75 mg l^{-1} IBA and 1.0 g l^{-1} potassium and sodium humate. Herewith, in cultivar Festivalnaya number of rooted microshoots was up to 84%, Raduga - 78%, Michurinka - 75% and Lany - 74% (Fig. 1D). In the cultivar Festivalnaya microshoots formed 4-6 roots 4.0-6.0 cm long, in Michurinka cultivar - from 2 to 4 roots 2.5-4.0 cm long,

in Raduga – 6-8 roots 0.5-2.5 cm long, in Lany - from 4 to 6 roots 3.0-5.0 cm long. Regenerants were transferred to adaptation *ex vitro*.

Cultivated rose regenerants under *in vitro* conditions were 2.4-5.6 cm height. Data comparisong in that index demonstrated that maximum microshoot height (3.6-4.2 cm) was characteristic for Lany cultivar and the shortest microshoots (2.4-3.2 cm) were noticed in Raduga cultivar.

To estimate morphogenic capacity of essential oil rose cultivars under *in vitro* conditions we analyzed the amount and size of formed leaves. It was found out that cultivars Raduga, Iskra and Festivalnaya formed by an average from 4 to 7 leaves per microshoot. Leaves were ternate, pinnate venation, leaf edge - toothed. Large leaves (0.63 × 0.87 cm) and maximum leaf shape index (0.73) was characteristic of Lany cultivar. Number of leaves facilitated improvement of the studied cultivars plasticity and their further adaptation to different factors of culture. These results are in agreement with the research findings by E.B. Kirichenko and his colleagues (1993).

For structural analysis, leaves from regenerants cultivated on the optimized Murashige and Skoog medium with 1.5 mg l^{-1} BAP for 4 months were collected. Leaf blades in essential oil rose under *in vitro* conditions were bifacial and they had both general and specific anatomical characteristics. The leaves were thin, their average thickness was 79-122 μm (Table 1).

Table 1. Quantitative indexes of leaf anatomical structure in some essential oil rose cultivars *in vitro* (M ± SE, μm).

Anatomical structure indexes		Cultivar		
		Raduga	Festivalnaya	Lany
Leaf blade thickness		121.52±19.69	107.69±4.22	79.30±9.35
Epidermis thickness	Adaxial Abaxial	13.70±1.24 15.89±1.19	12.56±0.60 12.01±0.78	11.83±0.51 9.85±0.97
Mesophyll thickness	Palisade	34.58±8.28	37.24±4.55	20.11±3.11
	Spongy	57.28±12.83	38.09±4.69	36.03±5.43
Palisade index		0.37	0.50	0.36
Indexes of leaf blade epidermis anatomy				
Epidermal cells size	Adaxial Abaxial	27 × 18 20 × 17	27 × 16 30 × 15	21 × 19 27 × 5
Stoma pore size (length×width)		19 × 5	20 × 7	19 × 8
Stomata number per 1mm^2 surface		87.78±15.50	79.82±4.00	76.61±5.63

Common to all the studied samples were single-layer epidermis, hypostomatic type, stomatal apparatus was of anomocytic type. Adaxial epidermal cells were larger than abaxial ones and of irregular flexuous shape.

Cuticular layer was thin (1-3 μm), glandular hairs absent. Adaxial epidermis thickness was equal or slightly greater than the thickness of abaxial one. Analysis of leaf covering tissues molds made immediately after the opening of the culture vessels demonstrated that most of stomata were open. According to the degree stomatal apparatus activity studied cultivars can be divided as follows: over 85% of open stomata in leaves of Lany cultivar, 60-85% - in Raduga and Festivalnaya.

Mesophyll was bilateral, cells of palisade and spongy tissues located loosely. One (rarely two) layers of cylindrical cells formed palisade mesophyll. Spongy mesophyll in the studied essential oil rose cultivars consisted of two or three layers of elongated or round-shaped cells. Chloroplasts arrangement in chlorenchyma cells was diffuse or line trim, they were oval shaped, their amount: 8-19 units/cell. Spongy parenchyma thickness in all studied cultivars was greater than palisade one thickness. In the cultivar Lany, despite reduced thickness of the sponge layer, the cells were arranged more compact, and number of layers was up to 4. There were significant differences between cultivars in the thickness of palisade mesophyll. Thus, one or two-row leaf palisade tissue in the cultivar Festivalnaya provided maximum palisade index (0.50), which represented the ratio of palisade thickness to the total mesophyll.

The main vein of the leaf was a closed collateral beam. Xylem vessels were arranged in regular rows, surrounded by mechanical and parenchymal plates, in the cultivars Raduga and Festivalnaya at the top and bottom of the beam initials of corner collenchyma were noticed (Fig. 2). Stomata were small, their length was 19.00-20.14 μm. Stomata number per unit area (1 mm^2) did not demonstrate any significant differences between the studied cultivars, but the highest number of stomata was observed in the cultivar Raduga (87.78±15.50 stomata/mm^2). E. Bunning and H. Sagromsky (1948) supposed that stomata functioning under aseptic culture was flawed due to disturbances in the mechanism of their opening and closing. Changes in culture conditions facilitated stomata closure (Smith et al., 1986). Thus, smaller stomata number may indicate lower evaporation of water, on the one hand, and on the other - low ability to regulate transpiration under hydrothermal and light stress, which can be triggered by adaptation of regenerants to *in vivo* conditions.

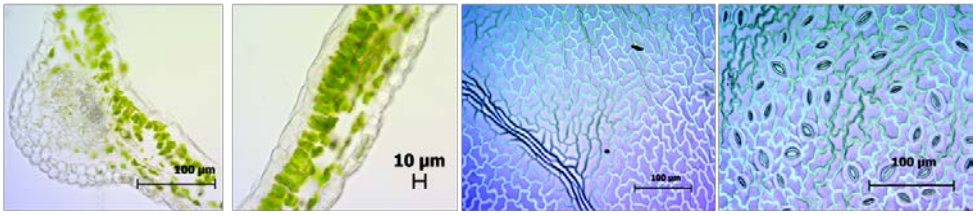


Fig. 2. Structure of leaf in cultivar Festivalnaya *in vitro*

Plants adaptation to the culture conditions resulted in required intensity of productive processes (Stirbet, A., Govindjee, 2011). Photosynthetic activity is

closely related to quantitative indexes plastid apparatus functioning. It can be determined by the method of chlorophyll fluorescence induction (CFI) based on Kautsky curve analyses.

Fluorescent signal increase from F_0 to F_m denoted in the values of variable fluorescence (F_v). Its relation to the maximum level (F_m) indicates photoinhibition at the level of light-harvesting complexes of photosystem 2 (Stirbet, A., Govindjee, 2011). Values of this index in the studied cultivars were in the normal range (from 0.53 relative units in the cultivar Lany to 0.68 – in the cultivar Raduga). Some authors (Bajron et al, 2000; Stirbet, A., Govindjee, 2011) supposed there was correlation between CFI kinetics and photosynthetic assimilation of carbon dioxide. So, photosynthetic activity can be evaluated as $(F_m - F_{st})/F_m$. Normally its value is 0.6 or higher but under pathologies of different origin it reduced proportionally to the weakening of photosynthetic function (Smith et al., 2008). Viability index – F_m/F_{st} in rose was 2.05 - 2.57 relative units. All those data suggest normal functional state of the photosynthetic apparatus in the investigated rose cultivars under *in vitro* conditions.

CONCLUSIONS

Morphogenetic potential was studied and the way of direct microshoot regeneration from the meristems was demonstrated for 6 essential oil rose cultivars. Possibility of multiple shoot formation, depending on the genotype and hormone factors was revealed. Significance of BAP and GA_3 for the induction of microshoot development and regeneration was determined. It was found that complex use of humates and IBA in 1/2 MS medium increased rooting efficiency and quality of roots.

Morphological, anatomical and physiological studies essential oil rose microshoots, as a part of complex biotechnological research, revealed informative structural indexes of plant adaptability and stress-resistance. Due to its anatomical characteristics of palisade tissue, number of chloroplasts in photosynthetic tissue cells and bundle sheath sophistication cultivar Festivalnaya was selected.

Comparative analysis of chlorophyll *a* fluorescence induction demonstrated that functional state of leaves in all studied cultivars wais normal. Maximum values of photosynthetic activity index and vitality index were observed in the cultivar Lany.

ACKNOWLEDGMENT

This study was funded by a research grant № 14-50-00079 of the Russian Science Foundation.

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DOI: 10.17707/AgricultForest.62.4.10

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DETERMINANTS OF CREDIT RATIONING: A STUDY OF FORMAL CREDIT GROUPS IN EKITI STATE FARMERS DEVELOPMENT UNION

SUMMARY

Failure of investment, failure of farmers to use borrowed funds for production and the refusal to return borrowed funds are some of the causes for low repayment performance: hence the need for credit rationing. The study was therefore aimed at determining factors affecting credit rationing among groups in Farmers Development Union (FADU) of Ekiti State.

The study was carried out in four local government areas (Ikere, Ado-Ekiti, Ayedun and Ijan Ekiti) in Ekiti State, Nigeria. A total sample size of 33 farmer groups within the union comprising of 10 members each were selected via sampling proportionate to size. Secondary data obtained from records of the groups spanning a single year period was used for the study in addition to interview schedule.

Findings, however, revealed that loan beneficiaries of FADU must be registered members of the organization and must have at least 25% loan request as savings before loans could be granted to them. Also, 66.7% of the beneficiaries were in their active working age and 84.8% of the groups consisted mainly of women with trading as their major occupation. All the beneficiaries were married with 36.4% of the women having a mean household size of 6 people. The regression analysis showed that educational level and income were socio-economic characteristics that positively affect repayment performance. Also, the probit model revealed that income, outstanding debt and leverage of beneficiaries were significant determinants of loan rationing. However, to fully harness the benefits inherent in credit rationing by microcredit institutions; proper monitoring of borrowed funds was encouraged as repayment performance and credit rationing are interwoven.

Keywords: Credit rationing, FADU, Probit model, Repayment performance and Leverage

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

INTRODUCTION

Credit is essential in poor rural economies to foster economic development. It is required to finance working capital and investment in fixed capital, particularly among farmers too poor to accumulate much saving. Agricultural credit is therefore expected to play a critical role in agricultural development (Duong and Izumida, 2002). Farm credit has for long been identified as a major input in the development of the agricultural sector in Nigeria. The decline in the contribution of the sector to the Nigeria economy has been attributed to the lack of a formal national credit policy and paucity of credit institutions, which can assist farmers among other things. The provision of this input is important because credit or loan-able fund (capital) is viewed as more than just another resource such as labour, land, equipment and raw materials.

In Nigeria for instance, a maize farmer harvests his crops once or twice a year, whereas his consumption is continuous. For a livestock farmer, the interval between the realization of income and the act of expenditure is shorter and his income is more or less continuous, provided he has enough breeding stock and ready access to market facilities. There is also the problem of indivisibility of fixed capital – for instance construction of wells, purchase of pump sets, farm implements, bullocks, tractors and the improvement of soil and moisture availability all require large expenditures that cannot be divided into smaller payments unless credit is available.

Rationing means either a part of the credit applied is granted or sometimes the full amount applied for is disapproved. Credit rationing therefore, results from a situation where the demands for loans exceed supply at the prevailing interest rate. The credit rationing of farmers often results in credit constraint condition that leads to low productivity (Akinterinwa, 2005). Therefore, according to Von Pischke (1991), small holders may be perpetually trapped in poverty due to lack of finances needed to undertake productive investments. At a given interest rate, lenders may refuse to give credit to some applicants, while rationing or fully agreeing to the loan amount demanded by other applicants. Credit rationing policy is, however, regressive to the small–holder farm households as it has serious implication for growth and equity objectives of development policy. This is because when credit is rationed some borrowers cannot obtain the amount of credit they desire at the prevailing interest rate, nor can they secure more credit by offering to pay a higher interest rate.

In essence, the rationing of credit occurs when lenders grant the loans demanded by applicants who are identified as credit worthy borrowers while granting loans smaller than demanded to some applicants and completely rejecting other applicants willing to pay the interest rate demanded. Credit rationing behavior of financial institutions may theoretically be influenced by a number of factors which include the borrower's characteristics (age, gender, wealth, experience, credit history), firm characteristics (business experience, risk profile, earnings), and loan characteristics (amount demanded, loan maturity, collateral offered, interest rate). The reasons for low repayment were classified

into three: the failure of the investment, the failure of farmers to use borrowed funds for production and the refusal to repay. Each microfinance institution tries to maximize its repayment performance whether it is profit oriented or not.

It is therefore worthy of note that non-governmental organizations (NGOs) dominate the microfinance market and their services are directed at individuals and household of the lower income strata. The clients of microfinance institutions are predominantly small traders as well as small and medium sized entrepreneurs. For the purpose of this research work, attention is focused on the Farmers Development Union (FADU). FADU is a multisectorial membership organization, using the instrument of microfinance to organize the Nigerian low income informal entrepreneurs, largely women, around social issues (health, literacy, environment, family planning and youth development) to achieve better living conditions for its members.

Loan beneficiaries of FADU are guided by certain conditions which include; being a registered member of the organization, paying dues and savings regularly in the various groups they are involved with and having at least 25 percent loan request as savings among others. Rationing of loan becomes necessary because resources are limited and there are high covariant risks involved especially in agricultural production. Hence, it becomes imperative to carry out a study on the determinants of credit rationing among formal credit groups in Farmers Development Union (FADU) with the specific aim of highlighting key determinants in their decision making process and accessing factors influencing credit rationing decisions and loan repayment.

MATERIAL AND METHODS

Ekiti state is located in south western Nigeria. It is located between longitudes 4, 5 and 45^0 East of the Greenwich meridian and latitude 7, 15 and 8, 5^0 North of the Equator. It is bounded in the South by Kwara and kogi States. In the east by Osun State and Ondo State in the South. Ekiti State has sixteen (16) local government areas. However, because the people of Ekiti state live mainly in towns, four (4) towns (Ikere, Ado-Ekiti, Ayedun and Ijan-Ekiti) were purposively selected within the FADU framework for the purpose of this study. The main occupation of the people in this area is mainly trading and agriculture which comprises of livestock and crop production. Data used for this study were mainly secondary data from 2010/2011 records of the groups kept at the head office of FADU and information extracted from interview with staff and management of the establishment. The information gathered include; (mean age, mean income etc.), loans issuing criteria, loan repayment performance status, loan repayment period for each borrower, loan repayment (₦), Savings (₦), Amount demanded (₦) and Amount supplied (₦) from a total of 33 farmer credit groups comprising of 10 members each within the FADU framework. Data collected were analysed using descriptive statistics, regression analysis and the probit model. Regression analysis was carried out to determine the effect of socio-economic characteristics of beneficiaries on repayment rates

The regression empirical model is expressed as:

$Y = b_{o+} b_1X_1 + b_2X_2 + b_5X_5 + b_6X_6$

Where Y is the rate of repayment performance, bo = intercept

Xi = independent variables (i = 1, 2, 5 and 6) as defined below:

X_1 = Age

X_2 = Educational status

X_5 = Household size

X_6 = Income

However X_3 and X_4 which represent occupation and years of involvement respectively, were not included due to their invariance.

The Probit model was used to determine the factors of credit rationing

The model is expressed as:

$Y = \beta_o + \beta_1 X_1 + \Sigma_i$ where β_o = intercept, Σ_I = error term, X_1 = independent variables (I = 1, 2, 3 ----------11), β_1 = regression co-efficient and Y = dependent variable having values of 1 or 0, if the groups credit were rationed or not.

Where; Y = 1, if the group's credit was rationed

Y = 0, if the group's credit was not rationed

X_1 = Mean age of beneficiaries (Yrs.)

X_2 = Mean Educational level

X_3 = Occupation

X_4 = Years of involvement

X_5 = Mean Household size

X_6 = Mean income of beneficiaries (₦)

X_7 = Loan repayment (₦)

X_8 = Mean savings of groups (₦)

X_9 = Share of Women (%)

X_{10} = Group's outstanding debt (₦)

X_{11} = Mean leverage of the group (measured as outstanding debt/income)

RESULTS AND DISCUSSION

The socioeconomic characteristics of the respondents as shown in Table 1 revealed that more (84.8%) female groups were beneficiaries of FADU in Ekiti state than their male counterparts. Also, it was revealed that 66.7% of the beneficiaries were between the age bracket 35-39 years, showing that most of the beneficiaries are adults and in their economically active years. The average household size was 6 people with 69.7% of the beneficiaries having between 4-6 members. This reveals that there is a low tendency among the beneficiaries to consume the loans meant for production/investment in business thus enhancing loan repayment. All (100%) group beneficiaries of FADU were married indicating that there was a high degree of responsibility expected in implementation of loan and repayment subsequently.

The results from Table 1 further revealed that 66.7% of the FADU beneficiaries had basic primary education implying that they exhibited a certain degree of literacy, thereby making communication flow easy and enable the beneficiaries grasp the intricacies of loan utilization and repayment. An appreciable proportion (81.8%) of FADU beneficiaries in Ekiti state are traders while a minute 18.2 percent invested in farming. This occupational pattern might not be unconnected with the fact most of the FADU beneficiaries were females. Also, 66.7% of the beneficiaries earned an average monthly income of ₦46, 000 - ₦58, 000. This implies that most of the loan beneficiaries of FADU in Ekiti state were low income earners hence, the need for credit facilities to help boost their production/investment capabilities.

Table 1. Socio-economic characteristics of FADU beneficiaries in Ekiti state, Nigeria

Variables	Frequency	Percentage (%)
Gender		
Male	5	15.2
Female	28	84.9
Age		
30 – 34	2	6.0
35 – 39	22	66.7
40 – 44	6	18.2
45 - 49	3	9.0
Household size		
≤ 3	4	12.1
4 - 6	23	69.7
≥ 7	6	18.2
Mean household size - 6		
Marital status		
Married	33	100
Educational status		
5 – 7	22	66.7
8 – 10	8	24.1
11 and above	3	9.0
Occupation		
Trading	27	81.8
Farming	6	18.2
Income (₦)		
33,000 – 45,000	6	18.2
46,000 – 58,000	22	66.7
59,000 and above	5	15.2

Source: Secondary data (FADU, 2012)

Determinants of Credit Rationing

The probit model was employed to analyze factors that determine the rationing of credit for FADU beneficiaries. The results of the probit model show that income of the beneficiaries, outstanding debt and leverage are significant variables that determine the rationing of credit among FADU beneficiaries in Ekiti state, Nigeria. The result in Table 2, shows that as income of FADU beneficiary increases, the probability of credit being rationed decreases by 0.0004.This implies that as the average income of loan beneficiaries within FADU increases, there is a higher probability of loan repayment and a low tendency for the group's loan to be rationed. This agrees with the study carried out by Sjostrom and Morelli (2002), that a decrease in wage (income) would increase the probability of credit rationing.

Furthermore from Table 2, the outstanding debt and leverage of the groups were significant at 10% level. This implies that a unit increase in the outstanding debt of the beneficiaries of FADU, will increase the probability of the group's loan being rationed by 0.00015. The leverage of the groups (outstanding debt service/income) was significant but negative denoting an inverse relationship. Therefore, as leverage of the groups reduces, the probability of credit rationing increases. The modal leverage was put at 1.10, which is relatively higher than 1, depicting that non-equity was greater than equity of the beneficiaries. A contrary view however from the study by Zeller (1994), is that the higher the leverage, the higher the probability of being constrained.

Table 2. Probit Analysis showing determinants of Credit Rationing

Explanatory Variables	Description of variables	Regression coefficient	T-Value	Mean of variable (X)
*	Constant	29.481**	2.600	
X_1	Age	-0.100	-1.118	38,788
X_2	Education	-0.157	-0.742	7.888
X_5	H/H size	0.452	1.199	5.303
X_6	Income	-0.000**	-2.212	51354.545
X_8	Savings	-0.000	-0.667	1160.606
X_{10}	Outstanding debt	0.000*	1.864	71060.758
X_{11}	Leverage	-7.928*	-1.958	1.432

Source: Data analysis, 2012

** - Significant @ 5% (t > 1.96 < 2.58)

*- Significant @10% (t > 1.64 < 1.96)

Effect of Socio-Economic Characteristics of Respondents on Repayment Rate

The value of the coefficient of determination (R^2) is 0.87, implying that, 87 percent of the total variation in rate of loan repayment is explained by the explanatory variables included in the model. The F-value (47.29) is significant at 0.05 level depicting that the exogenous variables jointly explain the endogenous variable. Therefore, the regression model is significant/ is a good fit. Educational level and income status of the beneficiaries were significant at 5% level. The results from Table 3 clearly show that as educational level of the groups increase, so will repayment rate increase by 0.535. This implies that as more people within

a group get educated and enlightened, repayment performance of such a group will be enhanced as they won't see the loan granted to them as their share of the "national cake".

Ahlin and Townsend (2007) found that more productive groups, measured by their education, have better repayment performance. An explanation for our results may lie in the fact that highly educated groups are less credit rationed. FADU typically begins by lending small amounts to groups and then increasing the loan size for groups with satisfactory repayment performance. If a group faces a high degree of credit rationing it implies that this group has unfulfilled credit demand. Also, Bhatt and Tang (2002) conducted a study to investigate the determinants of loan repayments in microcredit programmes that applied the group lending approach and found that a higher education level was significant and positively related to better repayment performance.

Furthermore from Table 3, income of the beneficiaries was found to be positively significant to repayment performance @ 5 percent level.

Table 3. Regression analysis showing the effect of Socio-Economic Characteristics of Respondents on Repayment Rate

Variables	Description of Variables	Regression Coefficient	Standard Error	T-Value
*	Constant	-7.955**	3.069	-2.592
X_1	Age	0.007	0.044	0.168
X_2	Education	0.535***	0.108	4.970
X_5	Household size	0.145	0.210	0.691
X_6	Income	0.000**	0.000	2.168

Source: Data analysis, 2012
R2 = 0.87, S.E = 0.84, DW = 1.05, F = 47.29
** - Significant @ 5% (t > 1.96 < 2.58)
*** - Significant @1% (t > 2.58)

Therefore as income of the beneficiary increases, the rate of loan repayment also increases. In relation to the study carried out by Bhatt and Tang (2002), income was shown to contribute positively to the groups repayment potentials. Also, Mohammad and Hooman (2009) in their study revealed income as having a significant and positive effect on repayment performance of farmers in Iran.

CONCLUSIONS

Income, outstanding debt and leverage of the beneficiaries were significant factors that determined the rationing of credit among groups in Farmers Development Union.

Information gathered from interview with the staff of the organization revealed that collaterals such as physical assets: land were not required unlike other formal or informal institutions, notice of any default was made to the village head in that local government area and if the response is negative, legal action would be taken to recover the loan.

Hence, the results show that formal lenders (FADU) use locally available information about the credit worthiness of groups in loan rationing such as debt servicing obligations (repayment performance/outstanding debt) and income.

Income was a significant factor in determining whether credit was to be rationed among groups in FADU, it is therefore expedient that the income generating activities of these groups be encouraged in other to boost their economic viability. Leverage of the groups was an important element of credit rationing, it is therefore necessary that checks and balances on loan utilization be put in place by the group members in other to raise their equity and reduce outstanding debt.

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DOI: 10.17707/AgricultForest.62.4.11

Hakan ENGIN and Zeliha GÖKBAYRAK[1]

IN VITRO POLLEN VIABILITY AND GERMINATION OF BISEXUAL AND FUNCTIONAL MALE FLOWERS OF SOME TURKISH POMEGRANATE CULTIVARS

SUMMARY

The evaluation of pollen viability and germination are two essential criteria for characterization of pollinating cultivars in pomegranate which is an andromonoecious species. With this point of view, pollens were collected from both bisexual and functional male flowers of 'Aşınar' and 'Caner II' cultivars to determine the pollen viability and germination. Colorimetric test of 2,3,5-triphenyl tetrazolium chloride (TTC) was used to estimate the pollen viability. Pollen germination was tested in vitro on a medium containing 1% agar and 20% sucrose. Results showed that pollen viability was not dependent on the type of the flowers. The highest pollen viability ratio (78%) was obtained in the pollen collected from functional male flowers of 'Aşınar'. Pollen germination ratio, however, varied with regard to the flower type in the same cultivar. Bisexual and male flowers in 'Aşınar', respectively, had 43.5% vs 22.3% pollen grains germinated. Pollens from two sexual morphs of pomegranates show different germination and viability ratios. Investigation on viability and germination capability of pollen grains coming from both bisexual and functional male flowers in pomegranate can enable growers and breeders to better select best crossing pollinators.

Keywords: *Punica granatum* L., Pollen, Viability, Germination.

INTRODUCTION

Pomegranate (*Punica granatum L.)* is characterized by having two types of flowers, bisexual flowers and functional male flowers, developing on the same tree. Each type of flowers can affect productivity and yield separately. Functional male flowers drop and generally fail to set. Fruits develop exclusively from bisexual flowers. Nevertheless, functional male flowers can be a way to spread genes, because pollen spread is more efficient.

Fertility of the pollen depends on various factors such as environmental factors, nutrition, varieties and plant regulators (Gökbayrak and Engin, 2015). Pollen viability and germination are important parameters in the analysis of gene flow in plant studies, highlighting the male reproductive potentiality of the

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

species and contributing to taxonomic, ecological, genetic and palynological studies (Gadze et al., 2011; Piccinini et al., 2012). To investigate pollination potential, estimates should be made on pollen quantity and viability, as well as on pollen germination capability. The previous studies on pomegranates showed that in vitro pollen germination ratio was affected by genotypes or methods used and chemical concentration in the medium (Gözlekçi and Kaynak, 2000, Melgarejo et al., 2000; Engin and Hepaksoy, 2003). Studies on in vitro pollen viability show that it is also possible to differentiate the aborted pollen grains from non-aborted ones. Aborted pollen grains do not have a nucleus, thus staining only the cellulose in the wall (Biondo and Battistin, 2001; Gökbayrak and Engin, 2015).

A comparison of the form and function of flower parts in the two sexual morphs, including pollen viability and germination, would provide information useful to enhance fruit production. This study was conducted to determine viability and in vitro germination ability of pollens derived from functional male and bisexual flowers of some Turkish pomegranate cultivars.

MATERIAL AND METHODS

Both functional male and bisexual flowers of the pomegranate cultivars, 'Aşınar and Caner II' were collected from the collection orchard located at the Dardanos campus of Çanakkale Onsekiz Mart University, Çanakkale, Turkey. Flowers of different sexes were hand-picked and taken to the laboratory. Their anthers were detached slowly using a forceps and then put on a paper at room temperature of 22°C for about 12-18 hours to waste some of their moisture, split and release pollens.

Colorimetric test of 2,3,5-triphenyl tetrazolium chloride (TTC, 1%) were used to estimate pollen viability. The analysis was immediately performed after two hours under a light microscope, considering pollen grains viable with dark-red tonality and non-viable with light-red tonality, lacking protoplasm. Ratio calculations (%) were made with viable pollens divided to non-viable ones.

Pollen grains obtained from the two sexual morphs of the pomegranate cultivars, 'Aşınar' and 'Caner II', were cultured in a medium containing 20% sucrose and 1% agar at 26±1°C under 8 hours dark and 16 hours daylight conditions. Twenty-four hours later, germinated and ungerminated pollens as a percentage were counted using a light microscopy (Olympus CX-41) at 10X magnification from a random selection of four-field views.

The statistical analysis was performed using MINITAB statisctical package software (Minitab Inc., ver.16), and the significant means were compared using Tukey's test.

RESULTS AND DISCUSSION

Pollen viability and *in vitro* germination of flowers with different sexes were tested on Turkish pomegranate varieties 'Aşınar and Caner II' (Table 1).

Table 1. Comparison of pollen sources (bisexual and functional male flowers) in the pomegranate cultivars ‘Aşinar’ and ‘Caner II’ for their pollen viability and in vitro germination ratios (%).

Cultivars	Pollen viability (%)			Pollen germination (%)		
	Bisexual	Functional male	*p* value	Bisexual	Functional male	*p* value
Aşınar	71.49	78.41	*0.476*	43.48 a	22.25 b	*0.002*
Caner II	50.36	64.18	*0.221*	30.53	20.46	*0.097*

TTC test of pollen viability of the bisexual and functional male flowers did not exhibit any statistically significant differences, although male flowers had slightly higher viable pollens. As opposed to comparatively higher pollen viability, germination was markedly low in the cultivars. These results corroborate those of derin and eti (2001) who reported that percentage of viable pollens in Turkish pomegranates ranged from 75.2 to 82.4% by TTC test and fluorescein diacetate (FDA) test, respectively. Thus, TTC can be recommended for testing pollen viability in pomegranates.

In terms of germination, there was a significant difference in the functional male flowers compared to the bisexual flowers in the cultivar ‘Aşinar’. Pollens of the cultivar ‘Caner II’ showed no differences. In general, two-thirds of the pollens of the both cultivars did not germinate in the male flowers. On the other hand, at least 60% of the pollens from the female flowers germinated.

Based on the observations made under light microscope, it was noted that pollen germination ratios for the different pomegranate cultivars were not synchronous, but heterogeneous. Pollen germination assays conducted at different temperatures showed that pollen germination is markedly influenced by temperature (Gaaliche et al., 2013). Yang et al. (2013) did not find significant differences in the sucrose ratio in the basal medium in ‘Taishanhang’ pomegranate. Malgarejo et al. (2000) showed that the male flowers of me15 clone of pomegranate gave a higher germination in vitro than the bisexuals when low sucrose concentrations were used. In the present study, germination capability of pollen grains from functional male flowers was not higher in each cultivar.

This means that genotype is a factor regulating this effect. In pomegranate, Derin and eti (2001) reported that there was a significant difference in germination ratio between bisexual and functional male flowers the cultivar of ‘33 n 26’, but not in the cultivar of ‘Hicaz’.

Gözlekçi and Kaynak (2000), in support of our study, in cultivar of ‘Caner II’ found that there was no difference in the germination ratios of the pollens derived from the different types of flowers. In general, there is a linear relation between pollen viability and germination capability in many fruit species (Stanley and Linskens, 1974), but in our study there is no linear relation between pollen viability and germination capability in both functional male and bisexual flowers of the pomegranate cultivars.

CONCLUSIONS

Pollens from two sexual morphs of pomegranates show different germination and viability ratios. Investigation on viability and germination capability of pollen grains coming from both bisexual and functional male flowers in pomegranate can enable growers and breeders to better select best crossing pollinators.

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DOI: 10.17707/AgricultForest.62.4.12

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CARCASS AND MEAT QUALITY IN RELATION TO THE POLYMORPHISM IN PORCINE *MYF4* GENE

SUMMARY

The aim of this study was to analyse the associations between polymorphism in porcine myogenin gene (*MYF4*) and economically important traits in relation to the carcass and meat quality of pigs. The genomic DNA samples were taken from in total 180 crossbreeds (Large White x Landrace). The detection of polymorphism in *MYF4* gene were performed in order to evaluate its effect on back fat thickness, proportion of valuable meat parts, MLT area and proportion of thigh. The genotyping of analysed individuals was carried out by means PCR-RFLP method and restriction endonuclease *Msp*I. The allele frequencies were as follows: A 0.75 and B 0.25. A prevalence of AA genotype (59%) compared to AB (33%) and BB genotypes (8%) were detected in analysed crossbreed population. The observed average value of heterozygosity (0.33) and positive value of the Wright's F_{IS} index (0.12) similarly reflected the higher proportion of homozygous genotype in populations. The effect of *MYF4* gene polymorphism on selected phenotypic traits has been tested using one-way ANOVA procedure. The statistical analysis showed only non-significant results. Due to the polygenic character of selected phenotypic traits the involvement of other candidate genes and increase of sample size could clarify the role of *MYF4* gene in porcine carcass and meat quality regulation. The study, which is based on molecular variability of livestock genetic resources, is necessary for the genetic improvement and understanding of relations between markers and trait of interest.

Keywords: genetic variability, myogenin gene, meat quality, SNP, pig.

INTRODUCTION

The pig breeding plays currently a key role in meat production, and the high consumption of pork corresponds to this. The high efficiency of pig breeding is absolutely necessary for competitiveness under today's difficult economic conditions (Weisz *et al.*, 2011). One of goals of animal genetics is to locate and identify loci that are responsible for economical important traits such

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

as meat performance, reproduction, and product qualities which are the most significant factors for pork production efficiency (Bulla *et al.*, 2007).

Variation in meat quality is likely to be caused by differences in many genetic and non-genetic (environmental) factors, which interact together and determine the course of metabolic processes in muscle tissue and in the *postmortem* conversion of muscle into meat (Kapelañski *et al.*, 2005). Intensive selection for lean growth in pigs may have caused a considerable genetic change in fibre type composition, which resulted in a higher proportion of glycolytic fibres and in an increase in fibre diameter in domestic pigs compared to native breeds. Changes in fibre type ratios affect metabolic properties of a muscle and thereby meat quality (Kłosowska *et al.*, 2004).

Histochemical and biochemical muscle fibre properties are the factors that influence the quantitative and qualitative characteristics of pork meat (Stupka *et al.*, 2014). Muscle fibre formation takes place during embryonic development in mammals and is controlled by the *MyoD* gene family, which consists of four genes, *MyoD1* (*MYF*3), myogenin (*MYOG* or *MYF4*), *MYF5* and *MYF6* (*MRF4*) (Horák *et al.*, 2004; Zhang *et al.*, 2007; Ujan *et al.*, 2011). The expression of the *MYF5* and *MYOD1* (*MYF3*) genes plays a fundamental role during myoblast proliferation, while the expression of the *MYOG* (*MYF4*) and the *MYF6* (*MRF4*) genes is linked to the differentiation and maturation of myofibres (Stupka *et al.*, 2012). The myogenin (*MYF4*) gene, which controls the start of myoblast fusion and myofibres formation, was identified on the 9th autosome, in the 9q2.1-2.6 area (Soumillion *et al.*, 1997; Ernst *et al.* 1998). The myogenin is a transcription factor specific to skeletal muscle and fulfils a key function in muscle differentiation by controlling the onset of myoblast fusion and the establishment of myofibres. Genetic variation in the *MYF4* gene can be associated with differences in myoblast and myofibril numbers (Weisz *et al.*, 2011). Ernst *et al.* (1993) and Soumillion *et al.* (1997) detected using endonuclease *Msp*I gene three polymorphic sites in the *MYF4*, in the promoter region, the second intron and the 3' side of the gene.

The aim of this study was to analyse the effect of polymorphism in porcine *MYF4* gene on economically important traits in relation to the carcass and meat quality in population of Large White x Landrace crossbreeds.

MATERIAL AND METHODS

The biological samples were collected in 2014 from in total of 180 crossbreeds of Large White x Landrace (86 boars and 94 sows) from the Experimental Centre of Farm Animals (Department of Animal Husbandry, Slovak University of Agriculture in Nitra, Slovakia). Each of selected individuals included in present study have been farmed in the same conditions and fed with standard feed mixtures.

To extract of genomic DNA from blood samples the protocol according to Miller et al. (1988) has been used. Subsequently, the concentration and purity of genomic DNA were analysed based on the spectrophotometry measurements by

the optical density at wave length of 260 nm. The polymorphism in *MYF4* gene was analysed according to Soumillion *et al.* (1997) using restriction endonuclease *Msp*I. The products of PCR reaction and restriction fragments have been separated and visualised using horizontal electrophoresis in 2% agarose gels (130 V for 50 min) and stained with day GelRed.

The genotypic structure of population and allele frequencies have been determined using Genalex version 6.1 (Peakall and Smouse, 2012). The Hardy-Weinberg equilibrium in population was tested based on the significance between observed and expected genotype frequencies using Chi-square test. The diversity indices including observed (H_o) and expected heterozygosity (H_e), effective allele numbers (N_e), polymorphic information content (PIC), and F_{IS} index was calculated using Genalex version 6.1 (Peakall and Smouse, 2012).

The effect of *MYF4* has been studied in relation to the back fat thickness (BFT), lean meat percentage (LM), thigh percentage (TP) and MLT area. Each of these carcass and meat quality indicators was measured according to Slovak standard technical norm (STN 466164). The associations between locus in *MYF4* gene and selected production parameters have been analysed using One-way ANOVA procedure incorporated in SAS software environment (SAS Institute Inc., 2009).

RESULTS AND DISCUSSION

In analysed population of Large White x Landrace crossbreeds the prevalence of homozygous AA genotype was found (59%), whereas the AB heterozygotes reached the level of 33%. The lowest proportion was observed for BB homozygotes (8%). The A allele was more frequent (0.75) than B allele (0.25). The higher proportion of homozygotes across all evaluated individuals was reflected in the decrease of H_o and H_e heterozygosities (0.33 and 0.39). The differences between observed and expected genotype frequencies have been only non-significant ($P<0.05$) that indicated the Hardy-Weinberg equilibrium in population. The value of N_e (1.59) signalised unbalanced alleles activity and observed value of polymorphic information content (0.38) showed medium level of polymorphism according to Botstein et al. (1980). The positive value of F_{IS} index (0.12) and also relative high value of observed homozygosity (0.67) indicated the deficiency of heterozygous animals compared to the Hardy-Weinberg expectations. The value of F_{IS} index, which is also considered as molecular equivalent of individual inbreeding coefficient with respect to the population, reflected in analysed population the increase of homozygosity resulting probably from the higher animal's relatedness and signalized the risk of inbreeding increase in the future generations.

The effect of *MYF4* gene polymorphism has been analysed in association to four indicators reflected carcass and quality of pork meat: back fat thickness, lean meat percentage, thigh percentage and MLT area. Table 1 shows the observed average values of analysed traits in relation to the *MYF4* genotypes. Contrary to the expectations resulting from previously published studies (Verner

et al., 2007; Civaňova and Knoll, 2007; Stupka *et al.*, 2012) all of the *MYF4* genotypes showed only non-significant (P<0.05) impact on selected production traits.

Table 1. Average values of measured traits in relation to MYF4 polymorphism genotypes

Genotypes	N	Traits (in average)							
		BFT (mm)	P	LP (%)	P	MLT area(cm^2)	P	TP (%)	P
AA	106	17.30±3.51	ns	55.35±1.90	ns	43.76±3.79	ns	22.62±1.24	ns
AB	59	17.40±3.53	ns	55.08±1.77	ns	44.56±4.85	ns	22.62±1.28	ns
BB	15	16.71±3.11	ns	55.56±2.35	ns	43.53±4.03	ns	22.82±1.42	ns

ns – not significant, BFT – back fat thickness, LP – lean meat percentage, MLT area – area of *musculus longisimus thoracis*, TP – thigh percentage (%)

The associations of *MYOD* genes family with pork quality has been previously investigated in various pig populations by several studies (Soumillion *et al.*, 1997; Kapelañski *et al.*, 2005; Cinar *et al.*, 2012; Stupka *et al.*, 2014). The *MyoD* genes were tested as candidate genes with an expected, significant effect principally on the muscle deposition (Kłosowska *et al.*, 2004). The genetic variation in the *MYF4* gene has been associated mainly with differences in myoblast and myofibre numbers (Soumillion *et al.*, 1997; Kim *et al.*, 2009). Te pass *et al.* (2004) revealed that the level of *MYF4* gene mRNA expression is localized mainly in red muscles in animals at slaughter and is associated with muscle fibre type. Gerześ *et al.* (2010) and Stupka *et al.* (2014) reported significant effect of *MYF4* polymorphism on the number of muscle fibres per area unit in pigs. Some authors also showed that the *MYF4* gene had also a tendency to regulate backfat thickness (Civaňova and Knoll, 2007). The animals with AA genotype were associated with increase birth weight, growth rate and content of lean meat (Te Pass *et al.*, 1999). In the Czech Large White population was found the significant association between *MYF4* gene polymorphism and back fat thickness (Verner *et al.*, 2007). Based on this and due to the polygenic character of selected phenotypic traits the involvement of other candidte genes mainly from *MYOD* family and increase of sample size is in the future studies needed in order to clarify the role of *MYF4* gene in porcine carcass and meat quality regulation.

CONCLUSIONS

In analysed population of Large White x Landrace crossbreeds the prevalence of homozygous AA genotype was found, whereas the lowest proportion was observed for BB homozygotes. The lower proportion of heterozygotes within population was reflected in the decrease of genetic variability represented by FIS index and heterozygosity. Moreover, the FIS index as molecular equivalent of inbreeding coefficient signalized for the analysed

population the risk of animal's relatedness increase in the next generations. In the contrary to the expectations resulted from the previously reported studies the MYF4 genotypes showed only non-significant ($P<0.05$) impact on all of the analysed production traits. In the future, the involvement of other candidate genes and increase of sample size could clarify the role of MYF4 gene in porcine carcass and meat quality regulation.

ACKNOWLEDGEMENT

This work has been supported by the Slovak Research and Development Agency (Contract No. APVV-14-0054 and No. APVV-0636-11).

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DOI: 10.17707/AgricultForest.62.4.13

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ENERGY AND ECONOMIC ASSESMENT IN TILLAGE AND SOWING FOR ROTAVATORS, CONVENTIONAL AND NO-TILL WHEAT ESTABLISHMENT

SUMMARY

Rice-wheat is major crop of IGP covering around 10 Mha areas and contributing about 40% to national food grain production. Rice residue management in combine harvested fields, for wheat sowing, is performed primarily through intensive tillage. This demands more energy input leading to higher production cost and lesser benefit-cost ratio. Indian government is promoting rotavators for speedy seedbed preparation in rice-wheat system. No-till wheat sowing is also quite popular amongst the farmers. This study compares energy input and benefit-cost ratio of six treatments viz. T_1 (RM_1 x 2 + sowing), T_2 (RM_2 x 2 + sowing), T_3 (RM_3 x 2 + sowing), T_4 (RM_4 x 2 + sowing), T_5 (No-till sowing) and T_6 (Disc harrow x 6 + Planking x 2 + sowing). Result revealed maximum time and fuel consumed in T_6 (10.13 h/ha and 59.85 l/ha) and minimum for treatment T_5 (1.39 h/ha and 6.19 l/ha). Energy saving was maximum (89.57%) in no-till wheat sowing (T_5) followed by rotavator treatments (47.08-62.65%) compared to treatment T_6. The energy productivity was highest (13.06 kg/MJ) for no-till sowing (T_5). It ranged from 2.73-4.20 kg/MJ for rotavator treatments (T_1-T_4) and was minimum (1.59 kg/MJ) for T_6. The benefit-cost ratio was found 2.99 for treatment T_6 and 6.35% higher for no-till wheat sowing (T_5). It ranged from 2.91-3.53 for treatments (T_1-T_4). Based on the results, T_5 was found most energy efficient treatment followed by T_3, T_4, T_2 and T_1. Conventional method (T_6) was found to be most energy intensive method of wheat establishment.

Keywords: Energy requirement, rotavator, wheat establishment, cost of production, no-till

INTRODUCTION

Agriculture has been the life line of Indian economy and provides livelihood to about 65% of the total population. It has largest arable land (160 Mha) sharing 11.2 percent arable land of the world. Rice-wheat are major crops grown in Indo-Gangetic Plains (IGP) covering around 10 Mha area and contributing about 40% of the country's total food grain production. During

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

2014-15, these two crops together contributed more than 76% to the total food grain production of the country (Anonymous, 2016). In regions where lowland rice is cultivated during the rainy season, tillage operation for preparing seedbed for wheat sowing, in combine harvested rice fields, is considered most difficult and time-consuming. Conventionally farmers, in tarai region of Uttarakhand, use 6-8 operations, sometimes even more, of disc harrow followed by planking twice to prepare the seedbed for wheat sowing in combine harvested rice fields. This not only increases cost of cultivation but also results in delayed sowing and marginalize benefit-cost ratio. No-till technique or reduced tillage could help the farmers in earning more profit from the same land by reducing the cost of cultivation. No-till wheat sowing, introduced during 1995 (Singh and Singh, 1995), is now limited to progressive farmers of this region. It has been reported to save operational energy and cost of cultivation over traditional method (Sharma et al., 2007).

Rotavators (rotary tillers) have been reported to produce smaller clod-mean-weight diameter, better residue incorporation as well as most energy and cost effective for seedbed preparation (Singh, et al., 2006; Prasad, 1996). These are being promoted by Indian government by providing 50% subsidy to the farmers on its purchase. Due to government support and demand by the farmers, a large number of manufacturers are manufacturing and supplying various sizes of rotavators. However, the data on energy requirement by various sizes of rotavator is lacking. Considering this in view, study was undertaken to assess energy input and economics for various sizes of rotavators, conventional and no-till system of wheat establishment.

MATERIAL AND METHODS

The experiment was conducted at University Farm in combine harvested rice field for consecutive two years (2013-14 and 2014-15). Four sizes of rotavators with rotor lengths as 115, 148, 172 and 195 cm fitted with L-shape blades were selected for seedbed preparation whose technical details are presented in Table 1. On an average, the initial residue load was 6.03 t/ha with average height of stubbles as 36.19 cm. The initial soil moisture ranged from 23.2-25.8%. An area of 1.25 ha was selected and was divided into 18 equal plots (size 60 m x 10 m) to accommodate all the 6 treatments (Table 2) with three replications. In conventional method of seedbed preparation (T6), double action trailed type disc harrow (8 x 8 disc) with disc diameter as 610 mm and weighing 500 kg was used. A wooden plank, 300 cm long weighing about 65 kg, was used for clod crushing and levelling of the field. No-till ferti-seed drill (11 rows) was used to sow wheat, at 110 kg/ha seed rate, in all the treatments including T5. A tractor of 37.3 kW was used for operating the various implements. Other cultural practices, after sowing, were performed similar in all the treatments to minimize experimental variation. The data related to soil, machine and crop parameters were determined as per the standard procedures. Energy as well as economic analysis was made by adopting the standard methods and energy equivalences

(Kumar, 2013; Asodiya, 2014 and Anonymous, 1970). The data was analyzed using Completely Randomized Design (CRD).

Table 1: Specifications of various sizes of rotavator

Machine Parameters		RM_1	RM_2	RM_3	RM_4
Overall	Length, cm	142	181	195	225
	Width, cm	90	65	95	90
	Height, cm	115	115	115	115
Length of rotor shaft, cm		115	148	172	195
Number of flange		6	6	7	9
Number of blades per flange	On first flange	3	6	6	3
	On other flanges	6	6	6	6
	On last flange	3	6	6	3
Total number of blades		30	36	42	48
Rotor radius, cm		19.50	19.50	20.50	18.75
Width of blade, mm		90	85	80	85
Length of the blade, mm		280	280	280	285
Weight, kg		394	416	445	448

Table 2. Details of the treatments used for the study

Treatments	Description	Number of replications
T_1	RM_1 x 2 + sowing	3
T_2	RM_2 x 2 + sowing	3
T_3	RM_3 x 2 + sowing	3
T_4	RM_4 x 2 + sowing	3
T_5	No-till sowing	3
T_6 -control	Disc harrow x 6 + Planking x 2 + sowing	3

RM1…4 represents the four sizes of the rotavators

RESULTS AND DISCUSSION

The results of clod size and residue incorporation has been presented in Table 3 which revealed minimum clod mean-weight-diameter (CMWD) of 14.9 mm in treatment T1 followed by 15.8, 16.7 and 17.2 mm in treatments T4, T3 and T2. Largest clod size of 17.6 mm was observed in case of treatment T6 (control). Smaller size of clods in treatments T1-T4 may be due to better slicing action by the rotavator blades as compared to discs of disc harrow. The clod size for all the treatments varied significantly from each other at 5% level of significance. Maximum residue incorporation (87.56%) was found for T1 followed by 87.40% (T4), 86.40 (T2) and 85.74% (T3). Treatment T6 showed minimum, 80.63%, residue incorporation (Table 3). Higher residue incorporation in treatments T1-T4 might be due to better cutting (because of higher peripheral velocity of rotavators blades in case of active tillage tools) and mixing of residue by the rotavators blades. Another reason could be the blade orientation with respect to direction of travel that might have helped in cutting the residues into smaller pieces consequently better mixing with soil. In treatment T6, the

peripheral speed of disc cutting edge remains same, as in case of passive tillage tools, as that of tractor forward speed resulting in poor slicing action and hence poor residue incorporation. The percentage residue incorporation in all the treatments was found to vary significantly from each other at 5% significance level.

Table 3: mean values of soil and machine parameters for various treatments

Treatments	CMWD, mm	Residue inco-rporation, %	Observed speed , km/h	Field capacity, ha/h	Time required, h/ha		
					tillage	sowing	total
T_1	14.9[a]	87.56[a]	4.36	0.44	4.53	1.39	5.92[a]
T_2	17.2[b]	86.40[b]	4.14	0.54	3.71	1.39	5.10[b]
T_3	16.7[c]	85.74[c]	4.23	0.68	2.96	1.39	4.35[c]
T_4	15.8[d]	87.40[d]	4.33	0.79	2.52	1.39	3.91[d]
T_5	-	-	4.21	0.72	-	1.39	1.39[e]
T_6	17.6[e]	80.63[e]	5.65	0.81	8.74	1.39	10.13[f]

Same letter – non-significant at 5%

Machine parameters namely speed of operation for rotavators, treatment t1-t4, was found in the range of 4.14 - 4.36 km/h showing very little variation (table 3). This was due to the fact that the tractor was operated at the same forward speed to minimize the experimental variations. The speed of operation for disc harrow and planker in treatment t6 was comparatively higher which was probably due to lesser draft requirement. However, all the implements were operated within their recommended speed of operation. The field capacity, amongst the treatments t1-t4, was found maximum for t4 and minimum for treatment t1 which is obvious (table 3) as known that field capacity is a function of width of cut and speed of operation of an implement. Similarly field capacity was found more for disc harrow (0.81 ha/h) and planker (1.48 ha/h) which was again due to higher width and speed of operation.

Time required in various treatments revealed maximum time requirement of 10.13 h/ha (table 3) for conventional method of tillage and sowing (t6). This was due to more number of disking and planking operation. No-till method of wheat sowing (t5) recorded minimum time of 1.39 h/ha which was due to elimination of seedbed preparation. In treatments t1-t4, the minimum time requirement of 3.91 h/ha was recorded for treatment t4 in which largest size of rotavators was used. It was followed by treatments t3, t2 and t1 respectively. This was due to the fact that smaller rotavator would require more time to till a given area than a larger size of rotavator. Also the field capacity, ha/h, is inversely proportional to time requirement (h/ha). Almost similar pattern was observed for cumulative fuel consumption, l/ha, in various treatments. This was again due to

lesser time requirement by a larger size of implement and vice-versa. Statistical analysis indicated significant variation in total time requirement for various treatments at 5% significance level.

The fuel consumption for each treatment was determined (table 4) which revealed minimum fuel consumption of 6.19 l/ha in t5 which is due to elimination of seedbed preparation. Treatment t6 consumed maximum diesel fuel which is due to repeated operation of disc harrow and planker. Among rotavator treatments, treatment t1 consumed more fuel followed by t2, t4 and t3 which is due to more time required in seedbed preparation. Statistical analysis shows that fuel consumption in each treatment vary significantly from each other at 5 percent significance level.

Table 4: mean values of soil, machine and crop parameters for various treatments

Treat-ments	Fuel consumption, l/ha			Number of plant / m^2	Average plant height, cm	Grain yield, q/ha	Straw yield, q/ha
	Tillage	Sowing	Total				
T_1	25.44	6.19	31.63[a]	322	51	49.09[a]	75.09
T_2	21.61	6.19	27.80[b]	328	48	51.24[b]	78.32
T_3	15.60	6.19	21.79[c]	308	50	46.76[c]	70.61
T_4	16.13	6.19	22.32[d]	369	51	53.20[d]	84.59
T_5	-	6.19	6.19[e]	314	50	46.25[ec]	70.66
T_6	53.66	6.19	59.85[f]	319	49	54.09[f]	83.85

Fuel consumption for various sizes of rotavators (T_1-T_4) was also determined on the basis of per meter rotor length and unit volume of soil worked and same is presented in Fig. 1 which indicated minimum fuel consumption (3.08 l/h) for treatment T_3 followed by T_4 (3.28 l/ha), T_2 (3.93 l/ha) and T_4 (4.90 l/ha). Fuel consumption, on the basis of unit volume of soil worked, also followed the similar trend. This indicated superiority of the rotavator used in treatment T_3 over other rotavators. In other words, rotavator with rotor size of 172 cm performed better than other three rotavators in respect of fuel consumption. This may also be due to the fact that the rotavator with rotor length of 172 cm was a better match, as compared to other rotavator size, for the size of tractor being used for operating it.

Wheat yield and its attributes has been presented in Table 4 which revealed that number of plants/ m^2 was observed highest (369) for treatment T_4 followed by treatments T_2, T_1, T_6 and T_5. The same was found minimum (308) for T_3 treatment. The plant height ranged between 48 and 51 cm for all the treatments under the experiment. The result also indicated maximum wheat yield of 54.09 q/ha under treatment T_6. It was observed as 53.20, 51.24, 49.09, 46.76 and 46.25 q/ha for treatments T_4, T_2, T_1, T_3 and T_5 respectively. The yield result

for various treatments was found to vary significantly from each other at 5% significance level, however, yields of treatments T_3 and T_5 did not vary significantly. The higher yield in T_6 may be due to the higher weight of grains per ear head. The straw yield was observed maximum as 84.59 q/ha for treatment T_4 followed by treatments T_6, T_2, T_1, T_5 and T_3 respectively.

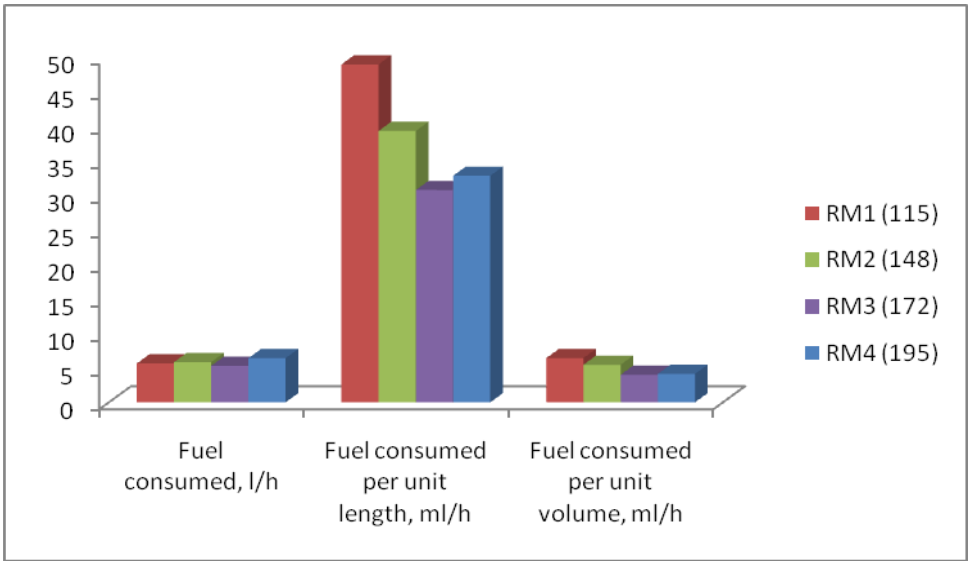

Figure 1. Fuel consumed by various sizes of rotavators

Energy analysis was performed for all the treatments and same has been presented in Table 5 that showed minimum total direct input energy (tillage +sowing operation) of 0.35 GJ/ha for treatment T_5 indicating as most energy efficient treatment. Amongst the rotavator treatments (T_1-T_4), input energy was observed minimum for treatment T_3 as 1.24 GJ/ha. Treatments T_4, T_2 and T_1 consumed 2.34, 27.45 and 45% more energy respectively compared to T_3 which was due to more time and fuel requirements in tillage operation because of comparatively smaller rotor length. Conventional method (T_6) recorded maximum, 174% higher, input energy as compared to treatment T_3. The energy saving, in terms of treatments T_6, was observed highest (89.57%) for treatment T_5 followed by treatments T_3, T_4, T_2. Minimum saving in input energy (47.08%) was observed for treatment T_1. The energy output to input ratio revealed minimum (45.95) for treatment T_6 and maximum 373.80 for treatment T_5 (no-till sowing). The total energy input and energy input-output ratio varied significantly for all the treatments at 5% level of significance. The energy productivity was also observed maximum (13.06 kg/MJ) for treatment T_5 followed by treatments T_4, T_3, T_2 and T_1. Treatment T_6 recorded lowest energy productivity of 1.59 kg/MJ. The statistical analysis indicated significant difference among the values of various treatments, however, treatment T_1, T_2 and T_2, T_3 and T_4 did not vary significantly among themselves at 5% level of significance.

Table 5. Energy input and output for various tillage treatments

Treatments	Human energy, MJ/ha			Fuel energy, GJ/ha	Total input energy, GJ/ha	Energy saving over T_6, %	Energy output, GJ/ha			Energy input-output ratio	Energy productivity, kg/MJ
	tillage	sowing	total				From grain	From straw	Total		
T_1	8.88	5.45	14.33	1.78	1.80[a]	47.08	71.08	69.46	140.54	78.28[a]	2.73a
T_2	7.27	5.45	12.72	1.57	1.58[b]	53.48	74.20	72.45	146.64	92.92[b]	3.25[ba]
T_3	5.80	5.45	11.25	1.23	1.24[c]	63.50	67.71	65.31	133.02	107.43[c]	3.78[cb]
T_4	4.94	5.45	10.39	1.26	1.27[d]	62.65	77.03	78.25	155.28	122.53[d]	4.20[db]
T_5	0.00	5.45	5.45	0.35	0.35[e]	89.57	66.97	65.36	132.33	373.80[e]	13.06[e]
T_6	17.13	5.45	22.58	3.37	3.39[f]	-	78.32	77.56	155.88	45.95[f]	1.59[f]

Economic analysis was performed for all the treatments included in the experiment (Table 4). The cost of cultivation was observed highest (451.57 USD/ha) for treatment T_6 which is 23.04% higher compared to T_5 which recorded minimum (367.01 USD/ha) input cost for cultivation.

Table 4. Economic analysis for various treatments

Treatment	Total input cost, USD/ha			Total return, USD/ha				B:C ratio
	Cost of tillage and sowing (A)	Other inputs cost (B)	Total input cost (A+B)	From wheat grain	From straw	Total return	Net profit	
T_1	56.41	354.51	410.92[a]	1105.79	521.30	1627.10	1216.18[a]	2.96[a]
T_2	49.42	354.51	403.92[b]	1154.22	543.73	1697.95	1294.03[b]	3.20[b]
T_3	40.42	354.51	394.92[c]	1053.31	490.20	1543.51	1148.59[c]	2.91[a]
T_4	39.40	354.51	393.91[dc]	1198.38	587.26	1785.63	1391.72[d]	3.53[c]
T_5	12.51	354.51	367.01[e]	1041.82	490.55	1532.37	1165.36[e]	3.18[bd]
T_6	97.06	354.51	451.57[f]	1218.42	582.12	1800.54	1348.98[f]	2.99[a]

1USD = 67.70 INR

Amongst rotavator treatments (T_1-T_4), T_4 and T_3 were having almost same cost of cultivation. Treatments T_1 and T_2 recorded marginally higher, 4.32 and 2.54%, cultivation cost compared to treatment T_3. Cost of cultivation for treatment T_4 was found 14.64% less compared to T_6.

This was mainly due to more time and fuel consumption per unit area basis. The net profit was observed highest for treatment T_4 followed by T_6, T_2, T_1, T_5 and T_3 respectively.

The statistical analysis indicated significant variation for all the treatments at 5% significance level. The benefit-cost ratio was found maximum (3.53) for treatment T_4 followed by T_2 (3.20), T_5 (3.18), T_6 (2.99), T_1 (2.96) and T_3 (2.91)

respectively. Treatment T_4 resulted in 18.06% and 11.01% higher benefit-cost (B: C) ratio compared to treatment T_6 and T_5 respectively.

The statistical analysis indicated significant difference between the treatments T_1, T_2, T_4 and T_5, however, it did not vary significantly for rest of the treatments at 5% significance level.

CONCLUSIONS

In terms of direct energy requirement, T5 (no-till sowing) was found most energy efficient treatment for wheat establishment. Among rotavator treatments (T1-T4), treatment T3 and T4 showed similar result and were found energy efficient next to T5. The energy productivity was found again higher for T5, T4 and minimum for treatments T1, T6. B:C ratio was found higher for T4 followed by T2 and T5 treatments. Based on study, it is concluded that larger size of rotavator (195 cm rotor length) could be used as a substitute to conventional method of wheat establishment. Amongst all the treatments, no-till is most energy efficient method of wheat cultivation.

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DOI: 10.17707/AgricultForest.62.4.14

Danguolė JUŠKEVIČIENĖ, Rasa KARKLELIENĖ, Audrius RADZEVIČIUS, Audrius SASNAUSKAS [1]

PRODUCTIVITY AND MORPHOLOGICAL FEATURES OF GARLIC (*ALLIUM SATIVUM L.*) GROWN IN LITHUANIA

SUMMARY

Garlic (*Allium sativum* L.) is the second most popular vegetable in the genus *Allium* in Lithuania. The field collection of garlic in the LRCAF Institute of Horticulture was established in 1992. New accessions enter the collection continually.

The aim of the research was to determine the productivity and structure of yield, to assess the morpho-biological features of 24 hardneck and the 14 of softneck garlic clonal accessions. Colour of external scale of bulbs, number of cloves in a bulb, arrangement and skin colour were estimated according guidelines for DUS test. The data of yield was statistically processed by the ANOVA method and analysis of principle coordinates within SPSS.

Results showed the differences in productivity and morphological features between both of types, populations and cultivars. The yield of hardneck garlic bulbs reached 16.8 $t \cdot ha^{-1}$, and lower yield 12.1 $t \cdot ha^{-1}$ was found of softneck garlic**.** Local populations of hardneck garlic showed higher possibility of yield formation compared with the foreign cultivars. More cloves per bulb were observed from softneck garlic accessions, while hardneck garlic distinguished with the formation of heavier cloves.

Three garlic cultivars Žiemiai, Dangiai and Vasariai are included in the National List of Plant varieties and Common catalogue EU of vegetable species varieties.

Keywords:, garlic bulb, morphobiological variation, yield, cultivar

INTRODUCTION

Garlic (*Allium sativum* L.) is an important vegetable in Lithuania which takes one of the main positions beside onions, carrots, cabbages, red beets. Growing area of garlic takes about 500 ha according presented reports of Association of vegetable growers and personal contacts. Lithuanian Institute of Agrarian Economics informs that local growers do not provide garlic demand in Lithuania. Therefore garlic should be grown on a larger scale.

Two types of cultivar representing softneck and hardneck garlic different

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".

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

according their productivity and morphological features are cultivated in Lithuania. The softneck (not forming flower stalk) garlic is planted early in spring (March-April) and hardneck (forming flower stalk) garlic in autumn (October) traditionally. Planting of garlic in spring was more popular growing method in the past, while planting of cloves in autumn is becoming more popular at present. Local populations and cultivars are mainly grown, but many of new foreign cultivars are available from seed companies and retailers. Using of planting material from nonlocal sources can be result of an unpredictable yield of bulbs. Bulb size, shape, colour of scales, number of cloves can be not display the characteristics that are presented in the catalogs. This is a result of large variability of bulb size, colour of scale, yield, and flavour influenced by genetic factor, climatic conditions, growth environment and production year (Grégrova, 2013, Moravčević et al., 2011, Volk and Stern, 2009, Waterer and Schmitz, 1994).

Many reports concerning with morphological features of various garlic cultivars are presented (Al-Zahim et al., 1997, Castellanos et al. 2004, Engeland, 1995, Ipek, 2003, Maaβ and Klass, 1995, Waterer and Schmitz, 1994). Identification of uniform and stable traits can afford a ground for indication of typical morphotypes of garlic cultivars in different climatic conditions and growth regions.

Lithuanian cultivars are positively assessed by experts and consumers. But regarding of assortment increasing of imported cultivars it is hard to choose one suitable to the growing demands for obtaining high quality yield and the profitability of production.

The aim of this investigation was to assess and to compare differences among garlic morphotypes, national and foreign populations and cultivars according their productivity, to evaluate morphobiological features. Results of the investigation give a possibility to determine of important qualitative parameters of garlic populations and cultivars and to optimize of garlic growing strategy. Cloves of the best samples will be used for the creation of new cultivars as breeding material.

MATERIAL AND METHODS

Investigation was carried out in crop rotation of the experimental field at the Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry (IH-LRCAF) in 2013 – 2015. Soil type: sandy light loamy *Calc(ar)i-EpihypogleyicLuvisol* (*LVg-p-w-cc*) (Buivydaitė *et al.*, 2001).

Total amount of investigated cultivars and populations reached 38 from which 24 samples representing hardneck morphotype and 14 softneck. Garlic representing of hardneck morphotype involved 8 cultivars of different origin: Žiemiai (Lithuania), J. Gribovo (Russia), Ducat and Unicat (Czech Republic), Liubasha (Ukraine), Teodor (Germany), Chinese (China), Spring Violeta (Spain) and 16 local populations: No.4, No.5, No.6, No.7, No.11, No.15, No.16, No.24, No.27, No.28, No.30, No.36, No.37, No.39, No.40, No.41. Samples representing

of softneck garlic involved 2 cultivars: Vasariai (Lithuania) and Jarus (Poland) and 12 local populations: No.8-2, No.9, No.14, No.15, No.19-1, No.19-2, No.22, No.25, No.26, No.27, No.28, No.40.

Cloves of hardneck garlic with an average 7 g of weight were planted on the last decade of October (in 2013 and 2014) and respectively softneck garlic with an average weight 2 g on the first decade of April (in 2014 and 2015). Planting of cloves was carried out by hand in 1m wide furrows. Distances of 25 cm between lines and 7 cm between plants were left. Area of record plot was 0.7 m². Experiment was carried out in three replications. During growing season fertilization, weeding, chemical plant protection and top removing were carried out typically according to the standard recommendations. Harvesting of hardneck garlic bulbs was carried out on the second decade of July, when the lower leaves of 30- 50% had dried. Sofneck garlic was harvested on the first decade in 2014 and on the second decade in 2015 of August when leaves had dried 80% and fall down. Bulbs were dried by using of active ventilation about two weeks. After drying bulbs were grated into two size fractions and total yield of garlic and its structure was determined.

Marketable yield was consisted of well developed, good quality bulbs with diameter above 4.0 cm of hardneck garlic and above 3.0 cm of softneck garlic. The rest bulbs were characterized as non-marketable, that includes small with diameter below 4.0 cm and 3.0 cm and damaged by diseases and pests. Ten marketable bulbs and ten cloves were weighted for the determination an average weight of a bulb and clove. Morphobiological features of 5 representative bulbs of each population and cultivar were estimated according guidelines for distinctness, uniformity and stability (DUS) test.

Such characterististics were included: colour of external scale of bulbs, number of cloves in a bulb, arrangement and skin colour.

The data of yield was statistically processed by the ANOVA method according to Fisher's and Duncan's multiple range for mean separation at 5% significance level and analysis of principle coordinates within SPSS (Statistical Package for the Social Sciences, 2002) SoftwareV.II.5.

Meteorological conditions: Good system of roots is one of the most important factors influencing productivity of garlic. The mean temperature was 5.4 and 4.9°C in October of 2013 and 2014, respectively the amount of precipitation reached 45.2 and 48.8 mm. In November the temperature reached 2.6 and 3.8°C and amount of precipitation was 45.8 and 43.3 mm. Meteorological conditions were favourable for rooting of hardneck garlic. The mean temperature in April of 2014 and 2015 had not exceeded 8.1°C therefore conditions were optimal for softneck garlic rooting. Air temperature in May, June and July of both year investigations was similar, but amount of precipitation was more than twice higher in 2014 than at the same period in 2015. This influenced better formation and growing of bulb in 2014. June was dry in 2015, but there were raining often in July, therefore was not missing of drought for softneck garlic growing.

RESULTS AND DISCUSSION

Lithuanian consumers prefer hardneck garlic bulbs with high dry weight and white colour or other light coloured of external scale. Desirable diameter of bulb it is about 4 cm and above.

The total yield of the investigated hardneck garlic populations and cultivars varied from 4.5 to 16.8 t·ha-1 in 2013–2015 (Table 1). Population No.30 distinguished with the significantly highest productivity.

The total yield of national cultivar Žiemiai reached 12.9 t·ha-1. According results of three years investigations it was determined that marketable yield of all populations and cultivars constitutes an average 87% in the total yield.

The significantly highest marketable yield 15,8 t·ha-1 was obtained from population No.30. It was determined 11 samples that formed the significantly heaviest bulbs. The average weight of a bulb of these samples reached from 72 to 82 g. Cloves number of hardneck garlic reached from 7.1 to 11.1 per bulb. Obtained results proved that number of cloves per bulb is affected by genetically factors. According Mahadeen A.Y. (2011) and Reghin et al.(2004) small (<1 g) seed cloves produced the least number of cloves, while Ahmed et al. (2007) disagreed with this position.

Colour of external scale of bulb is an important morphological parameter describing their economic value, because it is determined by the requirements of consumers. In the investigation bulbs and cloves was observed of variegated colour, sometimes with the anthocyanin stripes on dry external scales. Colour of external scales of local populations and foreign cultivars was constant, while some researchers present high variability among cultivars across different growth locations (Waterer and Schmitz, 1994, Volk and Stern 2009).

Investigated samples showed their hardneck phenotype and formed flower stalk from the central part of bulb, except cultivar Teodor that did not produce the flower stalk. Cloves of all samples showed single arrangement in a bulb.

The total yield of softneck garlic varied from 4.5 to 12.1 t·ha-1 (Table 1). Population No.40 formed the significantly highest total, marketable yield and amount of cloves per bulb.

Marketable yield reached 11.2 t·ha-1, respectively the number of cloves 24.8. Statistically significant differences of cloves number among other populations were not determined, except a few samples. Populations No.9, No.14, No.15, No.28 and No.40 formed the heaviest bulbs respectively from 21 to 26 g.

All investigated samples of softneck garlic did not form the flower stalks and cloves were arranged into two-three layers in a bulb.

External colour of dry scale was white or cream and colour of cloves skin varied among tested cultivars and populations from light purple to white or white with the anthocyanin stripes.

Table 1. Garlic productivity and morphological features 2013–2015

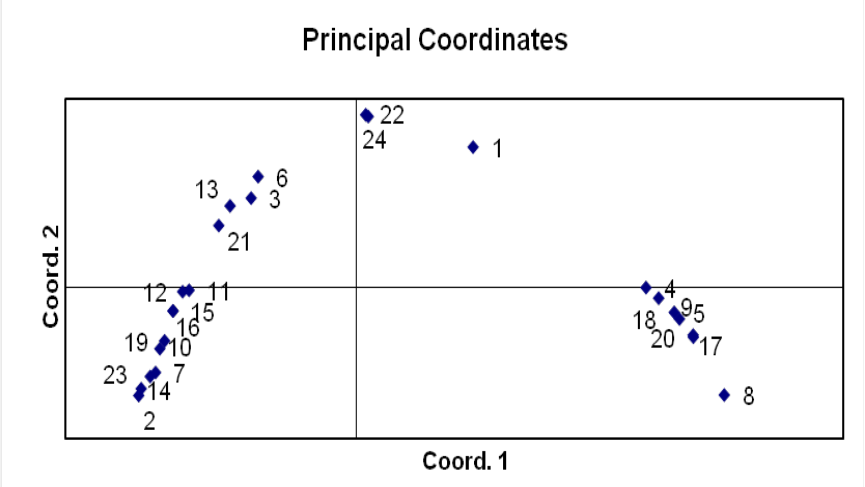
Cultivar/ Population	Total yield ($t \cdot ha^{-1}$)	Marketable yield ($t \cdot ha^{-1}$)	Average weight of marketable bulb (g)	Colour of bulb external scales	Number of cloves	Colour of cloves skin	Arrangement of cloves
Hardneck garlic							
Žiemiai	12,9	11,9	55cd	White	11,1a	White cream	Single
No.24	14,4	13,9	82a	White+stripe	7,1cd	Cream purple	Single
Gribovo	8,5	7,4	70b	Purple	8,2c	Purple	Single
Ducat	7,2	6,0	44e	Cream	7,3cd	White	Single
Unicat	9,4	8,0	41e	purple	8,3c	White purple	Single
Liubasha	12,8	12,1	69ab	Purple	9,4b	Light purple	Single
Teodor	7,8	5,8	80a	White	10,4a	White crem	Single
Chinese	4,5	3,8	27d	White	7,4cd	White	Single
Spring	7,5	6,2	41e	Pruple	8,3c	Prple	Single
Violeta							Single
No.4	9,6	9,2	78a	White	7,2cd	Crem	Single
No.5	8,7	6,9	75b	Light purpule	8,0c	Purple+stripe	Single
No.6	14,3	17,1	71b	White	8,7e	White	Single
No.7	11,6	10.8	71b	White+stripei	7,1ed	Lght purple	Single
No.11	9,1	7,8	80a	White	7,1ed		Single
No.15	10,9	9,7	76ab	White	9,4b	White	Single
No.16	14,4	13,1	76ab	Crem	7,8c	Creme	Single
No.27	6,8	5,3	39e	White+stripe	7,8c	Light purple	Single
No.28	7,4	6,0	43e	White	8,2c	White	Single
No.30	16,8	15,8	78a	Crem	9,8ab	White cream	Single
No.36	7,8	6,1	39e	Light Purple	9,1b	White+stripe	Single
No.37	10,3	8,9	72b	White+stripe	10,6a	Light purple	Single
No.39	10,9	10.0	62c	White+stripe	7,6cd	Purple	Single
No.40	13,9	12,5	81a	White	11,0a	Cream	Single
No.41	12,8	11,8	62c	Dark purple	8,8c	Dark purple	Single
LSD_{05}	2,3	2,0					
Softneck garlic							
No.8-2	5,8	5,4	19b	White	19,6b	White	2 layers
No.9	4,4	3,9	21ab	White+stripe	14,8c	Light purple	3 layers
No.14	9,4	8,9	21ab	White	14,1c	Cream	2-3 layers
No.15	7,2	6,0	21ab	White	15,1c	White cream	2 -3layers
No.19-1	9,4	9,0	16b	Cream	14,9c	Cream	2 layers
No.19-2	8,9	8,7	17b	Cream	14,6c	Cream	2 layers
No.22	5,4	4,4	14bc	White	16,1bc	White	2 layers
No.25	6,2	5,0	16b	Cream	13,1c	White	2-3 layers
No.26	5,5	4,1	14bc	White	12,6e	White+purple	2-3 layers
No.27	6,7	5,6	18b	Crem	13,3ed	Cream	2-3 layers
No.28	5,8	5,0	22a	White	13,4ed	White+purple	2 layers
No.40	12,1	11,2	26a	White	24,8a	White	2-3 layers
Jaris	9,8	9,0	18b	White	15,0e	White cream	2 layers
Vasariai	9,9	8,9	17b	White	14,6c	Cream	2 layers
LSD_{05}	1,4	1,2					

Means followed by the same letter within the column do not differ significantly at P = 0.05 (Duncan's multiple range test).

The principal coordinate analysis (PCA) showed different ability of all investigated populations and cultivars for the productivity. According obtained results it is clear that it is possible to classify the investigated samples of hardneck garlic into several groups with different potential possibilities for yield and bulb formation (Fig. 1A). Garlic cultivar Žiemiai, populations No.39 and No.40 were located into one group on the PC1 scatter plot with high positive value and their yield reached from 10.9 to 12.9 t·ha^{-1}and weight of bulb from 55 to 62 g. Cultivar Teodor and nine local populations were located in one big group at the PC scatter plot. Samples on this group distinguished with ability for the heaviest bulb formation and averaged weight of bulb reached 75 – 82 g. Cultivars from Spain, Czech Republic, China and three local populations were located in another group on the front side of the scatter plot. The yield and weight of a bulb of these samples were obtained the lowest and varied respectively from 4.5 to 9.4 t·ha^{-1}and from 27 – 44g.

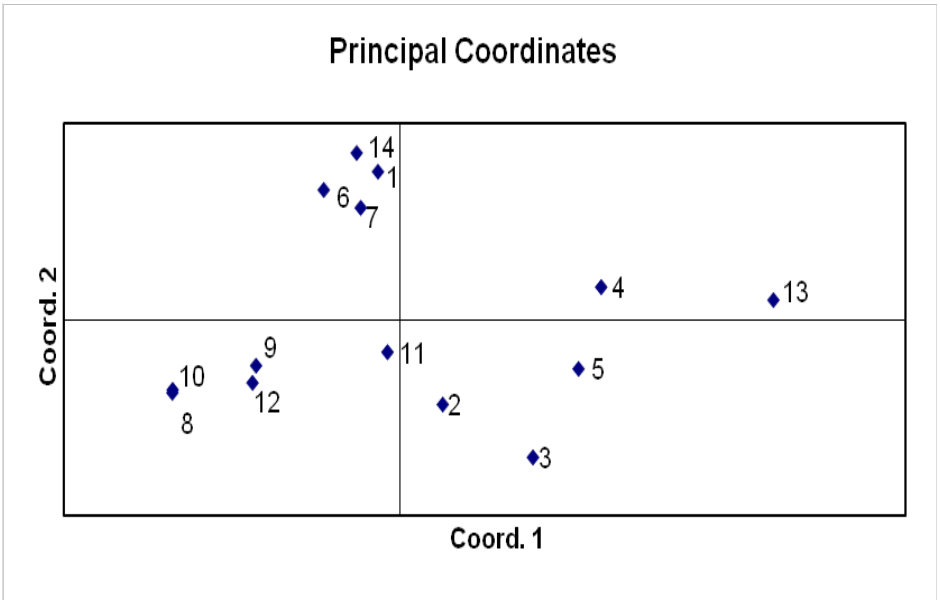
These results confirm the conclusions of other authors that garlic is very sensitive to the climatic conditions and its growth environment (Volk and Stern, 2009, Engeland, 1991, Waterer and Schmitz, 1994).

PCA of softneck garlic yield and bulb weight confirms a wide location of investigated populations and cultivars. Populations No.14 and No.40 were located separately from other samples at the PC scatter plot with high positive value (Fig.1B). These populations distinguished with the highest yield formation, respectively 9.4 and 12.1 t·ha^{-1}.



1 - Žiemiai; 2 - No.24; 3 - J.Gribovo; 4 – Ducat; 5 – Unicat; 6 – Liubasha; 7 – Teodor; 8 – Chinese; 9 - Spring Violeta; 10 – No.14; 11 – No.5; 12 – No.6; 13 – No.7; 14 – No.11; 15 – No.15; 16 – No.16; 17 – No.27; 18 – No.28; 19 – No.30; 20 – No.36; 21 – No.37; 22 – No.39; 23 – No.40; 24 – No.41.

Fig.1 A. Scatter plot of various hardneck garlic populations and cultivars according to the results of principled coordinate analysis (PC) of total yield and weight of bulb data in 2013–2015



1 - Vasariai; 2 – No.2; 3- No.9; 4 – No.14; 5 – No.15; 6 – No.19-1; 7 – No.19-2; 8 – No.22; 9 – No.25; 10 – No.26; 11 – No.27; 12 – No.28; 13 – No.40; 14 – Jarus.

Fig.1B. Scatter plot of various softneck (B) garlic populations and cultivars according to the results of principled coordinate analysis (PC) of total yield and weight of bulb data in 2013–2015

The results obtained in our investigation and reports of other researchers prove that cultivar is an important factor that affects plant productivity (Karklelienė *et al.* 2015, Volk and Stern, 2009, Waterer and Schmitz, 1994).

Comparison of different cultivars and hybrids creates a presumption for practical purpose to choose the most valuable breeding product.

CONCLUSIONS

Investigated populations and cultivars of garlic were differed according their productivity and morphological features. Bulbs of local and foreign samples retained their hardneck or softneck morphotype. The yield of hardneck garlic bulbs reached 16.8 $t \cdot ha^{-1}$, and lower yield 12.1 $t \cdot ha^{-1}$ was observed of softneck garlic. Local populations of hardneck garlic showed higher possibility of yield formation. More cloves per bulb were obtained from samples of softneck garlic, while hardneck garlic distinguished with the formation of heavier cloves.

ACKNOWLEDGEMENTS

This work was carried out within the framework of the long-term research program “Genetics and direct genotype development of agricultural and forestry plants” implemented by Lithuanian Research Centre for Agriculture and Forestry.

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DOI: 10.17707/AgricultForest.62.4.15

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THE REGROWTH CONSERVATION AFTER SELECTION CUTTING OF FOREST STANDS GROWING IN *HYLOCOMIOSA* SITE TYPE

SUMMARY

Clear cutting and selection cutting are final felling methods. Clear cutting are often used in Latvian forest. The disturbance of biological rhythm in clear cutting areas occurred for several years. Whereas selection cutting is more environmentally friendly, efforts should be made to apply it more in practice. Objectives of this research are to analyze the regrowth conservation after gradual felling in Hylocomiosa forest stands with a different species composition and determine tree health. For dendrometric indicators and gradual selective felling intensity characterization, twelve sample plots with an area of 500 m2 were established in both forest stands. Plots were surveyed before and after the gradual selective felling. In order to perform an analysis of regrowth, 92 small inventory plots with a radius of 2.82 m were evaluated. Regrowth trees were counted (conifers from 0.1 m height and deciduous - 0.2 m) and their height was measured, as well as root rot infection was verified. In the Norway spruce mixed stand (7S3P145) felling intensity was 52% but in the Scots pine mixed stand (5P5S105) respectively – 49%. After felling trees regrowth number of losses account are 53 and 68%. The average height of spruce regrowth trees in both stands after felling was fallen but average height of birch and Scots pine regrowth increased. The differences of regrowth preservation between tree species during selection cutting were significant ($p=0.016<\alpha=0.05$). Root rot was not detected in the mixed Scots pine stand, but in spruce stand it was found in 33% of cases.

Keywords: mixed stand, clear cutting, height, root rot

INTRODUCTION

Selection cutting as final cutting methods are known and used for a long time in Latvia but popularity in state and private forests is low. Nowadays clear cutting are recognized and more often used when all stand is cut down in one time. Naturally developed forest stand borders often are disassembled using clear cut and instead new forest stand borders in forest array structure are brightly visible and inherited in future (Lūkins and Nikodemus, 2011). Selection cutting can be used more often in future therefore more research is needed. In 2015 final

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Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

felling area in Latvia was 38876.77 ha; selection cutting was used in 14.4% of cases or 5594.56 ha and distribution shows that state forest manager used selection cutting in 6.9% (386.48 ha) and other forest managers in 93.1% (5208.08 ha) of cases (Forest statistics, 2015).

At this moment are different opinions regarding to optimal time period between cutting repetition; most mentioned is 4 – 6 years. Mainly it depends from stand condition after cut and young tree growth rate. Openings after selection cut in stand create different light conditions depending from angle of sun incidence and remaining tree height (Canham et all., 1990). Sometimes is needed urgent separate tree felling before time due to rapid quality decreasing (Rozītis and Lūkins, 2008). Natural stand regeneration is contributed by light condition under tree canopy (Gray and Spies, 1996; Lieffers et all., 1999). Opening enlargement incenses light amount in its center as function of stand opening diameter and surrounding stand tree height (Lieffers et all., 1999). Ecological and visual changes are less significant if selection cutting is managed correctly. Develops continuous forest array with sparse and thick different tree species groups if forest stands are managed using selection cutting and without brightly visible forest stand borders as using clear cutting methods. Selection cutting compared to clear cutting has minimal influence on forest ecosystem as different age and height trees make forest stand stable and resistant as possible (Rozītis and Lūkins, 2008). Using selection cutting in forest stand trees can be felt down in quantity until stand basal area is larger than critical basal area (LRMK, 2012) and owner has no need in artificial stand regeneration (Mangalis, 2004).

Selection cutting usage increases forest stand resistance from pest and disease damage who can endanger certain age specific tree species stands.Ground cover and its biodiversity is preserved; rare moss and arthropods continuous development needed conditions are created (Zviedris, 1949).

Rozītis and Lūkins (2008) have listed main mistakes and their description. Most frequent mistakes are related to windthrow risk incensement due to large intensity selection cutting. Wrong chosen cut down trees change stand and species structure. Selection cutting done in wrong time of year can lead to remaining tree logging damage and pest and disease damage distribution. Too small stand openings decreases natural stand regeneration rate.

Areas in Latvia where clear cutting is forbidden by regulations are used selection cutting methods and they are solution between ecologic, economic and social forest management disagreement (Zdors and Donis, 2011). Selection cutting mainly is used to gain forest stand natural regeneration in forest site type Hylocomiosa with Scots pine or with Norway spruce mix; also in above mentioned forest ecosystem deciduous stands with spruce as regrowth or in second floor in compartments that are planned as spruce and deciduous species mixed stands (Bisenieks, 2003).

Forest as ecosystem has been developing in long way of evolution depending on different natural conditions and processes. To gain success in forest

management it has to be coherent and adjusted to natural conditions and ecosystem natural structure and dynamics specialty (Bušs, 1981). In this way is provided better forest stand resistanceto windthrow possibility and larger stand durability against pest and disease damage for certain tree species in different stand age. In basis of above mentioned has been highlighted aim to analyze the regrowth conservation after selection cutting of forest stands growing in Hylocomiosa site type.

To realize research aim whereset following objectives: 1) to analyze dendrometric indicators of Scots pine stands before selection cutting; 2) to analyze dendrometric indicators and sanitary state of stand after selection cutting depending on intensity of cutting.

MATERIAL AND METHODS

Research data was collected in Scots pine (P) *Pinus sylvestris* L. and Norway spruce (S) *Picea abies* (L.) H. Karst. two mixed stands of *Hylocomiosa* forest site type in Vidzeme region (in area of 2.2 ha with species composition $7S3P_{144}$ (Lat: 57.413770; Long: 25.938024) and 10.9 ha – $5P5S_{104}$ (Lat: 57.528539; Long: 27.069571)) with gradual selective felling done in them.

In both forest stands are installed two type of temporary sample plots (hereafter SP); rectangular SP 20x25 m (1 SP on 1 ha of stand area and each 500 m^2) and round SP with radius 2.82 m (25 m^2 each and 4 SP on 1 ha). Larger temporary SP (12 over two stands together) established in stands characteristic places to measure dendrometric indicators before selection cutting - stand average breast height (1.3 m) diameter (DBH) and height, average tree cross-section, average tree volume, stand basal area and growing stock and after gradual selective felling - stand residual basal area, growing stock and cutting intensity. On both stand longest diagonal was established 92 round SP for regrowth registration and height measurement (coniferous from 0.1 m height and deciduous – 0.2 m) and also to evaluate sanitary state before and after gradual selective felling. Regrowth height measured with 5 m long metallic measuring tape (measurement accuracy ±1 cm). In each sample plot was cut down one regrowth tree and visually tested for rot in root collar level for determination of sanitary state in stand.

In larger SP with forest caliper was measured DBH (measurement accuracy ± 0.1 cm), and tree height was measured with VERTEX height meter (measurement accuracy ± 0.1 m), and trees was divided in Craft crown classes (Kraft, 1884; Miezīte et al., 2013). Empirical data was mathematically treated with Microsoft Office Excel 2013. Tree cross-section (g) in registering sample plot was calculated by Eq. (1):

$$g = 0.7854 \cdot \frac{D^2}{10000}, \quad (1)$$

where *D*– DBH (cm). Stand basal area (G) was calculated by Eq. (2):

$$G = \frac{G_{uzsk.PL}}{L} \cdot 10000 \text{ ,(2)}$$

where $G_{uzsk.PL}$ – summary tree cross-section of registering sample plots (m^2); L – summary area of registering sample plots (m^2). Average tree stem volume (v) was calculated by Eq. (3) (Liepa, 1996):

$$v = \psi * L^{\alpha} * D^{\beta \lg L + \varphi}, \quad (3)$$

where L – stem height, (m); D – DBH, (cm); ψ, α, β, φ – empirically established coefficients (Scots pine – ψ – $1{,}6541*10^{-4}$, α - 0,56582, β – 0,25924, φ – 1,59689; Norway spruce – ψ – $2{,}3106*10^{-4}$, α – 0,78193, β – 0,34175, φ – 1,18811). Growing stock (V) was calculated by Eq. (4):

$$V = \frac{V_{PL} \cdot 10000}{L} \text{ , (4)}$$

Felled stand volume ($V_{izc.}$) in selection cutting was calculated by Eq. (5):

$$V_{izc.} = G_{izc.} \cdot HF \quad (5)$$

where HF – form height (LRMK, 2014). Gradual selecting felling intensity was calculated by Eq. (6):

$$I = \frac{V_{izc.}}{V} \cdot 100 \quad (6)$$

All above mentioned calculations were done with Microsoft Excel 2013 and for description of tree dendrometric indicators and regrowth was used Descriptive statistics function. For comparison of regrowth tree species samples was used ANOVA analysis (Arhipova and Bāliņa, 1996).

RESULTS AND DISCUSSION

Woodland natural structure bigger maintaining opportunities are to carry out and consummate nature friendly forestry methods, for example, using selection cutting to maintain diversity, stability and provide forest functions in collective nature and social system which is one of sustainable forest resource management preconditions (Lindenmayer et al., 2006). During first felling time was cut down withered and pest infected trees, windthrow endangered terrain depression spruce trees, also trees shading regrowth and lower Kraft crown classes trees. Mixed stand dendrometric indicators were calculated before and after selection cutting and gathered in table 1 where we can see that in Hylocomiosa Pine mixed stand (5P5S105) average tree cross-section was 0.0630±0.00416 m3 but mixed stand basal area 37.2 m2 ha-1, average tree volume – 0.7184 m3 and stand growing stock 427 m3 ha-1. Felled stand basal area 16.8 m2 ha-1 and felled volume – 207 m3 ha-1. In Hylocomiosa Spruce mixed stand (7S3P145) average tree cross-section was 0.0823±0.00752 m2, stand basal area 49.3 m2 ha-1 and growing stock 631 m3 ha-1. Felled stand basal area was 26.1 m2 ha-1 and felled volume – 325 m3 ha-1. Felling intensity was 49 –

52% with what can be achieved sufficient regrowth natural regeneration and growth (Erefur, 2010).

Table 1. Mixed stand dendrometric indicators before and after selection cutting.

Species composition before / after felling, Age	Before felling				Before/ after felling		I, %
	D, cm	H, m	$g_{vid,}$ m^2	v, m^3	G, m^2 ha^{-1}	V, m^3 ha^{-1}	
$5P5E_{105}$ / $10P_{105}$	28.3± 0.92	22.8± 0.67	0.0630± 0.00416	0.7184± 0.00623	37.2 / 17.4	427 / 220	48.5
$7E3P_{145}$ / $8P2E_{145}$	32.3± 1.43	25.1± 0.62	0.0823± 0.00752	1.0779± 0.01548	49.3 / 23.2	631 / 306	51.5

Captions: d – average dbh ± standard error; h – average tree height ± standard error; gvid. – average tree cross-section± standard error; v – average tree stem volume ± standard error; v – stand growing stock; i – felling intensity, %.

Craft crown classes are used to describe tree growth in research stands where trees are divided in 5 classes depending on tree height, diameter and crown projection. All five craft crown classes (pre-dominant (i), dominant (ii), co-dominant (iii), suppressed (iv) and completely suppressed trees (v)) are represented in both research stands before gradual selecting felling. In both stands scots pine represent i, ii un iii craft crown class trees but norway spruce in 5p5s105 mixed stand represent ii, iv and v craft crown class trees and in 7s3p145 mixed stand spruce represent even i craft crown class trees. After selective cutting remaining trees in both mixed stands represent i, ii and iii craft crown classes or dominant trees from whom larger part are ii craft crown class trees. During gradual selective cutting were all iv and v craft crown class and damaged trees felled and also part of i craft crown class trees with large crown that decreased stand openings and reduced sufficient stand regeneration (rozītis and lūkins, 2008).

Regrowth are small trees below stand canopy that will form future stand in specific growth conditions (Bisenieks, 2003), see figure 1.

Mixed hylocomiosa pine stand (5p5s105) overall regrowth tree number before selective cutting was 1674 trees per ha (spruce, pine, birch) which is two times smaller than in spruce mixed stand but after selective cutting regrowth tree number was only 540 trees per ha (spruce, pine, birch). In spruce hylocomiosa mixed stand (7s3p145) before selective cutting were counted 3600 regrowth trees per ha (spruce, birch) and after selective cutting 1707 regrowth trees per ha (only spruce). There are significant difference between regrowth tree retention between tree species after gradual selective cutting ($p = 0.030$).

Regrowth trees are situated irregularly in mixed stands before and after selective cutting. Pine hylocomiosa mixed stand (5p5s105) 11% sample plot didn't contain regrowth trees before gradual selective cutting but after already

42% of established sample plots. Spruce hylocomiosa mixed stand (7s3p145) 7% sample plot didn't contain regrowth trees before gradual selective cutting but after cutting 20% of established sample plots. Regrowth decreasing during forest logging is significant and in pine (5p5s105) mixed stand reaches 68%, while by species: s – 3/5, p – 4/5, but in spruce (7e3p145) mixed stand reaches 53% (s ~ 1/2). In pine (5p5e105) mixed stand birch regrowth is located in bio groups and not inhibit pine development.

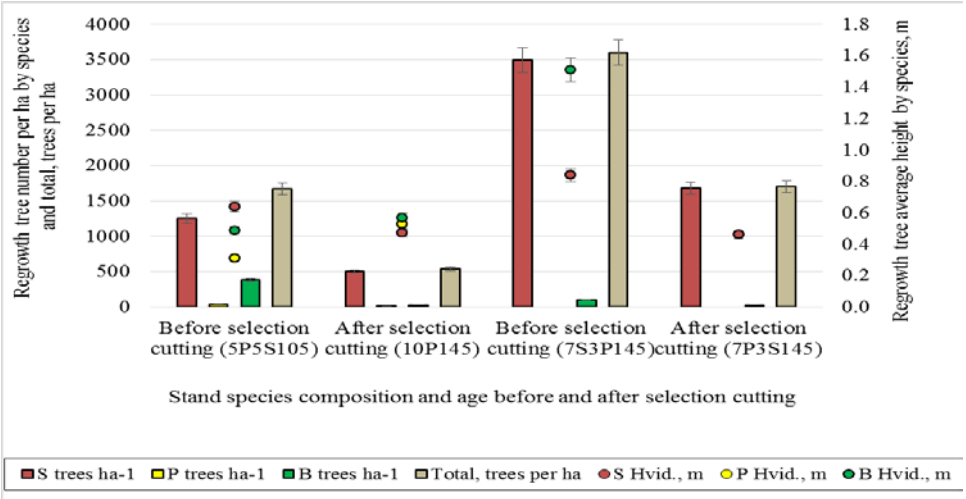

Figure 1. Regrowth trees per ha in Hylocomiosa mixed stands before and after gradual selective cutting and regrowth tree average height

Pine ($5P5E_{105}$) mixed stand spruce regrowth tree average height before gradual selective felling was 0.64±0.038 m but after felling 0.47±0.032 m, birch regrowth average height before gradual selective cutting was 0.49±0.038 m and after felling 0.57±0.181 m. Pine regrowth tree average height before gradual selective cutting was 0.31±0.055 m but after cutting 0.53±0.182 m. Spruce regrowth tree average height decrease after gradual selective cutting is result of taller regrowth tree damage during stand logging. Pine and birch regrowth tree average height increased after gradual selective cutting.

Spruce ($5P5E_{105}$) mixed stand spruce regrowth tree average height before gradual selective felling was 0.84±0.073 m but after felling 0.46±0.029 m, birch regrowth average height before gradual selective cutting was 1.51±0.59 m but after cutting birch and pine regrowth trees was not found – they were destroyed during logging process.

During research was estimated occurrence of root rot. In Pine ($5P5E_{105}$) mixed stand 77 sample plots were no root rot cases but from 15 Spruce ($7E3P_{145}$) mixed stand sample plots in 5 of them (33 %) was estimated root rot cases and it can be explained by stand species composition –relatively more spruce trees who are less resistant to root rot than pine trees.

CONCLUSIONS

In Pine (5P5E105) *Hylocomiosa* mixed stand gradual selective cutting intensity was 52% but in Spruce (7E3P145) *Hylocomiosa* mixed stand – 49%. There are significant difference between regrowth tree retention between tree species after gradual selective cutting ($p = 0.030 < \alpha = 0.005$), in Pine (5P5S105) mixed stand regrowth tree number decrease reaches 68 %, while by species: S – 3/5, P – 4/5, B - 9/10, but in Spruce (7S3P145) mixed stand reaches 53 % (S ~ 1/2).

Spruce regrowth tree average height is decreased after gradual selective cutting in result of taller regrowth tree damage during stand logging but pine and birch regrowth tree average height increased after gradual selective cutting because more damage was done to shorter regrowth trees.

Regrowth trees are situated irregularly in both mixed stands before and after selective cutting.

In Pine (5P5S105) mixed stand were no root rot cases but in Spruce (7S3P145) mixed stand root rot was recognized in 33 % of cases.

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DOI: 10.17707/AgricultForest.62.4.16

Halil BOLU [1]

SOUTHEASTERN ANATOLIA REGION INSECT FAUNA I (COLEOPTERA I: CARABOIDEA; DYTISCOIDEA; BOSTRICHOIDEA; CHRYSOMELOIDEA; CLEROIDEA; CUCUJOIDEA) OF TURKEY

SUMMARY

The aim of this study was to determine Insect fauna Southeastern Anatolia Region. Surveys on insect species in various ecologies have been conducted in the provinces (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) of Southeastern Anatolia region between the years 1948-2013. Almost 2600 species and subspecies among 180 families belonging to 13 insect orders are defined owing to these studies. Coleoptera species formed about 20% of the collected insects. Coleoptera included 32 families were recorded. During this study totally 268 species were found in 13 families and in 6 superfamilies of Coleoptera. Those superfamilies are Caraboidea, Dytiscoidea, Bostrichoidea, Chrysomeloidea, Cleroidea and Cucujoidea. The distribution of determined insect types according to the provinces, plant hosting and feeding type is also done. Information about their identification, host plants, and distribution in Southeastern Anatolia Region was presented as detailed.

Keywords: Southeastern Anatolia Region, Insect Fauna, Coleoptera, Turkey.

INTRODUCTION

Insects (Insecta) are the most numerous group of animals in the world, with over one million species that have been described (Price, 1997). Insects are difficult to study because they represent the most species-rich, yet one of the least known, of all taxa of living organisms, a problem that is compounded by a dearth of skilled entomologists. Although the number of described insect species is uncertain due to synonyms and the lack of a global list, most authorities recognize 900000–1000000 named morpho-species, representing 56% of all species known on Earth (Groombridge, 1992; Anonymous, 2003). Sensible estimates of the number of insects yet to be discovered range from another 1 million to 30 million species (Erwin, 1982; 1991), although most predict around 2–8 million more species (May, 1990; Gaston, 1991; Stork, 1997; Ødegaard, 2000). Conservative estimates suggest that 50–90% of the existing insect species on Earth have still to be discovered, yet the named insects alone comprise more than half of all known species of organism.

Insects constitute the most diverse form of animal life in terrestrial ecosystems. Most species are innocuous and essential components of natural

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

ecosystems. Because they are cold-blooded, the rates of key physiological processes in their life cycles are determined by environmental conditions, especially temperature and precipitation. In general they have short generation times, high fecundity and high mobility (Moore and Allard, 2008). Turkey in fact seems to be like a small continent in terms of biological diversity. Despite the Anatolia is not a continent alone, it contains all properties of a continent that should have an ecosystem and habitat. Each of seven geographical regions in Turkey has a distinguishable climate, flora and fauna. The aim of this study is to make contribution to some faunistical records of Coleoptera of the Southeastern Anatolia Region of Turkey.

MATERIAL AND METHODS

Southeastern Anatolia Region (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) entomology studies on insect species in different ecological provinces were made between the years 1948-2013 (Figure 1).

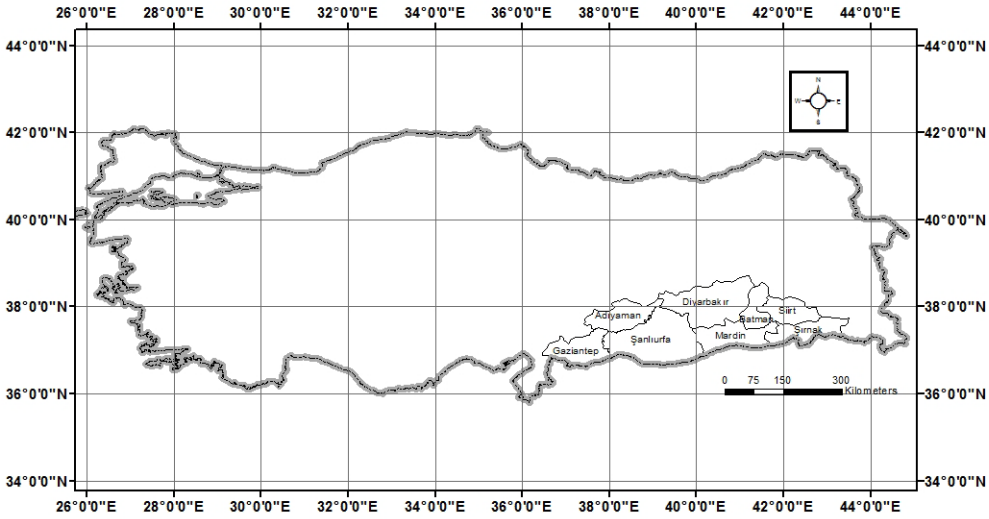

Figure 1. Sampling localities in Southeastern Anatolia Region of Turkey

In this study, we prepared for the inventory has reached the major advantage of the waterways:

-Currently in Turkey, published or unpublished entomology journals related to scanning,

-Giving more weight to faunistic studies, and in the meantime, the insect fauna of our country foreign scientific journals that publishes articles about scanning,

-Faculty of Agriculture, Faculty of Science and Regional Plant Protection Research Institute in the library of books on insect fauna and the screening of the booklet,

-The doctor and the master's thesis of entomology in the region on the scanning,

-Review of other studies on the insect fauna in the area.

In this study, we evaluated the information as described above were obtained.

RESULTS AND DISCUSSION

Surveys on insect species in various ecologies have been conducted in the provinces (adiyaman, batman, gaziantep, diyarbakir, mardin, siirt, şanliurfa, şirnak) of southeastern anatolia region between the years 1948-2013. Almost 2600 species and subspecies almost 180 families belonging to 13 insect orders are defined owing to these studies. During this study totally 268 species were found in 13 families and in 6 superfamilies of coleoptera. Those superfamilies are caraboidea, dytiscoidea, bostrichoidea, chrysomeloidea, cleroidea and cucujoidea. The number and percentages of the species of the families are given on Table 1.

Table 1. Number of species of order Coleoptera on Southeastern Anatolia Region

Superfamily	Family	Number Species	%
Caraboidea	Carabidae	18	6,71
	Cicindellidae	6	2,23
Dytiscoidea	Dytiscidae	1	0,37
Bostrichoidea	Bostrichidae	3	1,12
	Dermestidae	9	3,36
Chrysomeloidea	Cerambycidae	60	22,39
	Chrysomelidae	82	30,60
	Bruchidae	17	6,34
Cleroidea	Trogossitidae	1	0,37
Cucujoidea	Coccinellidae	66	24,63
	Nitidulidae	2	0,75
	Phalacridae	2	0,75
	Silvanidae	1	0,37
Total	13	268	100,0

The distribution of insect species in the Southeastern Anatolia Region as a result of studies carried out in different provinces ecology, information on the host status and feeding has been given below.

Order Coleoptera (beetles)
Suborder Adephaga
Superfamily Caraboidea
Family Carabidae

Amara aenea (De Geer, 1774), Distribution: Diyarbakır Host plant: Karacadağ mountain

Amara (Amara) eurynota (Panzer, 1797), Distribution: Gaziantep Host plant: unknown
Chlaenius festivus (Panzer, 1796), Distribution: Southeastern Anatolia Region Host plant: unknown
Corsyra fusula (Fischer von Waldheim, 1820), Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.
Daptus vittatus Fischer von Waldheim, 1823, Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.
Harpalus sp. nr.aeneus F., Distribution: Diyarbakır Host plant: Karacadağ mountain
Harpalus sp. nr.distinguendus, Distribution: Gaziantep Host plant: unknown
Harpalus (Harpalus) distinguendus distinguengus (Duftschmid, 1812), Distribution: Gaziantep Host plant: unknown
Harpalus (harpallophanus) haspesarmenus Schaubeger, 1926, Distribution: Adıyaman Host plant: unknown
Harpalus saxicola Dejean, 1829, Distribution: Southeastern Anatolia Region Host plant: unknown
Harpalus (s.str.) serripes (Quensel, 1806), Distribution: Adıyaman Host plant: unknown
Pseudoophonus rufipes (De Geer, 1774), Distribution: Diyarbakır Host plant: unknown
Parazuphium chevrolati (Castelnau, 1833), Distribution: Southeastern Anatolia Region Host plant: unknown
Zabrus blapoides Creutz, 1799, Distribution: Diyarbakır Host plant: unknown
Zabrus ruqulosus Kraatz, 1884, Distribution: Siirt Host plant: unknown
Zabrus sp. nr. socialis Sch., Distribution: Diyarbakır Host plant: Karacadağ mountain
Zabrus spinipes (Fabricius, 1798), Distribution: Batman, Diyarbakır, Siirt Host plant: unknown
Zabrus tenebrioides (Goeze, 1777), Distribution: Adıyaman, Diyarbakır Host plant: unknown

Family Cicindellidae

Cicindella campestris Linnaeus, 1758, Distribution: Diyarbakır Host plant: unknown
Cicindela herbacea Klug, 1832, Distribution: Adıyaman, Gaziantep Host plant: unknown
Calomera fischeri fischeri (Adams, 1817), Distribution: Adıyaman, Gaziantep, Siirt, Şanlıurfa Host plant: unknown
Homodela ismenia kilikiensis (Mandl, 1961), Distribution: Adıyaman, Gaziantep Host plant: unknown
Lophyridia fischeri (Adams, 1817), Distribution: Adıyaman, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: unknown

Lophyridia littoralis (Mandl, 1967), Distribution: Mardin, Siirt, Şanlıurfa Host plant: unknown

Superfamily Dytiscoidea
Family Dytiscidae

Deronectes evelynae Fery and Hosseinie, 1998, Distribution: Adıyaman, Gaziantep Host plant: unknown

Suborder Polyphaga
Superfamily Bostrichoidea
Family Bostrichidae

Rhyzopertha dominica (Fabricius, 1792), Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Triticum* spp., Flour, Storage pest
Sinoxylon sexdentatum (Olivier, 1790), Distribution: Adıyaman, Batman, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L.
Stenomera assyrica Lesne, 1895, Distribution: Adıyaman, Batman, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L.

Family Dermestidae

Attagenus (s.str.) orientalis Reitter in Schneider et Leder, 1878, Distribution: Mardin, Siirt, Şanlıurfa Host plant: Fruit, *Pistacia vera* L.
Attagenus (s.str.) quadrimaculatus Kraatz, 1858, Distribution: Gaziantep Host plant: unknown
Anthrenus (s.str.) delicatus Kiesenwetter, 1851, Distribution: Gaziantep Host plant: unknown
Anthrenus (s.str.) rotundulus rotundulus Reitter, 1889, Distribution: Gaziantep Host plant: unknown
Anthrenus (s.str.) simonis Reitter, 1881, Distribution: Diyarbakır Host plant: Light trap
Anthrenus (Nathrenus) pubifer Reitter, 1899, Distribution: Diyarbakır, Siirt Host plant: unknown
Anthrenus verbasci (Linnaeus, 1767), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Cucurbita* spp.
Trogoderma granarium Everts, 1898, Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., Storage pests, *Cucurbita* spp.
Trogoderma versicolor (Creutzer, 1799), Distribution: Diyarbakır Host plant: *Zea mays* L.

Superfamily Chrysomeloidea
Family Cerambycidae

Agapanthia dahli (Richter, 1821), Distribution: Gaziantep, Siirt Host plant: unknown

Agapanthia cardui (Linnaeus, 1767), Distribution: Diyarbakır, Siirt Host plant: unknown
Agapanthia (s.str) lais Reiche & Saulcy, 1858, Distribution: Mardin Host plant: unknown
Agapanthia kirbyi (Gyllenhal, 1817), Distribution: Siirt Host plant: unknown
Agapanthia walteri Reitter, 1898, Distribution: Mardin Host plant: unknown
Arhopalus syriacus (Reitter, 1895), Distribution: Gaziantep, Şanlıurfa Host plant: unknown
Aromia moschata (Linnaeus, 1758), Distribution: Adıyaman Host plant: unknown
Blepisanis vittipennis (Reiche, 1877), Distribution: Adıyaman Host plant: unknown
Calamobius filum (Rossi, 1790), Distribution: Gaziantep Host plant: unknown
Calchaenesthes oblongomaculata (Guerin 1844), Distribution: Adıyaman, Gaziantep Host plant: unknown
Callimellum angulatum (Schrank, 1789), Distribution: Adıyaman Host plant: unknown
Cardoria scutellata (Fabricius, 1792), Distribution: Adıyaman Host plant: unknown
Cerambyx dux (Faldermann, 1837), Distribution: Adıyaman, Gaziantep Host plant: *Amygdalus communis* L.
Cerambyx cerdo Linnaeus, 1758, Distribution: Adıyaman, Şırnak Host plant: unknown
Cerambyx nodulosus Germar, 1817, Distribution: Adıyaman, Mardin Host plant: unknown
Certallum ebulinum (Linnaeus, 1767), Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Şanlıurfa Host plant: unknown
Chlorophorus hungaricus Seidlitz, 1891, Distribution: Gaziantep Host plant: unknown
Chlorophorus sartor (Müller, 1766), Distribution: Gaziantep Host plant: unknown
Chlorophorus varius (Müller, 1766), Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Fruit, *Vitis* spp.
Chlorophorus varius damascenus (Chevrolat, 1854), Distribution: Diyarbakır Host plant: *Prunus persica* (L.)
Clytus rhamni Germar, 1817 Distribution: Adıyaman, Gaziantep Host plant: *Amygdalus communis* L.
Coptosia bithyniensis (Ganglbauer, 1884), Distribution: Adıyaman, Diyarbakır Host plant: unknown
Coptosia compacta (Ménétries, 1832), Distribution: Adıyaman Host plant: unknown
Cortodera colchica Reitter, 1890, Distribution: Adıyaman Host plant: unknown
Dorcadion cinerarium (Fabricius, 1787), Distribution: Gaziantep, Şanlıurfa Host plant: unknown

Dorcadion formosum Kraatz, 1870, Distribution: Gaziantep Host plant: unknown
Dorcadion lameeri Théry, 1896, Distribution: Gaziantep Host plant: unknown
Dorcadion septemlineatum Waltl, 1838, Distribution: Gaziantep Host plant: unknown
Dorcadion infernale Mulsant & Rey 1863, Distribution: Diyarbakır Host plant: unknown
Helladia armeniaca Frivaldsky, 1878, Distribution: Adıyaman, Diyarbakır Host plant: unknown
Helladia humeralis (Waltl, 1838), Distribution: Adıyaman, Diyarbakır Host plant: unknown
Helladia millefolii (Adams, 1817), Distribution: Adıyaman Host plant: unknown
Lampropterus femoratus (Germar, 1824), Distribution: Adıyaman, Diyarbakır, Gaziantep Host plant: unknown
Mallosia herminae Reitter, 1890, Distribution: Şırnak Host plant: unknown
Musaria puncticollis (Faldermann, 1837), Distribution: Diyarbakır Host plant: unknown
Neomusaria balcanica (Frivaldsky, 1835), Distribution: Mardin Host plant: unknown
Neoplagionotus bobelayei (Brullé, 1832), Distribution: Adıyaman, Diyarbakır Host plant: unknown
Oberea oculata (Linnaeus, 1758), Distribution: Adıyaman, Şanlıurfa Host plant: unknown
Opsilia coerulescens (Scopoli, 1763), Distribution: Adıyaman, Diyarbakır Host plant: unknown
Osphranteria coerulescens Redtenbacher, 1850, Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Malus domestica* Borkh., *Prunus armeniaca* L., *Prunus avium* L., *Vicia sativa* L., *Prunus persica* (L.), *Pistacia vera* L., *Prunus domestica* L.,
Oxylia argentata (Ménétriés, 1832), Distribution: Adıyaman, Diyarbakır, Siirt Host plant: unknown
Oxylia languida Mulsant, 1862, Distribution: Siirt Host plant: unknown
Pachytodes erraticus (Dalman, 1817), Distribution: Gaziantep Host plant: unknown
Paraplagionotus floralis (Pallas, 1773), Distribution: Adıyaman Host plant: unknown
Phymatodes testaceus (Linnaeus, 1758), Distribution: Adıyaman Host plant: unknown
Phytoecia geniculata Mulsant, 1862, Distribution: Gaziantep Host plant: unknown
Phytoecia puncticollis Faldermann, 1837, Distribution: Diyarbakır Host plant: unknown
Phytoecia virgula (Charpentier, 1825), Distribution: Adıyaman Host plant: unknown
Pilemia annulata (Hampe, 1852), Distribution: Adıyaman Host plant: unknown

Pilemia hirsutula (Frölich, 1793), Distribution: Adıyaman Host plant: unknown
Plagionotus arcuatus (Linnaeus, 1758), Distribution: Diyarbakır Host plant: unknown
Plagionotus floralis (Pallas, 1773), Distribution: Adıyaman Host plant: Trifolium
Pseudovadonia livida (Fabricius, 1776), Distribution: Adıyaman, Gaziantep Host plant: unknown
Purpuricenus budensis (Götz, 1783), Distribution: Adıyaman, Gaziantep, Siirt Host plant: *Amygdalus communis* L.
Ropalopus clavipes (Fabricius, 1775), Distribution: Adıyaman Host plant: unknown
Stenopterus kraatzi (Pic, 1892), Distribution: Adıyaman Host plant: unknown
Stenopterus rufus (Linnaeus, 1767), Distribution: Adıyaman, Gaziantep Host plant: unknown
Stenurella bifasciata (O.F. Müller, 1776), Distribution: Gaziantep Host plant: unknown
Stictoleptura cordigera (Füssli, 1775), Distribution: Adıyaman, Gaziantep Host plant: unknown
Stictoleptura tripartita (Heyden, 1889), Distribution: Adıyaman Host plant: unknown
Stromatium unicolor (Olivier, 1795), Distribution: Gaziantep Host plant: unknown

Family Chrysomelidae

Altica deserticola (Weise, 1889), Distribution: Batman Host plant: unknown
Antipus macropus (Illiger, 1800), Distribution: Diyarbakır, Siirt Host plant: *Pistacia vera* L.
Aphthona atrocaerulea (Stephens, 1831), Distribution: Mardin, Siirt Host plant: unknown
Aphthona franzi Heikertinger, 1944, Distribution: Diyarbakır Host plant: unknown
Aphthona flaviceps Allard, 1859, Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Aphthona gracilis Fadermann, 1837, Distribution: Diyarbakır Host plant: unknown
Aphthona maculata Allard, 1876, Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Aphthona semicyanea melanopeza Jacob, 1900-1901, Distribution: Diyarbakır Host plant: unknown
Cassida sp., Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Cassida brevis Weise, 1884, Distribution: Siirt Host plant: unknown
Cassida linnavuorii Borowiec, 1986, Distribution: Mardin Host plant: unknown
Cassida margaritacea Schaller, 1783, Distribution: Diyarbakır Host plant: unknown
Cassida nobilis Linnaeus, 1758, Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Cassida palaestina Reiche, 1858, Distribution: Diyarbakır, Gaziantep Host plant: unknown
Cassida pannonica Suffrian, 1844, Distribution: Diyarbakır Host plant: unknown
Cassida prasina Illiger, 1798, Distribution: Gaziantep Host plant: unknown
Cassida saucia Weise, 1889, Distribution: Batman, Gaziantep Host plant: unknown
Cassida subferruginea Schrank, 1776, Distribution: Siirt, Şanlıurfa Host plant: unknown
Cassida viridis Linnaeus, 1758, Distribution: Diyarbakır, Şanlıurfa Host plant: unknown
Chaetocnema montenegrina Heikertinger, 1912, Distribution: Diyarbakır Host plant: unknown
Cheilotoma musciformis (Goeze, 1777), Distribution: Gaziantep Host plant: unknown
Chrysolina polita (Linnaeus, 1758), Distribution: Mardin Host plant: unknown
Chrysolina chalcites (Germar, 1824), Distribution: Diyarbakır Host plant: unknown
Chrysolina sahlbergi (Ménétriés, 1832), Distribution: Diyarbakır Host plant: unknown
Chrysomelina chalcites (Germar, 1824), Distribution: Diyarbakır Host plant: unknown
Cryptocephalus duplicatus Suffrian, 1847, Distribution: Siirt Host plant: unknown
Cryptocephalus moraei (Linnaeus, 1758), Distribution: Diyarbakır Host plant: unknown
Cryptocephalus octacosmus Bedel, 1891, Distribution: Diyarbakır, Mardin Host plant: unknown
Clytra cingulata Weise, 1898, Distribution: Diyarbakır, Gaziantep Host plant: unknown
Clytra bodemeyeri Weise, 1900, Distribution: Gaziantep, Mardin, Siirt Host plant: unknown
Clytra novempunctata Olivier, 1808, Distribution: Gaziantep, Mardin, Siirt Host plant: unknown
Colaphellus sophiae (Schaller, 1783), Distribution: Diyarbakır Host plant: unknown
Coptocephala gebleri Gebler, 1841, Distribution: Diyarbakır Host plant: unknown
Cyaniris limbata Steven, 1806, Distribution: Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: unknown
Cyaniris viridana Lacordaire, 1848, Distribution: Southeastern Anatolia Region Host plant: unknown
Entomoscelis adonidis (Pallas, 1771), Distribution: Diyarbakır, Gaziantep Host plant: unknown

Epitrix hirtipennis (Melsheimer, 1847), Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Exosome thoracica Rdt., Distribution: Şanlıurfa Host plant: unknown
Galeruca interrupta Illiger, 1802, Distribution: Mardin Host plant: *Prunus avium* L.
Gastrophysa polygoni (Linnaeus, 1758), Distribution: Diyarbakır Host plant: unknown
Gynandrophtalma limbata Steven, 1806, Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L.
Gynandrophtalma viridana Lacordaire, 1848, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Vitis* spp.
Gonioctena fornicata (Brüggemann, 1873), Distribution: Diyarbakır, Mardin Host plant: unknown
Hermaeophaga ruficollis (Lucas, 1849), Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Hispa atra Linnaeus, 1767, Distribution: Batman, Diyarbakır, Gaziantep, Siirt Host plant: unknown
Labidostomis asiatica Faldermann, 1837, Distribution: Diyarbakır, Gaziantep Host plant: unknown
Labidostomis brevipennis Faldermann, 1837, Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.
Labidostomis cyanicornis Germar, 1822, Distribution: Siirt Host plant: unknown
Labidostomis decipiens Faldermann, 1837, Distribution: Şanlıurfa Host plant: unknown
Labidostomis longimana (Linnaeus, 1761), Distribution: Siirt Host plant: *Pistacia vera* L.
Labidostomis mesopotamica Heyden, 1886, Distribution: Mardin Host plant: unknown
Labidostomis oertzeni Weise, 1889, Distribution: Diyarbakır, Mardin, Siirt Host plant: unknown
Labidostomis propinqua Faldermann, 1837, Distribution: Diyarbakır Host plant: unknown
Lema melanopa Linnaeus, 1758, Distribution: Diyarbakır, Siirt Host plant: unknown
Longitarsus sp., Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Longitarsus albineus (Foudras, 1860), Distribution: Diyarbakır Host plant: unknown
Longitarsus alfierii Pic 1923, Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Longitarsus anchusae (Paykull, 1799), Distribution: Diyarbakır Host plant: unknown
Longitarsus dichrous Iablokoff-Khnzorian 1962, Distribution: Diyarbakır Host plant: unknown
Longitarsus fallax Weise, 1888, Distribution: Diyarbakır Host plant: unknown

Longitarsus ganglbaueri Heikertinger, 1912, Distribution: Diyarbakır Host plant: unknown
Longitarsus lycopi (Foudras, 1860), Distribution: Diyarbakır Host plant: unknown
Longitarsus parvulus (Paykull, 1799), Distribution: Diyarbakır Host plant: unknown
Longitarsus salviae Gruev, 1975, Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Longitarsus suturellus (Duftschmid, 1825), Distribution: Diyarbakır, Şanlıurfa Host plant: unknown
Longitarsus ochroleucus (Marsham, 1802), Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Oulema melanopa (Linnaeus, 1758), Distribution: Mardin Host plant: *Prunus avium* L.
Pachybrachis fimbriolatus (Suffrian, 1848), Distribution: Diyarbakır Host plant: unknown
Phaedon cochleariae (Fabricius, 1792), Distribution: Diyarbakır, Gaziantep Host plant: unknown
Phratora vitellinae (Linnaeus, 1758), Distribution: Diyarbakır, Mardin Host plant: unknown
Phyllotreta corrugata Reiche, 1858, Distribution: Diyarbakır, Gaziantep Host plant: unknown
Phytodecta fornicatus Brüggem, 1873, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.
Plagiodera versicolora (Laicharting, 1781), Distribution: Diyarbakır, Mardin Host plant: unknown
Prasocuris junci (Brahm, 1790), Distribution: Diyarbakır Host plant: unknown
Psylliodes anatolica Gök & Çilbiroğlu, 2004, Distribution: Diyarbakır Host plant: *Pistacia vera* L.
Psylliodes hyoscyami (Linnaeus, 1758), Distribution: Diyarbakır Host plant: unknown
Psylliodes sophiae Heikertinger, 1914, Distribution: Diyarbakır Host plant: unknown
Rhaphidopalpa foveicollis Lucas, 1849, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.
Smaragdina limbata (Steven, 1806), Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Prunus avium* L.
Smaragdina scutellaris (Lefevre, 1872), Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.
Smaragdina unipunctata (Olivier, 1808), Distribution: Diyarbakır, Gaziantep, Mardin, Şanlıurfa Host plant: *Prunus avium* L.

Smaragdina viridana (Lacordaire, 1848), Distribution: Mardin Host plant: *Prunus avium* L.

Family Bruchidae

Acanthoscelides obtectus (Say, 1831), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchus ervi Frölich, 1799, Distribution: Diyarbakır, Gaziantep, Mardin, Şanlıurfa Host plant: *Lens culinaris* Medik.

Bruchus dentipes (Baudi, 1886), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchus lentis Frölich, 1799, Distribution: Gaziantep Host plant: *Lens culinaris* Medik.

Bruchus nubilus (Boheman, 1833), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Legumes and forage crops

Bruchus tetragonus (Baudi, 1886), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchus tristis Bohemann, 1833, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Legumes and forage crops

Bruchus ulicis Mulsant & Rey, 1858, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Legumes and forage crops

Bruchidius albopictus (Allard, 1883), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchidius anobioides (Baudi, 1886), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchidius bimaculatus (Olivier, 1795), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Legumes and forage crops

Bruchidius fulvus (Allard, 1883), Distribution: Diyarbakır Host plant: Licorice

Bruchidius foveolatus (Gyllenhal, 1833), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchidius trifolii (Motschulsky, 1873), Distribution: Southeastern Anatolia Region Host plant: unknown

Bruchidius quinqueguttatus (Olivier, 1795), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Legumes and forage crops

Caryedon palestinicus Southgate, 1979, Distribution: Şanlıurfa Host plant: Light trap

Callosobruchus maculatus (Fabricius, 1775), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Legumes and forage crops.

Superfamily Cleroidea
Family Trogossitidae

Tenebroides mauritanicus Linnaeus, 1758, Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., the pests of flour, *Cucurbita* spp.

Superfamily Cucujoidea
Family Coccinellidae

Adalia bipunctata (Linnaeus, 1758), Distribution: Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Gossypium hirsitum* L., *Zea mays* L. Host insect: Aphididae, Coccoidea

Adalia decempunctata (Linnaeus, 1758), Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L. Host insect: Aphididae, Coccoidea

Adalia fasciatopunctata revelierei (Mulsant, 1866), Distribution: Diyarbakır, Mardin Host plant: Unknown Host insect: Aphididae, Coccoidea

Adonia variegata (Goeze, 1777), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Zea mays* L., *Gossypium hirsitum* L., *Olea europaea* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous, Aphididae, Coccoidea

Chilocorus bipustulatus (Linnaeus, 1758), Distribution: Diyarbakır, Gaziantep, Mardin, Siirt Host plant: *Amygdalus communis* L., *Prunus avium* L., *Pistacia vera* L. Host insect: Coccoidea

Clitostethus arcuatus (Rossi, 1794), Distribution: Diyarbakır Host plant: unknown

Coccinella conglobata Linnaeus, 1758, Distribution: Gaziantep Host plant: *Pistacia vera* L.

Coccinella septempunctata Linnaeus, 1758, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: Trifolium, *Amygdalus communis* L., *Prunus avium* L., *Zea mays* L., *Gossypium hirsitum* L., *Olea europaea* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous

Coccinella undecimpunctata Linnaeus, 1758, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Trifolium, *Amygdalus communis* L., *Zea mays* L., *Gossypium hirsitum* L., *Olea europaea* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous

Coccinula sinuatomarginata (Faldermann, 1837), Distribution: Southeastern Anatolia Region Host plant: unknown

Coccinella quatuordecimpustulata Linnaeus, 1758, Distribution: Southeastern Anatolia Region Host plant: unknown

Exochomus flavipes (Thunberg, 1781), Distribution: Southeastern Anatolia Region Host plant: unknown

Exochomus nigromaculatus (Goeze, 1777), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: Trifolium, *Amygdalus communis* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous

Exochomus pubescens Küster, 1848, Distribution: Şanlıurfa Host plant: Trifolium,
Adalia fasciatopunctata revelierei Mulsant, 1866, Distribution: Diyarbakır, Mardin Host plant: *Pistacia vera* L. Host insect: Aphididae, Coccoidea
Exochomus quadripustulatus (Linnaeus, 1758), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous
Harmonia quadripunctata (Pontoppidan, 1763), Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L. Host insect: Polyphagous
Henosepilachna argus (Geoffroy, 1762), Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Henosepilachna elaterii Rossi, 1794, Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Hippodamia variegata (Goeze, 1777), Distribution: Diyarbakır, Mardin, Şanlıurfa Host plant: *Amygdalus communis* L., *Pistacia vera* L., Host insect: Polyphagous
Hyperaspis femorata (Motschulsky, 1837), Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Hypraspis quadrimaculata Redtenbacher, 1843, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Cicer arietinum* L., *Zea mays* L., *Gossypium hirsitum* L., *Lens culinaris*, *Pistacia vera* L., *Cucumis melo* L., *Citrillus lanatus* L., Host insect: Polyphagous
Hyperaspis repensis (Herbst, 1783), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Pistacia vera* L. Host insect: Polyphagous
Hyperaspis polita Weise, 1885, Distribution: Diyarbakır, Şanlıurfa Host plant: Vegetables Host insect: Polyphagous
Myrrha octodecimquttata (Linnaeus, 1758), Distribution: Diyarbakır, Mardin Host plant: Unknown Host insect: Polyphagous
Myzia oblongoquttata (Linnaeus, 1758), Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Nephus (Sidis) caneparii Fürsch &Uygun, 1980, Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L. Host insect: Polyphagous
Nephus (Sidis) hiekei (Fursch, 1965), Distribution: Adıyaman, Gaziantep, Mardin, Şanlıurfa Host plant: *Olea europaea* L. Host insect: Polyphagous
Nephus includens Kirsch, 1870, Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Nephus ludyi Weise, 1879, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L. Host insect: Polyphagous
Nephus nigricans Weise, 1879, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Cicer arietinum* L., *Zea mays* L., *Gossypium hirsitum* L., *Lens culinaris* L., *Cucumis*

melo L., *Olea europaea* L., *Pistacia vera* L., Trifolium, Vegetables, *Vitis* spp., *Citrillus lanatus* L. Host insect: Polyphagous

Nephus quadrimaculatus (Herbst, 1783), Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous

Oenopia conglobata (Linnaeus, 1758), Distribution: Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Zea mays* L., *Pistacia vera* L., *Prunus avium* L., Alfalfa Host insect: Polyphagous

Oenopia oncina (Olivier, 1808) Distribution: Diyarbakır, Mardin, Siirt Host plant: *Amygdalus communis* L., *Pistacia vera* L. Host insect: Polyphagous

*Pharoscymnus numidicus (*Pic, 1900), Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Pistacia vera* L. Host insect: Polyphagous

Pharoscymnus pharoides (Marseul, 1868), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Pistacia vera* L. Host insect: Polyphagous

Platynaspis luteorubra (Goeze, 1777), Distribution: Diyarbakır, Mardin, Şanlıurfa Host plant: *Amygdalus communis* L., Vegetables Host insect: Polyphagous

Propylea quatuordecimpunctata (Linnaeus, 1758), Distribution: Diyarbakır, Şanlıurfa Host plant: *Punica granatum* L., Vegetables, Host insect: Polyphagous

Psyllobora bisoctonotata (Mulsant, 1850), Distribution: Mardin Host plant: *Prunus avium* L. Host insect: Polyphagous

Psyllobora vigintiduopunctata (Linnaeus, 1758), Distribution: Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., Alfalfa Host insect: Polyphagous

Rodolia cardinalis (Mulsant, 1850), Distribution: Southeastern Anatolia Region Host plant: unknown Host insect: unknown

Scymnus apetzi Mulsant, 1846, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Gossypium hirsitum* L., *Olea europaea* L., *Pistacia vera* L. Host insect: Polyphagous

Scymnus (Pullus) araraticus Iablokoff-Khnzorian, 1969, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Zea mays* L., *Olea europaea* L., *Punica granatum* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous

Scymnus (Pullus) auritus Thunberg, 1795, Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L. Host insect: Polyphagous

Scymnus bivulnerus Capra & Fürsch, 1967, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Cucumis melo* L., *Avena sativa* L., *Pistacia vera* L., Vegetables, *Citrillus lanatus* L. Host insect: Polyphagous

Scymnus flagellisiphonatus (Fürsch, 1970), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Pistacia vera* L., Vegetables Host insect: Polyphagous

Scymnus flavicollis Redtenbacher, 1843, Distribution: Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Zea mays* L., *Gossypium hirsitum* L., Vegetables Host insect: Polyphagous

Scymnus frontalis Fabricius, 1787, Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous

Scymnus inderihensis Mulsant, 1850, Distribution: Diyarbakır, Mardin, Şanlıurfa Host plant: *Avena sativa* L. Host insect: Polyphagous

Scymnus interruptus (Goeze, 1777), Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Gossypium hirsitum* L., Vegetables Host insect: Polyphagous

Scymnus levaillantii Mulsant, 1850, Distribution: Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Zea mays* L., *Gossypium hirsitum* L., *Avena sativa* L., *Punica granatum* L. Host insect: Polyphagous

Scymnus marginalis (Rossi, 1794), Distribution: Diyarbakır, Şanlıurfa Host plant: Vegetables, Host insect: Polyphagous

Scymnus mediterraneus Iablokoff-Khnzorian, 1972, Distribution: Diyarbakır, Mardin Host plant: Unknown Host insect: unknown

Scymnus nigrinus Kugelann, 1794, Distribution: Diyarbakır Host plant: *Cucumis melo* L., *Citrillus lanatus* L. Host insect: Polyphagous

Scymnus pallipediformis Günther, 1958, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Zea mays* L., *Cucumis melo* L., *Pistacia vera* L., *Punica granatum* L., Alfalfa, Vegetables, *Vitis* spp., *Citrillus lanatus* L. Host insect: Polyphagous

Scymnus rubromaculatus (Goeze, 1777), Distribution: Adıyaman, Batman, Diyarbakır, Mardin, Şanlıurfa Host plant: *Zea mays* L., *Cucumis melo* L., Alfalfa, *Punica granatum* L., Vegetables, *Vitis* spp., *Citrillus lanatus* L. Host insect: Polyphagous

Scymnus suturalis Thunberg, 1795, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L., Host insect: Polyphagous

Scymnus subvillosus (Goeze, 1777), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Zea mays* L., *Pistacia vera* L., Alfalfa, Vegetables, *Vitis* spp., *Prunus avium* L. Host insect: Polyphagous

Scymnus syriacus (Marseul, 1868), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L., *Prunus avium* L. Host insect: Polyphagous

Scymnus quadriguttatus Fursch & Kreissl 1971, Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Prunus avium* L., *Cucumis melo* L., *Pistacia vera* L., *Punica granatum* L., Vegetables, *Vitis* spp., *Citrillus lanatus* L. Host insect: Polyphagous

Scymnus quadrimaculatus (Herbst, 1783) Distribution: Adıyaman, Gaziantep, Mardin, Şanlıurfa Host plant: *Olea europaea* L. Host insect: Polyphagous

Coccinella undecimpunctata Linnaeus, 1758 Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Stethorus gilvifrons (Mulsant, 1850), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Zea mays* L., *Gossypium hirsitum* L., *Olea europaea* L., *Pistacia vera* L., Alfalfa, Vegetables, *Vitis* spp., *Citrillus lanatus* L. Host insect: Polyphagous
Stethorus punctillum (Weise, 1891), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Gossypium hirsitum* L., *Pistacia vera* L., Host insect: Polyphagous
Synharmonia conglobata (Linnaeus, 1758), Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Olea europaea* L., *Pistacia vera* L., *Punica granatum* L., Alfalfa, Vegetables, Host insect: Polyphagous
Tea vigintiduopunctata (Linnaeus, 1758), Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous
Vibidia duodecimguttata (Poda, 1761), Distribution: Southeastern Anatolia Region Host plant: Unknown Host insect: Polyphagous.

Family Nitidulidae

Cybocephalus fodori minor (Endrödy-Younga, 1965), Distribution: Mardin, Siirt Host plant: *Vitis* spp.
Cybocaphalus sp., Distribution: Diyarbakır Host plant: *Pistacia vera* L.

Family Phalacridae

Olibrus flaviZea mays L.is (Sturm, 1807), Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.
Stilbus apicalis (Melsheimer, 1844), Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.

Family Silvanidae

Oryzaephilus surinamensis (Linnaeus, 1758), Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Host plant: *Amygdalus communis* L., Storage pests, the pests of flour, *Cucurbita* spp.

CONLUSIONS

The aim of this study is to make contribution to some faunistical records of Coleoptera of the Southeastern Anatolia Region of Turkey. This region has different climatic conditions and contains different habitats. Coleoptera contains both important phytophagous and also zoophagous insects.

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DOI: 10.17707/AgricultForest.62.4.17

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HABITAT CONDITIONS FOR WILDLIFE MANAGEMENT IN THE DINARIC REGIONS OF CROATIA AND MONTENEGRO

SUMMARY

Hunting is an important socio-economic activity, particularly in rural areas. Characteristics and quality of habitat are important in the sustainable management of game animals and wildlife conservation. Three hunting grounds were analysed and their habitat composition was compared with regard to abundance of main game animal species. Hunting ground VIII/2 "Bjelolasica" is located in Gorski kotar region; XVII/6 "Maglaj-Cista" in the inland of middle Dalmatia; and hunting ground "Dragišnica" is located in northern part of Montenegro.

Keywords: habitat, hunting ground, Dinarides, Croatia, Montenegro

INTRODUCTION

Habitat represents an area in which individual organism or population of plants and animals lives in interaction with their environment. The protection of habitats makes an integral part of the nature protection in the European Union, based on the Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora (The Habitats Directive).

The Dinarides mountain chain is extending in NW-SE direction over 650 km in length and over 150 km wide (Mihevc et al., 2010). It consists of more than 200 mountains shared among seven countries: Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Kosovo and Albania. The Dinarides mountain system is divided into three morphostructural zones: Inner, Central and Outer Dinarides (Bognar, 2008). The highest elevations occur in the Central zone: Snežnik (1,796 m), Risnjak (1,528 m), Velika and Mala Kapela, Plješivica (1,657 m), Velebit (1,758 m), Dinara (1,913 m), Vitorog (1,907 m), Vran, Čvrsnica (2,228 m), Prenj (2,155 m), Bjelašnica (2,067 m), Jahorina, Lelija, Maglić, Durmitor (2,522 m), Tara, Njegoš (1,721 m), Orjen (1,895 m), and Lovćen

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Paper presented at the International Conference "Sustainable development of mountain areas - Experiences, challenges and perspectives" 14-16 September 2016, Žabljak Montenegro.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

(1,749 m). The main part of the Dinarides is formed of Mesozoic and Tertiary rocks, mostly limestone and dolomite. Due to dissolution of these rocks, a typical karst landscape and relief, with numerous surface and underground features appear.

Characteristics and quality of habitat are important in the sustainable and cost effective management of wildlife, including those wild species classified as game animals. Three hunting grounds, situated in the Dinaric regions of Croatia and Montenegro, were selected for analyses and comparison of habitat structure and suitability for the management of main game species. From Croatia: VIII/2 "Bjelolasica", located in Gorski kotar region and XVII/6 "Maglaj-Cista" in the inland, continental part of middle Dalmatia; and hunting ground "Dragišnica" from Montenegro, located in northern part of the country, bordering to the National Park "Durmitor".

MATERIALS AND METHODS

Study area

Hunting ground VIII/2 "Bjelolasica" is located in the western Croatia, in the southern part of Gorski kotar region; XVII/6 "Maglaj-Cista" in the middle Dalmatia; and hunting ground "Dragišnica" from Montenegro is located in northern part of the country (Figure 1).

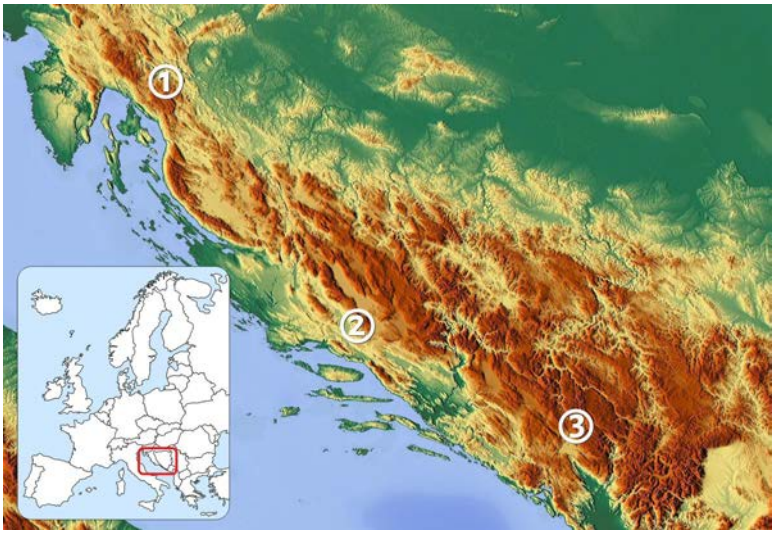

Figure 1. Map of the Dinarides mountain system with position of the surveyed hunting grounds in Croatia (1="Bjelolasica"; 2="Maglaj-Cista") and Montenegro (3="Dragišnica")

Hunting ground "Bjelolasica" (total surface 30,458 ha) is a typical Dinaric hunting ground. Altitudinal range is 350 m-1,534 m (Mt. Bjelolasica summit),

and mean altitude is 942 m. Mean air temperature is 7.2 ^{o}C; annual amount of precipitation is 1,999 mm and mean number of days with snow cover is 82 days.

Gorski kotar region with 60% forest cover is the most wooded part of Croatia. Some parts remained as primeval due to their inaccessibility and are protected at national level, such as Risnjak National Park (Ozimec et al., 2010).

Hunting ground "Maglaj-Cista" (total surface of 9,558 ha) is situated at a typical rugged karst plateau, with shaped flat karst fields, and surrounded by hills high ca. 800 m. Altitudinal range is 397 m-928 m (Mt. Maglaj summit), and mean altitude is 663 m. Mean air temperature is 13.2 ^{o}C; annual amount of precipitation is 1,300 mm and mean number of days with snow cover is 8 days.

Hunting ground "Dragišnica" (total surface of 9,795 ha) belongs into Northern hunting sector of Montenegro, comprising NW part of the Municipality of Šavnik. Altitudinal range is 651 m-1,998 m (summit Ogorjeli krš), and mean altitude is 1,890 m. Mean air temperature is 4.6 ^{o}C, annual amount of precipitation is 1,456 mm and mean number of days with snow cover is 58 days.

Data collection and analyses

Main sources of data were Hunting Management Plans of the surveyed hunting grounds. This is a document which contains data on following: land surface structure; description of vegetation and habitats; assessment of habitat quality for game animals; population structure and guidelines for management with game animals.

RESULTS AND DISCUSSION

Main game species

An inventory list of main game animals present in the surveyed hunting grounds is shown in Table 1.

Table 1. List of main game animals and their population size in the hunting grounds

Game species	Hunting ground		
	Croatia		Montenegro
	Bjelolasica	Maglaj -Cista	Dragišnica
Red deer (*Cervus elaphus*)	130		
Roe deer (*Capreolus capreolus*)	160	32	46
Wild boar (*Sus scrofa*)	110	24	25
Brown bear (*Ursus arctos*)	100		6
Grey wolf (*Canis lupus*)			15
Chamois (*Rupicapra rupicapra*)			38
European hare (*Lepus europaeus*)	70	180	180
European badger (*Meles meles*)	90	12	15
Beech marten (*Martes foina*)	60	10	60
Red fox (*Vulpes vulpes*)	30	10	50
Eurasian jay (*Garulus glandarius*)	150	20	
Western capercaillie (*Tetrao urogallus*)			12
Rock partridge (*Alectoris graeca*)		84	

Habitat composition and distribution

In the "Bjelolasica" hunting ground, forest habitats cover 24,279 ha, and dominate with 81% of total surface. Dominating is mixed Dinaric beech and fir forest (*Omphalodo-Fagetum*) which is distributed over 150,000 ha in the Croatian Dinarides area (Vukelić, 2012). It thrives at altitudes 600-1,300 m. European beech (*Fagus sylvatica*) is an important food source to large and small mammals. Beechnuts have about the same protein content as corn but five times more the fat content. Diverse shrubs and deciduous trees provides valuable food source to red deer and roe deer. Sub-alpine beech forest with platain leafed buttercup (*Ranunculo platanifolii-Fagetum*) thrives at altitude 900-1,400 m. Tree layer is completely dominated by the beech, which provides sufficient food source to wild animals. Acidophilic fir forest with hard fern (*Blechno-Abietetum*) grows on silicate rocks and podzolic soils at altitudes 600-900 m. The dense young-growth of conifers makes a good shelter for all big game animals. Calciphilic Dinaric fir forest on limestone blocks (*Calamagrostio-Abietetum*) growth at altitude above 1,100 m, on sunny and warm slopes consisting of large limestone blocks. Due to configuration and inaccessibility this is a place where brown bear (*Ursus arcto*s) usually makes his dense. Stands of dwarf mountain pine (*Pinus mugo*) are making the upper boundary of forest vegetation in Croatian Dinarides. It thrives at altitude above 1,400 m, where climate conditions are very harsh with high and long-lasting snow cover. This association *Hyperico grisebachii-Pinetum mugi* is characteristic for the Northern Dinarides in Slovenia and Croatia (Čarni and Mucina, 2015).

Grassland in the hunting ground "Bjelolasica" comprises meadows and pastures on surface of 4,724 ha which makes 16% of total surface. Mountain meadows of upright brome on calcareous substrate from the vegetation alliance *Bromion erecti* have high fodder value in diet of wild animals, mainly herbivores. Today, surfaces with natural or semi-natural meadows are largely reduced, and progression towards forest growth is present. Subalpine calcareous grassland from the vegetation class *Seslerietalia juncifoliae* is present on the highest peaks of the Dinarides mountains, above the vegetation belt of dwarf mountain pine.

In the hunting ground "Maglaj-Cista" forest habitats covers 8,057 ha and dominates with 78% of total surface. Thermophilous, deciduous forest and scrubs of pubescent oak (*Quercus pubescens*) and oriental hornbeam (*Carpinus orientalis*), classified as association *Querco-Carpinetum orientalis*, are specific for the lower submediterranean vegetation zone (Vukelić et al., 2008). This forest is rarely developed in shape of complete forest but mostly as degradation stage of tall or high scrubs. In the higher, Mediterranean-montane belt, the climate is even colder and relatively more humid. Mixed forest and scrub of pubescent oak and hop hornbeam (*Ostrya carpinifolia*), classified as association *Ostryo-Quercetum pubsecentis*, are present mostly as degradation stage of tall scrubs.

Thermophilous and xerophilous scrubs of eastern submediterranean region (*Rhamno-Paliuretum*), consists mainly of thorny and spinous plants, such as: the Jerusalem thorn (*Paliurus spina-christi*) and *Rhamnus intermedia*.

Grassland in the hunting ground "Maglaj-Cista" comprises meadows and pastures on surface of 614 ha which makes 7% of total surface. Dry rocky grasslands, classified into associations: *Stipo-Salvietum officinalis* and *Festuco-Koelerietum splendentis*, are among the most frequent grassland communities in the Mediterranean region, and widely distributed along the whole of the Croatian part of the Adriatic coast (Topić and Vukelić, 2009). They usually develops on hill slopes on sparse soil, surface of which is dominated by stable or unstable stone, and exposed to the impact of the wind, great oscillations in temperature and to a paucity of water, which is not retained in the permeable and skeletal soil. These grasslands give good food sources as well as for domestic (sheep and goats) as for the wild animals (European rabbit and rock partridge).

In the hunting ground "Dragišnica" forest habitats covers 3,057 ha and makes 31% of total surface. Thermophilous forest (*Ostryo-Quercetum petraeae*) of common (*Quercus petraea*) and hop hornbeam (*Ostrya carpinifolia*) thrives on steep terrain such as canyon of the Komarnica River. Mixed forest of moesian beech (*Fagus moesiaca*) and fir (*Abieti-Fagetum moesiace*) dominates in the altitudinal range 800-1,900 m, on calcareous substrate. Mixed forests of beech, fir and spruce (*Piceeto-Abieti-Fagetum* s. lat.) are reported from Montenegro (Čurović et al, 2011). Subalpine beech forest with sycamore (*Fageto-Aceretum visianii*) thrives in high altitudinal zone 1,400-1,800 m. Stands of dwarf mountain pine (*Pinus mugo*) are present at highest part of mountains, at altitude 1,800-2,400 m, where climate conditions are extremely harsh. According to recent findings (Čarni and Mucina, 2015), plant communities dominated by *Pinus mugo* in Bosnia and Herzegovina and Montenegro are classified into association *Lilio bosniacae-Pinetum mugo*.

Grassland in the hunting ground "Maglaj-Cista" comprises meadows and pastures on surface of 3,156 ha which makes 31% of total surface. Diverse alpine and subalpine calcareous grasslands are classified into associations: *Stachydi-Festucetum pseudoxanthynae*, *Genisto-Festucetum spadicaeae* and *Festuco-Agrostietum*.

Management of main game animals

Diversity of habitats in all three surveyed hunting grounds is most suitable for management of the following game species: roe deer, wild boar, European hare, European badger, beech marten, and red fox.

Good habitat conditions for managing population of red deer and particularly brown bear are present in the hunting ground "Bjelolasica" in Gorski kotar. In the hunting ground "Dragišnica" in Montenegro, habitat conditions are good for managing the population of brown bear, grey wolf and western capercaillie.

Submediterranean rocky grasslands in the hunting ground "Maglaj-Cista" in middle Dalmatia are the high quality habitats for managing the population of European hare and winged game, such is the rock partridge.

CONCLUSIONS

Characteristics and quality of habitats are important preconditions for protection, conservation and management of wildlife, as well as of free-living animal species as those classified as game animals. Structure and distribution of habitats supports rich biological diversity in the regions of Croatia and Montenegro which belongs into the Dinarides mountain chain.

Diversity of habitats and their good quality is essential for the sustainable and effective management of big and small game animals in the hunting grounds.

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DOI: 10.17707/AgricultForest.62.4.18

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THE ANALYSIS OF THE TAXATION STRUCTURE *Robinia pseudoacacia L.* STANDS IN THE FORESTS WHITHIN OF NORTHERN STEPPE, UKRAINE

SUMMARY

In the article, it was analyzed the main biometric indicators of modal forest stands with *Robinia pseudoacacia* L. within the Dnieper Steppe of Ukraine. Under conditions of the Dnieper Northern Steppe, forest stands with *Robinia pseudoacacia* occupy an area of 17683.7 ha, representing 26.9 % of total area covered with forests. The range of habitat conditions in *Robinia pseudoacacia* varied from A_0 to D_4. Age-class structure and distribution of *Robinia* wood stock cannot be evaluated as optimal because over-mature stands amounted the largest share 72.3 % of total *Robinia* area when small share of young stands. The trend of productivity increasing with age in *Robinia* forest stands is not established. Taking into account the *Robinia* normative maturity age amounted 26–35 years, it is necessary to take and implement some silvicultural measures to such type of forest stands growing at the present time.

Keywords: *Robinia pseudoacacia* stands, Northern Steppe of Ukraine, biometric parameters, growing stock

INTRODUCTION

The works of silvicultural researchers A. Belgard, A. Lokhmatov, O. Furdychko (Belgard, 1971; Furdychko, 2003; Gritsan, 2000; Kobets et al., 2015; Lokhmatov and Gladun, 2004) are dedicated on issues of forest functioning under conditions of Steppe zone, and searching the mechanisms to improve resistance and productivity of main forest-forming species. Within Steppe zone of Ukraine, forests mostly represented by artificial plantings, that is why the surveys of their current state, compliance physiological requirements to forest-forming species with their site conditions, and determination of main environmental factors influencing forest productivity are actual.

Forest stands in the Dnieper Steppe formed with environmentally significant species is *Robinia pseudoacacia* L. (black locust), introduced from Northern America. This species is economically and ecologically significant in Ukraine. The black locust plantations were created over a hundred years in the Steppe zone of country (Hensiruk, 1992; Gladun, 2005; Gritsan et al., 1997; Gritsan, 2005).

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

Firstly, *Robinia* plantations had been created in 1860–1880 in the steppe forest district; numerous shelter-belt forests were planted in 30th of XX century. Then *Robinia* was used in afforestation of gully systems and broken slopes. For the last 50 years, this species stands have been created in the form of forest shelter belts, protective plantations along water reservoirs, ravine and riverine slopes, watersheds or ravine-gully systems. *Robinia* stands are especially widely distributed along the Kakhovsky and Dneprodzerzhinsky reservoirs (Lokhmatov and Gladun, 2004). Currently, they dominate in total area of forest plantations within Steppe zone of Ukraine, and that perform a variety of protective, environmental-transforming and utilitarian functions (Gritsan et al., 2007; Sytnyk et al., 2015).

According to zoning by S. Hensiruk (Hensiruk, 1992), the studied black locust forest stands occurred within Northern Steppe. They are quite varied in relation to spatial forms, habitats and farming creating, functionality, protection; therefore, such stands require a differentiated silvicultural care. *Robinia* stands are divided three historically formed silvicultural groups (Lokhmatov and Gladun, 2004):

1-st group: plantings of the first and second vegetative generation (root shoots);

2-nd group: plantations established in 30s of XX century. These plantations include forest shelter belts, ravine-gully and slope forests. Such plantations are widely spread on the designated forest lands; their origin is mainly seed, mixed (seed + vegetative), and vegetative (from first generation root shoots);

3-rd group: forest stands planted in 1950–1990. These protective forest stands grow along river banks, and under condition of ravine and slopes, ravine-beam systems, watersheds, shelter belts, and fully stocked woods of the steppe forests. These stands are especially numerous in the Dnipropetrovsk area along reservoir banks, eroded slopes and ravine-gully systems.

The purpose of this study is analysis of current state of the modal *Robinia pseudoacacia* stands within the Dnieper Northern Steppe, Ukraine. In perspective, it may be used for dynamics simulation and forecast of growth and productivity by the main biometric indicators.

MATERIAL AND METHODS

In biometric analysis, it was used the information from stratum database ”Ukrderzhlisproekt“ (http://www.lisproekt.gov.ua/), which characterized pure and mixed stands with *Robinia pseudoacacia* within the Dnieper Steppe, Ukraine (Gulchak et al., 2011).

Total sample size of the database was 4739 stratums with total area of 17683.7 ha. In this case, the analysis was subjected to such taxation characteristics as well as stand area (S), average plantation age (A), average diameter (D), average height (H), stock per 1 ha, total stock on the wood stock (M), site quality class or bonitat (B), forest site type (FST). In data analysis, it

was used the general principles of mathematical statistics and conventional methods used in forest inventory (Gulchak et al., 2011; Migunova, 2014; Nikitin and Shvidenko, 2008; Sytnyk et al., 2015).

The classification schemes of trophotops and hygrotops is based on the edaphic factors, such as soil fertility and humidity. Soil fertility is characterized by trophogenic sequence and denoted by the letters A, B, C, and D. Each individual unit of trophogenic sequence called trophotope. Each trophotope is represented by the forest site with equal soil fertility within its boundaries, distinguished from the next by one gradation (Migunova, 2014).

Trophotope "A" or "bir" indicate very poor soil conditions, predominantly with sandy soil, sometimes loamy sands with a short rhizosphere zone; gritty consistency stipulate their poorness. It includes peaty soils, which occurred as a result of swamping by the sphagnum (raised) type. Vegetation is exclusively oligotrophic.

Trophotope "B" or "subir" is characterized by relatively poor soils. It is represented by loamy sand or sandy soil with thin sandy-loam or loamy layers, or with a heavy layers of that at a considerable depth. In the other cases, this trophotope is represented by sandy-loam and loamy soil with low thickness of soil layer, including skeletal soil on mountain slopes. It includes the peat soil with transitional swamping. The vegetation consists of pine-forest oligotrophes with admixed mesotrophes.

Trophotope "C" or "suhrud" has a relatively rich habitat conditions. Soils are represented by sandy-loam, sometimes sand with layers of loam and sandy-loam, or denuded shallow grey forest soil, sometimes brown soil with the little humus horizon, skeletal, derived from volcanic rocks and sandstones. Vegetation is composed of oligo-, meso- and megatrophes.

Trophotope "D" or "hrud" has the most fertile habitat conditions. The soils are loam with the heavy (greater than 0.8 m) rhizosphere, more rarely sandy and sandy-loam soils with the layers of loam and clay, available to plant roots. Sometimes the sandy and sandy-loam soils occur with shallow horizon of flow "mineralized" groundwater. This includes also the richest soils of lowland swamps. Megatrophes dominated in composition of climax forest vegetation.

Every trophotopes differs in the degree of soil moisture (hyhrotopes), with indicate in the range from 0 to 5. Hyhrotope of 0 range respond to very dry (xerophilic) conditions; 1 – dry-mesic (meso-xerophilic); 2 – mesic (mesophilic); 3 – wet-mesic (meso-hygrophilic); 4 – wet (hygrophilic); 5 – moist (ultrahyhrophilic).

Very dry types. The habitat conditions include the top of sand dune hills, denuded soil of the beam foreheads, and drained upland edaphotopes within the Steppe. Groundwater under these conditions is very deep, and the only source of water is precipitation, which is able to moisten the top of soil, usually dry, only for a short period of time.

*Dry types***.** Conditions for sandy soils with low water storage capacity; dryness of them depends on the groundwater depth; on the clay soils it depends

on dryness of climate, surface runoff (on the slopes), high evaporation (southern exposure), and low total water capacity (shallow and lithosolic clay soils of mountain slopes).

*Dry-mesic types***.** Sandy soil in well-moistened habitat is provided by higher groundwater level (2–4 m); in the loamy lands the groundwater is deeper than 4 m, often outside the layer available for plant roots. In southern regions, the satisfactory moistening is carried out by surface runoff reducing, increasing total soil water capacity, and more complete saturation of the soil by winter precipitation (steady winters).

Mesic types. In southern regions, good moisture is provided by the same conditions as in the previous type, and in Northern regions due to the better drainage (tops of hills, slopes). The groundwater level in sandy soils is 1–2 m, and 2–4 m in loamy and clay soils. The subsoil is blowdown, the land form is undulating or gently rolling.

Wet types. The overwetting habitats. Increasing of soil moisture depends on the higher groundwater level (at a depth of about 1 m in the sands, and 1–3 m in the sandy-loam soil).

Moist types. The habitats have excessive moisture and peat soils. Much of vegetative period, the groundwater level is near of the soil surface.

RESULTS AND DISCUSSION

Forests within the Dnieper Northern Steppe are subordinated to the State Forest Resources Agency the *Robinia pseudoacacia* forest stands occupy an area of 17683.7 ha, or 26.9 % of the total area covered with forest vegetation. These plantations have the following representation relative to functional categories (Gulchak et al., 2011):

- reserve forests with historical, cultural and scientific purposes: 1831.1 ha (18.8 % of the total forest area covered with this category);
- recreational forests: 7173.5 ha (22.9 % of the total area covered with recreational forests);
- protective forests: 8679.1 ha (35.2 % of the total area of protective forests).

Robinia pseudoacacia mainly forms pure stands, 82.5 % of the total area this species. 17.5 % of this stands occupied by black locust mixed stands with oak, ash and poplars.

Based on the thesis that uniform distribution of forest areas by age groups is biologically and economically expedient. The results of analysis of the age structure in stands this species allow us to ascertain the unequal distribution of areas by age groups. Distribution of area, total productivity, and average stock of *Robinia pseudoacacia* stands by age structure are given in Table 1. Distribution of stands by age groups is carried out depending on the main felling age. For the studied speciesfelling stage depends on regime of forest use: in forests with a special regime of use 31–36 years, in forests with a restricted regime of use 26–30 years. For the black locust as for fast-growing species, age class of such is 5

years. Furthermore, age groups consist of follow age classes: young growth – 1, 2; middle-aged – 3–5; premature – 6; mature – 7; overmature – 8 and above.

Table 1. Age structure of Robinia pseudoacacia stands

Index	Total	Age groups				
		Young growth	Middle-Aged	Premature	Mature	Over-mature
Area, ha	17683.7	370.2	1414.6	486.3	2626.6	12786.0
Total productivity, ths. m^3	2625.2	6.4	45.8	34.9	346.4	2191.6
Average stock per 1 ha, $m^3.ha^{-1}$	149	17	32	72	132	171

As it can be seen from the data in Table 1, that the smallest part of the area belongs to young stands, i.e. stands aged up to 10 years, which is 2.2 % of the total area of *Robinia* stands. Premature stands are 2.7 %, middle-aged and mature stands are 8.0 % and 14.8 %, respectively. Overmature stands occupy the largest part 72.3 % of the total area of this species stands within research area. For total productivity indexes, the lowest part is young stands 0.2 %. Premature stands amounted 1.3 %; middle-aged are 1.7 %, and mature stands are 13.2 %. Forest stands composed with overmature *Robinia* plantations have the largest total stock – 83.6 %. The average age of such stands is 43 years.

The present age-class composition and the distribution of average stocks in *Robinia pseudoacacia* forests of Dnieper Steppe is a consequence of insufficient number of the forest sanitary and cleaning fellings, and it cannot be considered as optimal.

The average stock per hectare by age classes was calculated in analysis of age structure in *Robinia* plantations. According to confidence index of approximation, it was selected the model described by polynomial function. Thus, to describe the dependence between productivity and age of forest stands, obtained model is presented in the chart (Figure 1).

The chart demonstrates that over-mature *Robinia pseudoacacia* stands aged 80–85 years (17^{th} age class) has the greatest average wood stock (194 m^3/ha), whereas both 8^{th} class (36–40 years, 141 m^3/ha) and 18^{th} class (86–90 years, 142 m^3/ha) have the smallest average wood stock within overmature age group. The data given in the chart demonstrate lack of dependence in forest stands productivity on age; there is a polynomial dependence was not detected

The bonitat class is the index of the forest stands productivity. The biggest of the area is occupied by stands with 1^{st} bonitat class. The II^{nd}, III^{rd} and IV^{th} classes occur. Very small proportion of poor stands (V^{th} class) demonstrates accordance with the regional site conditions. A third part of the stands with *Robinia pseudoacacia* with characteristics of 1^{st} bonitat class were formed mainly in the halogen variant of dry suhrud (4161.4 ha; or 35.3 % of total area occupied by the stands with such species of 1^{st} forest bonitat class).

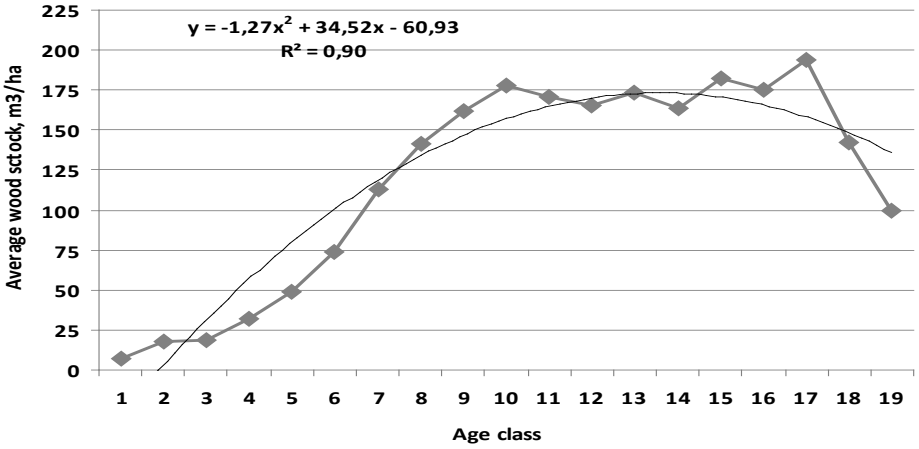

Figure 1: Dynamics of average wood stock Robinia pseudoacacia stands by age classes

Determination of habitat conditions makes possible not only to estimate sufficiently the forest site potential of different soil types, but also to determine the advanced species composition for them, the nature and methods of growing stands for various functional purposes (Nikitin and Shvidenko, 2008; Migunova, 2014).

The range of habitat conditions for *Robinia pseudoacacia* within Dnieper Northern Steppe of Ukraine is very wide, from A_0 to D_4. This species is located in seventeen edatopes: A_0–A_2, B_0–B_3, C_0–C_5, D_1–D_4 (Table 2).

Table 2. The distribution area *Robinia* stands by forest site types

Hyhrotops	Trophotops								Total for hyhrotops	
	A		B		C		D			
	ha	%	Ha	%	ha	%	ha	%	ha	%
0	4.6	0.02	38.9	0.22	990.7	5.60	–	–	1034.2	5.9
1	32.7	0.18	700.6	3.96	7471.0	42.20	5712.4	32.30	13916.7	79.0
2	102.3	0.58	437.3	2.47	1072.3	6.06	982.2	5.55	2594.1	14.7
3	–	–	38.6	0.22	46.8	0.27	29.2	0.16	114.6	0.7
4	–	–	–	–	1.2	0.01	8.4	0.05	9.6	0.1
5	–	–	–	–	14.5	0.08	–	–	14.5	0.1
Total	139,6	0.78	1215.4	6.87	9596.5	54.28	6732.2	38.07	17683.7	100.0

Area distribution by the variants of trophotop series leads to the conclusion that the largest area black locust stands belongs to mixed oak forest (9596.5 ha), which is 54.3 % of total area this species. The mixed oak forest occupy more than one third of the total area (6732.2 ha, or 38.1 %). *Robinia* stands growing under the poorest soil conditions of pine forest occupy the minimum area (139.8 ha, or 0.8 %) (Figure 2).

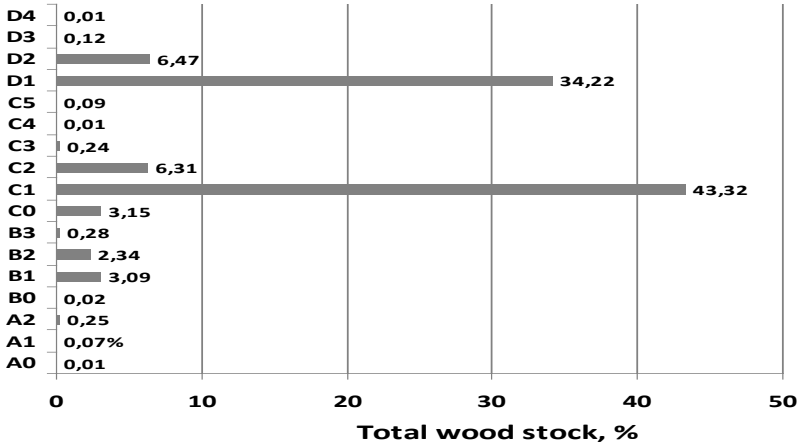

Figure 2: The distribution of *Robinia pseudoacacia* total growing stock by forest site types

Robinia pseudoacacia can form the forests within very dry, dry, dry-mesic, mesic, wet, and moist hyhrotopes. Most of the *Robinia* stands area are located on the dry hyhrotop (A_1, B_1, C_1, D_1): 13916.7 ha (78.7 %). 2594.1 ha (14.9 %) of the *Robinia* stands area grow in dry-mesic conditions (A_2, B_2, D_2), and a very small proportion of such stands occupied moist hyhrotope: 14.5 ha (0,1 %). Note that 55.5 % of the total *Robinia* stands area (9808.8 ha) grow under stressful environmental factors such as salinity, i.e. under halogen forest site conditions The forest site type is an important indicator effecting significantly on the forest productivity. Data on the total wood stock distribution by forest site types are shown in Figure 4. The data in Fig. 4 evidence that *Robinia* forest stands grow mainly on the relatively rich soils, and they formed follow shares of stock: C_1 – 42.2 %; D_1 – 32.3 %; C_2 – 6.1 %, C_0 – 5.6 %; the rest of stock belongs to mixed pine forests (6.9 %), and a very small proportion is concentrated within pure pine forests (0.8 %).

CONCLUSIONS

Robinia pseudoacacia stands occupy an area of 17683.7 ha, representing 26.9 % of the total area covered with forests, under conditions of Dnieper Northern Steppe. The range of habitat conditions for this spesies is very wide within Dnieper Northern Steppe of Ukraine: from A_0 to D_4. Black locust is located in seventeen edatopes: A_0–A_2, B_0–B_3, C_0–C_5, D_1–D_4. *Robinia pseudoacacia* mainly forms pure stands in 82.5 % of the total area. This species mixed forests with oak, ash, poplars occupy 17.5 % of the total area of black locust plantations. The present age-class structure and distribution of the total wood stocks in stands with this species are consequence of the insufficient forest sanitation, and it cannot be considered as optimal; young stands aged up to 10 years old occupy the smallest share of the total area, overmature stands account

for the largest share of 72.3 % of the total area within study region. The trend of productivity increasing with age in black locust stands is not established.The main part of the area is occupied by these stands with I-st classes of forest capacity. A third of *Robinia pseudoacacia* stands of the I-st bonitat class were formed mainly in the halogen variant of the suhrud.Taking into account the *Robinia* normative maturity age amounted 26–35 years, it is necessary to take and implement some silvicultural measures to the such type of forest stands growing at the present time.

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DOI: 10.17707/AgricultForest.62.4.19

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SALINITY INFLUENCE ON SURVIVAL, GROWTH AND NUTRIENT DISTRIBUTION IN DIFFERENT PARTS OF *MILLETTIA PINNATA* SEEDLINGS

SUMMARY

Millettia pinnata is a mangrove associate tree species in the Sundarbans and commonly used for soil stabilization along the embankment and riverbank of coastal area. This study examined the effect of different salinity levels on seedling survival, growth, nutrients (Nitrogen, Phosphorus, Potassium) and Sodium distribution in different parts (leaf, stem and root) as well as total chlorophyll and proline concentration in the leaves of *Millettia pinnata* seedlings. Survival and growth study was carried out in different saline treatments from 0 to 35 ppt and from 0 ppt to 25 ppt respectively at 5 ppt interval for six months. Highest survival (100%) was observed at 0 to 10 ppt salinity and then gradually decreased to 10% at 25 ppt salinity. No survival was observed at 30 and 35 ppt salinity. Biomass increment showed significantly negative correlation (r=-0.84) with salinity levels. Oven dried biomass increment was the highest (6.97 g/month) at 0 ppt salinity and gradually decreased to 0.75 g/month at 15 ppt salinity. Nitrogen and potassium concentration in different seedling parts and phosphorus in leaves showed significant negative correlation with salinity levels. However, sodium concentration in different parts showed significant positive correlation with salinity levels. Total chlorophyll concentration was the highest (0.62 mg/g) in the leaves of seedlings grown at 0 ppt and decreased to 0.29 mg/g at 25 ppt salinity. Highest proline concentration (6.88 µmoles/g) was measured at 25 ppt salinity and lowest (1.52 µmoles/g) at 0 ppt salinity.

Keywords: Salinity, Growth, *Millettia pinnata*, Nutrient, Chlorophyll, Proline

INTRODUCTION

Salinity appears to be a critical environmental factor in the mangrove ecosystem which regulates species composition and diversity in the Sundarbans

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

(Pethick, 2011). Salinity affects the availability of nutrients to plants which may influence metabolism and osmoregulation of plant cell (Taiz and Zeiger, 2006). Moreover, it regulates absorption and transportation of nutrients within different plant parts (Munns and Tester, 2008; Mahmood et al. 2014). Salt has a limiting effect on photochemistry of plants, negatively affects photosynthesis process by inhibiting chlorophyll synthesis, thereby reducing growth (Munns and Termaat, 1986). To cope with saline environment, mangrove species accumulate some compatible solute in their cytoplasm in order to maintain high osmotic potential (Parida et al. 2004). Among different compatible solutes, proline has been found to occur frequently in salt tolerant species (Popp et al. 1985). Salt tolerance varies with the species (Hossain et al. 2001; Ball, 2002). Mangrove associates can tolerate less salt compared to true mangroves (Tomlinson, 1986; Paliyavuth, 2001; Youssef, 2007).

Millettia pinnata is a fast growing multipurpose mangrove associate tree species (Allen and Allen, 1981) and found to occur in slight saline to moderate saline areas in the coastal regions of Bangladesh (Mahmood, 2015). Presently, this species has drawn much attention for its nitrogen fixing capacity, good coppicing ability and for the ability to tolerate water logging (Misra and Singh, 1987). It is also a potential source of biodiesel now a days (Shrinivasa, 2001; Sharma and Singh, 2008). Salt intrusion from the sea and reduced flow of fresh water from the upstream might have significant influence on plant community in the coastal regions of Bangladesh. Therefore, selection of suitable plant species are of great importance for plantation on the salt affected marginal land and embankment of the coastal areas. Considering the ecological and economic significance of *M. pinnata*, it may appear as a suitable species for plantation in the salt affected areas. However, scientific information on survival and growth of this species at different salinity levels is still scanty. Therefore, present study was designed to evaluate the influence of salinity on survival and growth, nutrients (Nitrogen, Phosphorus and Potassium), Sodium distribution pattern in different parts (leaf, stem and root), as well as chlorophyll and proline concentration in the leaf of *Millettia pinnata* seedlings.

MATERIAL AND METHODS

Description of the study area.

Sundarbans is located between 21° 30' and 22° 30' N latitudes and between 89° 00' and 89° 55' E longitudes. It is a unique habitat for a wide diversity of flora and fauna (Karim, 1995) and has been declared as world's heritage site by UNESCO in 1997 (Basar, 2012). Based on the level of soil salinity, Sundarbans is divided into less saline (LS), moderate saline (MS) and strong saline (SS) zones having salinity <2 dS/m, 2-4 dS/m and >4 dS/m respectively (Siddiqi, 2001). Rainfall is strongly seasonal (from May to October) with 87% of the mean annual rainfall (1500 mm). Temperature ranges from 18.50 to 35.20 in summer and from 12.20 to 28.80 in winter. Soil is silty to sandy clay loam, as well as bulk

density, particle density and porosity vary from 1.18 to1.27 g/cc, 2.31 to 2.52 g/cc and 46–52%, respectively. Soil pH is 7.8 (Siddiqi, 2001).

Seed collection and raising of seedlings

Mature seeds of M. pinnata were collected from mother trees in the Moderate saline zone of the Sundarbans. Collected seeds were sorted manually and defective seeds were discarded. Seeds were reddish brown in color. Its average length and width were 1.5 to 2 cm and 0.5 to 1 cm respectively. Seeds were sown on germination bed (5 m ×1.5 m) filled with 30 cm thick layer of coarse sand. Seedlings were raised under fresh water condition.

Experiment setup

A total of 160 polyethylene terephthalate (PET) bottles (20 cm height and 9 cm diameter of each) were taken. Three month old seedling was planted in each bottle filled with coarse sand. Twenty pet bottles with seedlings were kept in a plastic box (100 cm × 20 cm × 30 cm). Therefore, a total eight boxes were prepared. Eight treatments of salinity (0 to 35 ppt at 5 ppt interval) were applied randomly to the boxes. Sixteen liters of full strength modified Hogland solution was given in each box as a source of nutrient. Salinity in each treatment level was increased gradually at weekly interval following Mahmood et al. (2014). Nutrient solution was changed weekly. Salinity and water level of each treatment were checked and corrected at 24 hours interval. This experiment was conducted for 6 months in the glass house of forest nursery of Khulna University. Number of survived seedlings in each treatment was counted at the end of the experiment.

Growth study

A total of 82 polyethylene terephthalate (PET) bottles (20 cm height and 9 cm diameter were taken. Three month old seedlings were used for this experiment. Initial collar diameter, height and green biomass of each seedling were measured and recorded. One seedling was planted in each pet bottle filled with pre-gravel. Initial green biomasses of ten seedlings were measured individually. Then, it was dried in an oven at 80°C for 4 days to calculate green to oven-dried weight conversion ratio. No seedlings were survived at high saline treatments (30 to 35 ppt) during survival study. So, this experiment was set up with salinity ranging from 0 to 25 ppt salinity at 5 ppt interval with three replications. Each replication contained four pet bottles with seedlings kept in a plastic box (55 cm × 30 cm × 20 cm). Thus, 18 boxes were prepared. Eight litter of modified Hogland nutrient solution was added to each box to nourish the seedlings. Six levels of saline treatments (0 to 25 ppt at 5 ppt interval with three replications was applied to the seedlings. Initially the salinity of the nutrient solution was maintained zero to prevent the seedlings from sudden stress of salinity. Salinity level of each treatment was increased gradually following Mahmood et al. (2014). Distilled water was used for 0 ppt treatment level. The nutrient solution was replaced weekly. Salinity and water level of each plastic box were checked and corrected at 24 hours interval. The pH of each treatment was maintained 8. Salinity of the treatment solution and that inside the pet bottle were cheeked and found similar for both the cases. This experiment was

conducted for six month in the glass house of forest nursery of Khulna University. At the end of the experiment, seedlings were harvested. Their height, collar diameter, and green biomass were measured and recorded salinity treatment wise. Growth in term of height and diameter increment as well as oven-dried biomass was measured at the end of experiment.

Nutrients: nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) in seedling parts. Subsamples (100 g of each) of seedling parts (leaf, stem and root) were collected from the harvested seedlings of each treatment and dried in oven at 80°C for four days. Each oven dried sample was processed and acid digested following Allen (1989). Concentration of nitrogen and phosphorus in the sample extract was measured by using colorimetric method following Baethgen and Alley (1989) and Timothy et al. (1984) respectively in UV-VIS Spectrophotometer (HITACHI-2900U). Potassium and sodium concentrations in sample extract were measured by Flame Photometer (PFP7, Jenway LTD, England).

Chlorophyll and proline in leaf

At the end of the experiment, 3 fresh leaves from each saline treatment were taken randomly for the measurement of total chlorophyll. The leaves were cut into disks (0.83 cm diameter) using a disk cutter. Total chlorophyll was extracted from the said leaf disk following Dimethyl Sulphoxide (DMSO) method (Hiscox and Israelstam, 1979). Proline in leaf samples were measured following Bates et al. (1973).

Statistical analysis

Seedling survival percentage values of all treatments were transformed to arcsine and compared among saline treatments by one-way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) at $p<0.05$ by using SAS (6.12) Statistical software. Height, collar diameter, oven-dried biomass increment, chlorophyll and proline concentration of seedlings were compared among salinity treatments by one-way ANOVA followed by DMRT using the above Statistical software. Nitrogen, phosphorus, potassium and sodium concentration in different parts of seedlings were compared among the salinity treatments and also among different plant parts by using two-way ANOVA followed by DMRT using the above Statistical software. Moreover, correlations among all studied parameters were evaluated using SPSS-20 statistical software.

RESULTS AND DISCUSSION

Survival of seedlings.

Survival percentage of seedlings varied significantly among the saline treatments (Figure 1a). Survival of seedlings was the highest (100%) at 0 to 10 ppt salinity. It decreased from 85% to 10% among 15 ppt and 25 ppt salinity. No seedlings survival was observed at 30 ppt and 35 ppt salinity. Survival percentage showed strong negative correlation with salinity levels (Table 2).

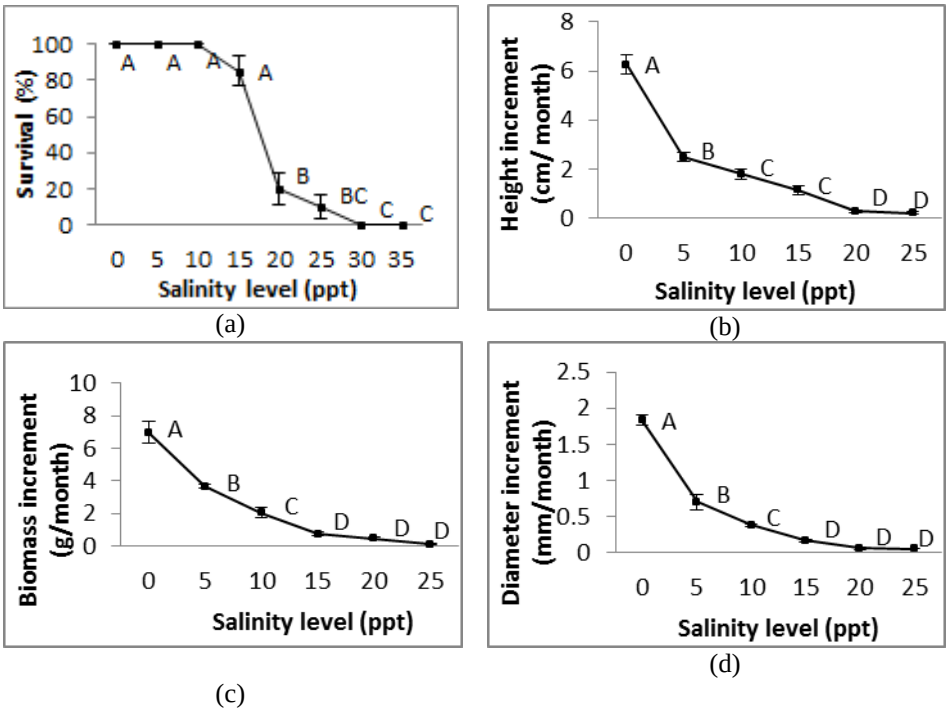


Figure 1: Salinity influence on *Millettia pinnata* (a) Survival of seedling (b) Height increment (c) Collar diameter increment (d) Oven-dried biomass increment. Similar alphabet along the line are not significantly ($p>0.05$) different. Vertical bars indicate standard error.

Growth of seedlings

Height, collar diameter, and oven-dried biomass increment separately varied significantly among salinity treatments (Figure 1b, 1c, 1d). Highest increment in height (6.26 cm/month), collar diameter (1.83 mm/month), and oven-dried biomass (6.97 g/month) were found at 0 ppt salinity whereas lowest increment in height (0.22 cm/month), collar diameter (0.06 mm/month), and oven-dried biomass (0.12 g/month) was measured at 25 ppt salinity. Sharp decrease in growth parameters were observed at 5 ppt salinity. Strong negative correlation was observed between salinity levels and height, collar diameter as well as between salinity levels and oven-dried biomass (Table 2).

Nutrients (N, P and K) and Na distribution in seedling parts

Significant variation in N, P, K and Na concentration was observed in different parts of seedlings of M. pinnata with different salinity levels. Comparatively higher concentration of N (2.63 to 2.10%), P (0.70 to 0.64%) and K (1.68 to 1.24%) was observed in leaves than other parts of seedlings at different salinity levels. On the other hand, higher Na concentration was found in roots (1.97 to 0.73%) than other parts of seedling at different salinity levels (Table 1).

Table 1: Concentrations (%) (Mean±Standard Deviation) of nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) in different parts *Millettia pinnata* seedlings.

	Nitrogen			Phosphorus			Potassium			Sodium		
Salinity level (ppt)	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
0	1.35±0.04	1.10±0.10	2.63±0.21	0.63±0.08	0.54±0.03	0.70±0.11	1.13±0.02	0.67±0.0	1.68±0.07	0.73±0.02	0.32±0.01	0.34±0.03
5	1.35±0.04	1.12±0.13	2.64±0.22	0.61±0.08	0.53±0.03	0.69±0.16	0.62±0.02	0.54±0.07	1.58±0.12	1.14±0.06	0.54±0.01	1.12±0.01
10	1.33±0.15	0.94±0.07	2.56±0.11	0.62±0.08	0.53±0.08	0.65±0.10	0.59±0.02	0.46±0.04	1.53±0.07	1.33±0.03	0.83±0.04	1.14±0.02
15	1.26±0.03	0.83±0.06	2.20±0.04	0.61±0.03	0.53±0.11	0.64± 0.08	0.58±0.02	0.41±0.05	1.40±0.06	1.35±0.06	1.05±0.01	1.32±0.02
20	1.27±0.06	0.8±0.1	2.19±0.05	0.60±0.13	0.53±0.03	0.64±0.11	0.56±0.01	0.35±0.01	1.30±0.07	1.42±0.01	1.10±0.02	1.35±0.09
25	1.25±0.13	0.8±0.1	2.1±0.1	0.60±0.05	0.53±0.03	0.64±0.08	0.46±0.09	0.32±0.03	1.24±0.08	1.92±0.20	1.16±0.01	1.51±0.06

Table 2: Correlation matrix of all studied parameters.

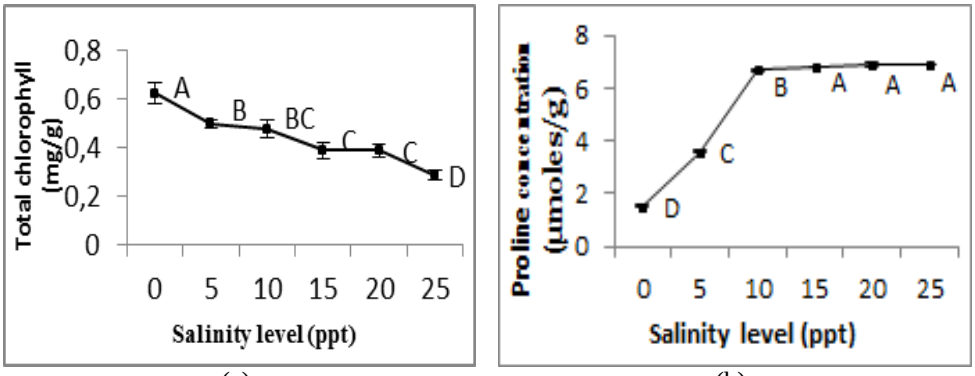
		Salinity	Survival	Biomass	Diameter	Height	N (Root)	N (Leaf)	N (Stem)	P (Root)	P (Leaf)	P (Stem)	K (Root)	K (Leaf)	K (Stem)	Na (Root)	Na (Leaf)	Na (Stem)	Chlorophyll	Proline
Salinity	r	1																		
	Sig																			
	N	160																		
Survival	r	-0.84																		
	Sig	0.00																		
	N	160																		
Biomass	r	-0.84	0.48																	
	Sig	0.00	0.00																	
	N	61	61																	
Diameter	r	-0.83	0.43	0.89																
	Sig	0.00	0.00	0.00																
	N	61	61	61																
Height	r	-0.84	0.47	0.87	0.89															
	Sig	0.00	0.00	0.00	0.00															
	N	61	61	61	61															
N (Root)	r	-0.47	0.22	0.40	0.43	0.40														
	Sig	0.05	0.39	0.10	0.08	0.10														
	N	18	18	18	18	18														
N (Leaf)	r	-0.87	0.61	0.76	0.75	0.65	0.45													
	Sig	0.00	0.01	0.00	0.00	0.00	0.06													
	N	18	18	18	18	18	18													
N (Stem)	r	-0.80	0.61	0.66	0.76	0.76	0.45	0.69												
	Sig	0.00	0.01	0.00	0.00	0.00	0.06	0.00												
	N	18	18	18	18	18	18	18												
P (Root)	r	-0.39	0.61	0.39	0.37	0.25	-0.22	0.47	0.23											
	Sig	0.11	0.01	0.11	0.14	0.31	0.38	0.05	0.37											
	N	18	18	18	18	18	18	18	18											

P (Leaf)	r	-0.59	0.46	0.67	0.56	0.55	0.23	0.60	0.38	0.44										
	Sig	0.01	0.06	0.00	0.02	0.02	0.36	0.01	0.12	0.07										
	N	18	18	18	18	18	18	18	18	18										
P (Stem)	r	-0.17	-0.01	0.22	0.25	0.21	0.29	0.26	0.30	-0.08	0.12									
	Sig	0.51	0.96	0.37	0.32	0.40	0.25	0.30	0.23	0.75	0.64									
	N	18	18	18	18	18	18	18	18	18	18									
K (Root)	r	-0.79	0.45	0.90	0.87	0.88	0.29	0.58	0.59	0.27	0.56	0.26								
	Sig	0.00	0.06	0.00	0.00	0.00	0.24	0.01	0.01	0.27	0.02	0.29								
	N	18	18	18	18	18	18	18	18	18	18	18								
K (Leaf)	r	-0.91	0.74	0.72	0.80	0.72	0.46	0.82	0.69	0.43	0.63	0.17	0.68							
	Sig	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.07	0.01	0.51	0.00							
	N	18	18	18	18	18	18	18	18	18	18	18	18							
K (Stem)	r	-0.94	0.64	0.90	0.88	0.92	0.41	0.78	0.72	0.36	0.71	0.20	0.86	0.88						
	Sig	0.00	0.01	0.00	0.00	0.00	0.09	0.00	0.00	0.14	0.00	0.43	0.00	0.00						
	N	18	18	18	18	18	18	18	18	18	18	18	18	18						
Na (Root	r	0.92	-0.69	-0.83	-0.83	-0.85	-0.40	-0.72	-0.67	-0.39	-0.55	-0.22	-0.83	-0.83	-0.89					
	Sig	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.12	0.02	0.38	0.00	0.00	0.00					
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18					
Na (Leaf)	r	0.86	-0.54	-0.91	-0.90	-0.91	-0.40	-0.66	-0.66	-0.34	-0.58	-0.20	-0.96	-0.79	-0.91	0.89				
	Sig	0.00	0.02	0.00	0.00	0.00	0.10	0.00	0.00	0.17	0.01	0.43	0.00	0.00	0.00	0.00				
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18				
Na (Stem)	r	0.96	-0.59	-0.92	-0.94	-0.90	-0.50	-0.85	-0.83	-0.31	-0.63	-0.19	-0.82	-0.87	-0.94	0.87	0.89			
	Sig	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.21	0.01	0.46	0.00	0.00	0.00	0.00	0.00			
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18			
Chlorophyll	r	-0.89	0.65	0.87	0.85	0.77	0.34	0.88	0.64	0.58	0.62	0.15	0.80	0.81	0.85	-0.87	-0.86	-0.87		
	Sig	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.01	0.01	0.54	0.00	0.00	0.00	0.00	0.00	0.00		
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18		
Proline	r	0.86	-0.43	-0.93	-0.94	-0.91	-0.41	-0.70	-0.77	-0.25	-0.65	-0.21	-0.87	-0.76	-0.90	0.82	0.89	0.95	-0.79	
	Sig	0.00	0.07	0.00	0.00	0.00	0.09	0.00	0.00	0.32	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	

N and K concentration in different parts (roots, leaves and roots) and P concentration in leaves showed significantly negative correlation with salinity levels. However, P concentration in roots and stems showed no significant correlation with salinity. Sodium concentration in different parts of seedlings showed significant positive correlation with salinity (Table 2).

Total chlorophyll and proline concentration in leaves

Total chlorophyll concentration of leaves varied significantly among salinity treatments (Figure 2a).



(a) (b)

Figure 2: Salinity influence on (a) Total chlorophyll concentration (b) Proline concentration in leaves of *Millettia pinnata* seedling. Similar alphabet along the line are not significantly ($p>0.05$) different, Vertical bars indicate standard error.

Highest concentration (0.62 mg/g) of total chlorophyll was measured at 0 ppt, and it decreased to 0.29 mg/g at 25 ppt salinity. Total chlorophyll concentration showed strong negative correlation with salinity levels (Table 2). Proline concentration in leaves varied significantly among salinity treatments, lowest proline concentration (1.52 µmoles/g) was measured at 0 ppt salinity which increased up to 6.67 µmoles/g at 10 ppt salinity (Figure 2b). However, no significant increase in proline concentration was observed at high salinity (from 15 to 25 ppt). Proline concentration showed strong positive correlation with salinity levels (Table 2).

Salinity gradient is recognized as a potential stressor and a critical environmental factor that regulates growth, height, survival, and zonation patterns in mangroves (Lin and Sternberg, 1992; Mahmood et al. 2014). Mangroves usually invest a large proportion of its photosynthetic product for the surviving in high saline environments (Lopez-Hoffman et al. 2006). Nucleic acid and protein synthesis are two early processes of seedling growth. However, these processes are suppressed by salinity (Bewely and Black, 1985). Moreover, high soil salinity affects plant growth by creating low water potential, ion toxicities, nutrient deficiencies or a combination of these factors (Khan et al. 2000). Similarly, these factors may be responsible for the survival of mangrove seedling

at high salinity (Mahmood et al. 2014). These could be the reason for comparatively high survival and growth of *M. pinnata* seedlings at low salinity level. Higher concentration of Na+ may disrupt nutrient transport which restricts plant growth (Grant et al. 1987). In this study, salinity did not show any significant implications with N and P concentration in different parts of seedlings except leaves. However, it showed significant influence on K+ and Na+ concentration at different parts of seedlings. Gorham et al. 1986 observed a drastic reduction of leaf $NO3^{-}$ when plants were grown in saline condition. However, there was no strong evidence on the relationship between N concentration in other parts of plants and salinity levels (Munns and Termaat, 1986). Influence of salinity on P concentration in plant parts is variable and depends on the species and experimental conditions (Champagnol, 1979). Salinity reduced the concentration of K+ in plant parts (Ratthert, 1982), and the excess NaCl leads to loss of potassium due to membrane depolarization by Na+ (Cramer et al. 1985). This could be the reason for antagonistic relation between K+ and Na+ concentration in different parts of seedlings of M. pinnata. According to Singh (1990) and Singh and Yadav (1999), P. pinnata failed to grow at ECe 32.5 dS/m and K contents did not exhibit any definite relationship with increasing salinity under 31 months experiment of 9 month old seedlings. However, we found that in case of seedlings survived up to 25 ppt salinity, K concentration decreased with increased salinity.High concentration of sodium can cause ionic imbalance (Rathert et al. 1981) that influences the osmotic adjustment (Marschner et al.1986) and enzyme inhibition in plants (Flowers et al. 1977; Greenway and Osmond, 1972).

Total chlorophyll concentration depends on the biological process and development stages of plants and concentration of salt (Khan, 2003; Iqbal et al. 2006; Mahmood et al. 2014). Decrease in chlorophyll content at high saline environment results in rapid maturing of leaves (Yeo et al. 1991). Furthermore, decrease in chlorophyll content at higher salinity might be due to changes in the lipid-protein ratio of pigment-protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996). Salt stress reduces the photosynthetic capability of plant (Dubey, 1997; Jamil et al. 2007; Bayuelo-Jimenez et al. 2002). The inhibition in photosynthesis under saline condition can be explained by the decline in chlorophyll content (Delfine et al. 1999; Jamil and Rha, 2007). In our study, the concentration of total chlorophyll decreased with increasing salinity levels. Low concentration of chlorophyll in plant leaves results lower uptake of CO2, which ultimately reduces photosynthesis (Francios and Mass, 1993; XinWen et al. 2008).

In another way, plants synthesize proline under arid and salinity stress conditions in order to protect themselves and to regulate their physiological process (Edreva, 1998). Genotypes with a high proline accumulation and chlorophyll content, high K/Na ratio and low Na+ and Cl^{-} accumulation are more tolerant to salt (Mane et al. 2011).

CONCLUSION

Millettia pinnata produced comparatively low proline and chlorophyll which make it less salt tolerant than other dominant mangrove species in the Sundarbans. This species can survive and grow up to 25 ppt salinity, but 0 to 5 ppt salinity appeared to be a favorable range of salinity for its growth and development.

ACKNOWLEDGEMENT

We are thankful to United States Department of Agriculture (USDA) for their financial support; Ministry of Education and University Grants Commission, Bangladesh for their monitoring and smoothing the project activities. We also acknowledge the Sundarbans East Forest Division, and Forestry and Wood Technology Discipline, Khulna University, Bangladesh for the logistic support.

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DOI: 10.17707/AgricultForest.62.4.20

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THE INFLUENCE OF THE PARENT MATERIAL ON THE TEXTURE AND WATER RETENTION CURVES IN THE SOIL FORMED UPON LIMESTONES AND DOLOMITES

SUMMARY

The paper present results from the research of the influence of the parent material on the soil texture and water retention curves of Rendzic Leptosols. Chromic Luvisol on hard limestones and Ferric Luvisol on hard limestones - Terra Rossa. The contents of the fine soil separates in the Rendzic Leptosols vary depending on the subtype. The physical sand fraction (coarse sand + fine sand) in the Amo horizon amounts 44.81% at the Rendzic Leptosols, haplic 40.13% at the Rendzic Leptosols-organogenic and Rendzic Leptosols, chromic luvic 36.52%. In the (B)rz horizon at the Rendzic Leptosols, chromic luvic amounts 32.64%. The contents (clay + silt) or physical clay in the Amo horizon amounts 55.19% at the Rendzic Leptosols, haplic 59.87% at the Rendzic Leptosols, organogenic and the highest content is at the Rendzic Leptosols, chromic luvic 63.48%. The average value of this fraction in the horizon (B)rz at the Rendzic Leptosols, chromic luvic amounts 67.36%. In the Chromic Luvisol on hard limestones the average content of the fraction physical sand in the Amo horizon amounts 33.43%, and in the cambic horizon (B)rz 22.50%. At the Terra Rossa the fraction physical clay is represented with a greater percentage related to the physical sand fraction. In the Amo horizon in the physical clay fraction the clay fraction is predominant, average 43.08% and 52.13% in the cambic horizon, i.e. 24.90% in Amo and 19.37% for the silt fraction. There is the highest retention capacity, with the average of 41.48% in the humus-accumulative horizon Amo in the Rendzic Leptosols subtype organogenic. The remaining subtypes of Rendzic Leptosols have lower retention capacity in this horizon. As with retention capacity of 0.33 required in other points of tension (6.25 and 15 bar) shows the same condition (mean value have Rendzic Leptosols organogenic (29.39 and 17.22% respectively), then Rendzic Leptosols, haplic (23.98 and 18.34% respectively) and Rendzic Leptosols, chromic luvic (21.70% and 16.60% respectively). The average retention values in the Amo horizon and the cambic horizon (B)rz regarding Chromic Luvisol on hard limestones amounts to 33.49% i.e. 33.24%. The average retention values (pressure of 6.25 and 15 bar) in the

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

Amo horizon and the cambic horizon (B)rz regarding Chromic Luvisol on hard limestones amounts to (25.27% and 17.06% respectively) i.e. (27.89% and 16.68% respectively). The average retention capacity in Terra Rossa, amounts to 39.05% in the Amo horizon, and 40.25% in (B)rz. The average retention values (pressure of 6.25 and 15 bar) in the Amo horizon and the cambic horizon (B)rz regarding Terra Rossa amounts to (29.92% and 20.93% respectively) i.e. (33.23% and 23.38% respectively). The results from our research show that in Amo and (B)rz horizon there is a positive correlation between the water retention curves at 0.33; 6.25 and 15 bars and contents of physical clay (clay and silt) and clay. There is also a high negative correlation between the retention curves at 0.33; 6.25 and 15 bars contents of physical sand fraction (coarse sand + fine sand; r = -0.47).

Key words: Soil, water retention, texture, limestones, dolomites.

INTRODUCTION

The soils formed on limestones and dolomites can be found in all mountain regions and regions which are fully or partially composed of limestones. It can be said that these soils are present on almost all continents due to their connection with the parent material. (Kochkin, 1967; Plaster et al. 1971; Bronger. et al. 1981; Bronger. et al. 1983). The hydrous and physical relations. in addition to the mineralogical composition of the soil, are also influenced by the mechanical content. Various researchers studied the content of organic matter (Hillel, 1980; Maclean and Yager, 1972; Jamison and Kroth, 1958; Shaykewich and Zwarich, 1968; Heinonen, 1971) and the influence of organic matter and the mechanical composition over the retention of moisture in several different soils in the USA, Europe and Asia. In the research of Hollist et al. (1977) it is confirmed that the soil moisture retention in Western Midland (Great Britain) depends mainly on the organic matter and mineralogical composition of soil. According to (Warkentin & Meada, 1974, 1975; Rausseaux & Warkentin, 1976), the retention of moisture at different tensions is strongly correlated with the content of humus, clay, silt and the mineralogical composition of the clay.

The hydro physical properties of soils is the water retention and the water permeability in the saturated and unsaturated zone, not only affect the water balance but also have a dominant influence on the conditions of growth and development of plants. They determine the availability of water to plants and leaching of nutrients dissolved to the deeper layers of the soil (Coquet et al. 2005; Hillel. 1998. Kutilek and Nielsen. 1994). The knowledge of the hydrophysical properties of the soil is therefore essential in the interpretation and prediction of changes of the vegetation cover, which occur as a result of a natural succession.

The intensity of the impact of the mechanical composition on the retention of soil moisture depends on the share of certain fractions of soil separates and the percentage. Particles of clay, due to the large inner and outer active surface, high cation exchange capacity (CEC) and mineralogical composition, represent the

most active fraction of the mechanical composition of the soil (Škorić. 1991). In our research, the main emphasis is on the dependence and impact of mechanical composition on the retention of water in the surveyed Rendzic Leptosols, Chromic Luvisol on hard limestones and Terra Rossa in the Republic of Macedonia. Due to the mentioned importance of the mechanical composition and organic matter of the other properties of soil, this paper investigates the impact on retention of soil moisture at different points of tension, ranging from 0.33 up to 15 bars, which correspond to the water constant, which is called permanent wilting point (PWP). The remaining moisture above 15 bars is unavailable to plants (Markoski. 2013).

MATERIAL AND METHODS

Field researches have been carried out for the soils formed on limestones and dolomites, on various locations on the territory of the Republic of Macedonia. Locations for the opening of the pedological profiles (a total of 52, out of which 34) are: Rendzic Leptosols (FAO Classification, 1988), WRB-Rendzic Leptosol (WRB classification, 2006); 13 Chromic Luvisol on hard limestones (FAO Classification, 1988), (WRB-Chromic Leptic Luvisol on hard limestones) (WRB classification 2006), and 5 profiles of Ferric Luvisol on hard limestones - Terra Rossa. WRB-Rhodic Leptic Luvisol on hard limestones (FAO Classification, 1988), (WRB CLASSIFICATION 2006). The field research was carried out standard soil science methodologies. The mechanical composition of the soil is determined according to the international A-method (Singh et al. 1999; Kadam. J. R. et al 2005).

Figure 1. Preparing soil and placing samples on
Bar extractor and Porous plate extractor

The fractioning of the mechanical elements has been performed according to the International Classification; the classification of the soils in texture classes has been performed according to the American triangle (Bormann, 2007; Christopher and Mokhtaruddin, 1996). The determination of moisture retention at a pressure of 0.33 bar (pF-2.54) and 1 bar (pF-3), was performed by a method of applying pressure with a Bar extractor. To determine the retention of soil

moisture at higher pressures, the method of Richards (1982), Porous plate extractor): 2.0 bar (pF-3.3); 6.25 bar (pF-3.90); 11 bar (pF-4.04) and 15 bar (pF-4.2).

An analysis of variance (ANOVA) has been made for samples of various sizes, for all analysed properties in both horizons. The influence of the substrate, the soil type and their interaction on the variability of all examined properties has been determined. The importance of the differences among the mean values of the analysed properties, for each parent material and soil type separately, has been determined with the Tukey test, for level $p<0.05$. All statistical analyses have been made with the software package R.

RESULTS AND DISCUSSION

The mechanical composition of the soils formed on limestones and dolomites varies extensively and depends on the mechanical composition of the residuum from which the mineral part of the soil is composed, on the character of the limestone and the dolomite (the degree of weathering), on the deposition of nearby materials (from the higher fields) and on the degree of erosion (Yaalon and Ganor, 1973; Jackson, et al., 1982; Mizota, et al., 1988; Borg and Banner, 1996; (Durn, et al., 1999; Durn, 2003; Muhs and Budahn, 2008). Chart 1 contains an overview of the parent materials on which Rendzic Leptosols, Chromic Luvisol on hard limestones and Terra Rossa are formed.

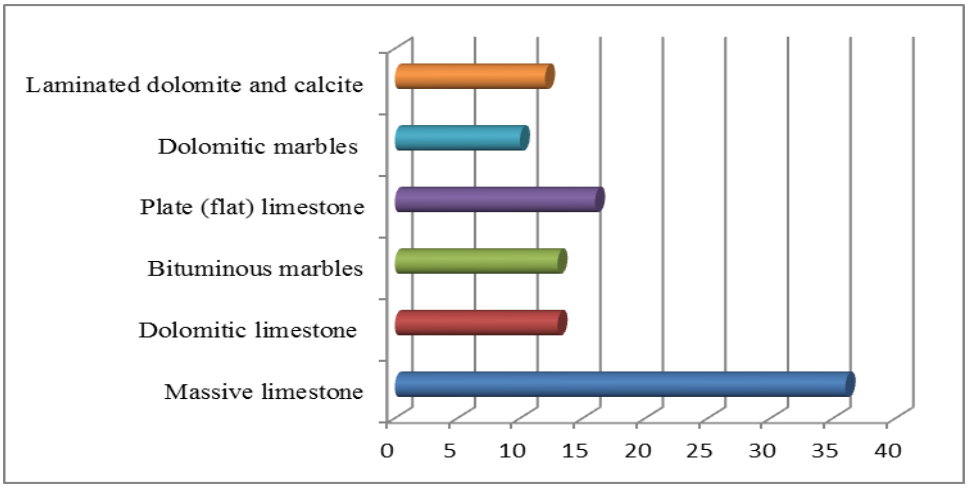

Chart 1. Parent materials on which Rendzic Leptosols. Chromic Luvisol on hard limestones and Ferric Luvisol on hard limestones Terra Rossa are formed

The chart shown that 36% of the soils are formed on massive limestone; 13% on dolomitic limestone and bituminous marbles; 16% on Plate (flat) limestone; 10% on dolomitic marbles; and 12% on laminated (plate) dolomite and calcite. On the basis of the above, it can be confirmed that the soils are formed from horn slate – hornfels facies and their composition has an influence

on the mechanical composition. This facia has a thickness of around 500 – 550 m, it is composed of dolomites, plate (flat) limestone with hornfels and massive limestone, and it represents a continuation in Anisian and Ladinian, and it strongly reflects on the morphology, the physical and physical-mechanical properties, water retention curves, the mineral composition and the chemical properties of the soils.

It also depends on the processes which take place within these soils during their paedogenesis and evolution, and which cause the texture differentiation. In order to have better understanding, hereunder are given the mean values for each fraction of the fine earth of the examined soils, by type, subtype and horizon. Table 1 and Table 2.

The content of particular fraction of the fine earth in Calcomelanosols varies depending on the subtype. The physical sand fraction (coarse sand + fine sand) in the Amo horizon amounts 44.81% at Rendzic Leptosols, haplic, 40.13% at Rendzic Leptosols organogenic and 36.52% at Rendzic Leptosols, chromic luvic. In the (B)rz horizon at the Rendzic Leptosols, chromic luvic it amounts 32.64%.

The content of physical clay fraction (clay + silt) in the Amo horizon amounts 55.19% at the Rendzic Leptosols, haplic 59.87% at the Rendzic Leptosols, organogenic, and the highest content is found at the Rendzic Leptosols, chromic luvic 63.48%. The mean value of this fraction in the horizon (B)rz at the Rendzic Leptosols, chromic luvic amounts 67.36%. In all profiles of Chromic Luvisol on hard limestones, the fine earth predominates over coarse.

In the fine earth, the physical clay fraction in both horizons, Amo and (B)rz is present as twice as more than the physical sand fraction. The average content of the physical sand fraction in the Amo horizon amounts 33.43%, and in the cambic horizon (B)rz 22.50%. At the Terra Rossa, the physical clay fraction is present in a greater percentage in relation to the physical sand fraction. At the same time, the physical clay fraction in the Amo horizon is predominated by the clay fraction, 43.08% in average and 52.13% in the cambic horizon, i.e. 24.90% in Amo and 19.37% for the silt fraction.

The differences in the mechanical composition are confirmed with the descriptive statistics and the analysis of variance made for each type, subtype and horizon. The analysis of variance demonstrated that in both horizons, the soil type has an important influence over the variability of the content of coarse, silt and the total content of clay. In the Amo horizon, the soil type has influence both over the physical sand fraction and the physical clay fraction.

The data on the mechanical composition of the examined soils formed on limestones and dolomites are similar to the data for these soil types presented by other authors: (Maric, 1964; Durn. 2003; Delgado. et al. 2003).

When it comes to the impact of the subsoil (limestone and dolomite) on the mechanical composition of the soil, care should be taken, because the soil is formed only from residual and it has the same mechanical composition in all locations on the same wall.

Table 1. Mean values for the mechanical composition of the soils in the Amo horizon

Soil type	N	Coarse > 2 mm		Coarse sand 0.2 – 2 mm		Fine sand 0.02 – 0.2 mm		Total sand 0.02 - 2 mm		Silt 0.002 – 0.02 mm		Clay < 0.002 mm		Silt + Clay < 0.02 mm	
		X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
1	7	12.20b	3.05	2.83	1.80	37.30	8.07	40.13ab	8.18	37.60b	4.95	22.27a	7.23	59.87ab	8.18
2	22	11.08b	6.43	10.81	14.70	34.00	15.21	44.81b	13.41	22.52a	9.56	32.68ab	9.50	55.19a	13.41
3	5	6.70a	3.42	10.28	8.33	26.24	4.88	36.52ab	6.33	26.88a	3.39	36.60ab	3.89	63.48ab	6.33
4	13	4.82a	3.56	6.18	5.20	27.25	9.27	33.43ab	9.96	28.38a	10.05	38.19b	6.77	66.57ab	9.96
5	5	3.18a	1.02	8.27	5.91	23.75	8.22	32.02a	14.00	24.90a	8.06	43.08b	10.40	67.98b	14.00
P.M	N	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
M.L	19	5.79a	3.30	6.76	6.43	28.41a	8.79	35.17ab	9.43	27.72ab	6.82	37.11	10.89	64.83ab	9.43
D.L	7	6.99ab	5.53	4.93	3.27	23.79a	7.19	28.71a	6.43	37.76b	6.06	33.53	8.20	71.29b	6.43
B.L	7	7.59ab	4.56	4.22	1.86	33.72ab	11.41	37.94ab	11.37	32.86b	8.75	29.20	12.69	62.06ab	11.37
P.L	8	8.48ab	2.88	12.16	4.76	30.19ab	5.09	42.35ab	6.45	23.84ab	4.12	33.81	4.08	57.65ab	6.45
D.M	5	14.52b	4.31	4.49	3.29	49.39b	15.99	53.88b	14.37	15.86a	8.44	30.28	8.79	46.12a	14.37
L.D.C	6	14.79b	9.90	19.76	26.34	30.40ab	18.73	50.16ab	14.36	15.93a	9.38	33.92	10.75	49.84ab	14.36

1. Rendzic Leptosols -organogenic; 2. Rendzic Leptosols, haplic; 3. Rendzic Leptosols, chromic luvic; 4. Chromic Luvisol on hard limestones; 5. Terra Rossa. P.M- Parent material; M.L.-Massive limestone; D.L-dolomitic limestone; B.L-bituminous Limestone; P.L-Plate limestone; D.M. Dolomitic Marbles; L.D.C- laminated (plate) dolomiteand calcite

The author (Markoski, 2013) found at two locations of massive limestone that residual has 17.2 and 30.2% secondary phyllosilicates (clay). In Tables 3 profiles are grouped according to texture classes for each of the parent substrate for both horizons.

For horizon Amo it can be concluded that 6 stem parent materials all have 4 textural classes, 2 of them have 3 classes and 1 has two textural classes. From Table 3 it can be seen that the horizon (B)rz is found in a small number of profiles, in most clay it is in mechanical composition.

(Ćorić. 2009) has an interesting consideration regarding the homogeneity of the textural classes in the formation of Terra Rossa.

He points out that the content of certain categories of particles in the homogenous soils, as Terra Rossa, should be indicated on the autochthony of the texture categories and simultaneously, it is a confirmation that the same has been formed at that place. On the contrary, if these categories are not homogenous, it means that the soils have polygenetic characters, and that the solum has a depositing character.

Water is one of the five environmental factors for normal growth and development of the plant, and it has strong influence on the quality and stability of the crop yield.

The retention of water in the soil is the result of two forces: adhesion (attraction of water molecules by soil particles) and cohesion (water molecules attract each other). Adhesion is much stronger than cohesion.

Table 2. Mean values for the mechanical composition of the soils / horizon (B)rz

Soil type	N		Coarse > 2 mm		Coarse sand 0.2 – 2 mm		Fine sand 0.02 – 0.2 mm		Total sand 0.02 - 2 mm		Silt 0.002 – 0.02 mm		Clay < 0.002 mm		Silt + Clay < 0.02 mm	
			X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
	3	5	4.39b	5.24	10.47	5.57	22.17	4.92	32.64	8.29	26.33ab	6.79	41.03a	3.11	67.36	8.29
	4	14												8.10	77.80	
			1.65a	2.06	5.31	4.27	16.89	5.02	22.20	7.72	31.80b	5.63	46.00ab			7.72
	5	7												8.71	71.50	
			1.42a	0.90	9.05	5.29	19.45	4.44	28.50	9.06	19.37a	5.49	52.13b			9.06
P.M	N	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
M.L	11	1.11a	0.84	8.89	6.63	17.68	5.57	26.56	11.52	20.54a	5.43	52.90	9.08	73.44	11.52		
D.L	4	1.03a	0.67	5.79	1.66	16.66	2.80	22.45	4.18	33.61ab	3.08	43.94	2.64	77.55	4.18		
B.L	4	0.94a	0.71	3.50	1.24	21.28	3.70	24.78	4.88	33.90ab	5.15	41.33	2.05	75.23	4.88		
P.L	5	4.79b	4.96	9.96	2.66	22.10	5.07	32.06	5.37	28.78ab	4.80	39.16	1.41	67.94	5.37		
D.M	1	8.15b	/	1.43	/	15.17	/	16.60	/	35.50ab	/	47.90	/	83.40	/		
L.D.C	1	2.80ab	/	3.94	/	11.56	/	15.50	/	37.10b	/	47.40	/	84.50	/		

3. Rendzic Leptosols, chromic luvic;
4. Chromic Luvisol on hard limestones;
5. Terra Rossa;P.M- Parent material; M.L.-Massive limestone; D.L-dolomitic limestone; B.L- bituminous Limestone; P.L-Plate limestone; D.M. Dolomitic Marbles; L.D.C-laminated (plate) dolomiteand calcite

The force with which water is retained in the soil is called capillary potential and is closely related to water content. Free water in the soil has capillary potential equal to zero, a condition when all the soil pores, capillary and non-capillary, are filled with water.

Earlier we pointed out that the mechanical composition (texture), water retention curves of the soils formed on limestones and dolomites depends both on the material, as well as the soil type i.e. pedogenesis and evolution. The differences in mechanical composition and statistical justification of the above can be best seen from the descriptive statistics and variance analysis made according to types, subtypes, horizons and parent material on which the soils are formed (Table 4) which is also the conclusion from our research.

Soil water potential can be determined indirectly by recourse to measurements of soil water content and soil water release or soil moisture characteristic curves that relate volumetric or gravimetric content to soil water potential. The measurement of water potential is widely accepted as fundamental to quantifying both the water status in various media and the energetics of water movement in the soil-plant-atmospheric continuum (Livingston, 1993; Markoski 2013) pointed out that by reducing the moisture content in the soil, the value of the capillary potential is increasing. For assessment of soil moisture by means of capillary potential, quantified by Schofield (Vucić, 1987), it was suggested pF values, where the force of water in the soil was expressed by the

soil was expressed by the height of the water column in cm (1 bar = 1063 cm water/cm^{-2}). The pF values are affected by the change of the mechanical composition and. according to the same author, the greater the share of the smaller fractions, the greater the pF values, especially at a pressure of 0.33 bars.

Table 3. Texture classes in horizon Amo and (B)rz in relation to parent material

Parent material	Total number of profiles	Loam	Sandy loam	Sandy clay loam	Clayey loam	Sandy clay	Clayey
Horizon Amo							
Massive limestone	19	4	–	3	4	–	8
Dolomitic limestone	7	1	–	–	4	1	1
Bituminous limestone	7	1	1	–	5	–	–
Plate limestone	8	–	–	3	5	–	–
Dolomitic marbles	5	–	1	2	1	1	–
Laminated (plate) dolomiteand calcite	6	–	–	3	1	–	2
Horizon (B)rz							
Massive limestone	9	–	–	–	1	–	8
Dolomitic limestone	4	–	–	–	1	–	3
Bituminous limestone	3	–	–	–	1	–	2
Plate limestone	5	–	–	–	4	–	1
Dolomitic marbles	1	–	–	–	–	–	1
Laminated (plate) dolomite and calcite	1	–	–	–	–	–	1

The degree the mineralogical composition affects the soil moisture retention depends on the percentage amount and fraction of the clay minerals present in a soil type. The clay particles represent the most active part of the fine earth because of their large external and internal active surface, cation-exchange capacity (CEC) and mineralogical composition.

Many authors, such as Barteli and Peters, Salter et al., Petersen et al. (Markoski 2012), state that the increase in the slightly smaller particles in their quantity increases the surface of tangency between the solid phase and soil moisture.

In addition to the mechanical composition explanation presented before, we studied the influence of the moisture retention, given the different levels of tension in soil (starting with 0.1 (pF-2) and going to 15 bar or (pF-4.2), which corresponds to the wilting point). The remaining soil moisture above 15 bars is not available to the plant (Bogdanovic 1973).

According to Filipovski (1996), soil water retention with different tension is in tight correlation with humus, clay and silt content and mineralogical composition of the clay. Maclean and Yager (1972); Jamison and Kroth (1958), Shaykewich and Zwarich (1968) and Heinonen (1971) studied the effect of the organic matter and the mechanical content over the water retention in several soils throughout the U.S. Europe and Asia.

Data for the water capacities of calcocambisols (pF 1; pF 2; pF 2.5; pF 3.5 and pF 4.2) can be found in the researches of González-Pelayo. et al. (2006).

Table 4. Analysis of variances of soil mechanical composition for the surface Amo and cambic horizon (B)rz

Hor.	Factors	Df	Mid-squares (Mean Sq) Coarse > 2 mm	Coarse sand 0.2 – 2 mm	Fine sand 0.02 – 0.2 mm	Total sand 0.02 - 2 mm	Silt 0.002 – 0.02 mm	Clay < 0.002 mm	Silt + Clay < 0.02 mm
Amo	Soil types	4	144.15***	106.49	258.48	355.85*	317.21***	418.90**	355.85*
	Parent material	5	95.56***	196.07	436.95*	478.22**	329.02***	95.24	478.22**
	Soil type x parent material	9	10.48	78.15	18.55	49.29	70.40	33.03	49.29
	error	33	16.85	109.47	130.15	105.43	39.49	75.04	105.43
(B)rz	Soil types	2	16.15***	63.43	54.97	233.05	363.99***	186.94*	233.05
	Parent material	5	19.11***	20.04	49.15	74.76	78.52*	114.39	74.76
	Soil type x parent material	2	23.33***	48.01	9.73	72.56	5.88	55.38	72.56
	error	16	1.72	20.81	17.38	63.95	23.32	41.53	63.95

* Significant level 0.05;
** Significant level 0.01;
*** Significant level 0.001

Table 5. Mean values for retention on the horizon Amo

Soil type	N	0.33 bars X	S.D	1 bars X	S.D	2 bars X	S.D	6.25 bars X	S.D	11 bars X	S.D	15 bars X	S.D
1	7	41.48b	10.04	36.93b	9.80	32.25b	4.94	29.39b	9.16	22.41a	7.09	17.22b	9.02
2	22	32.16a	7.27	28.89a	6.97	26.73a	6.54	23.98a	5.11	20.16a	5.22	18.34a	5.00
3	5	31.23a	6.81	27.74a	6.96	25.47a	6.26	21.70a	5.27	18.25a	4.35	16.60a	4.37
4	13	33.49a	3.57	30.17a	3.49	29.01ab	3.58	25.27ab	3.07	21.05a	2.07	17.06a	3.13
5	5	39.05ab	0.82	35.95b	1.30	32.81b	1.66	29.92b	2.66	25.17b	2.21	20.93ab	1.09
P.M	N	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
M.L	19	37.80	8.27	34.74b	7.94	32.21	7.11	28.28b	6.23	23.73	6.05	21.05	5.60
D.L	7	37.07	5.36	33.74b	5.62	30.36	5.41	25.59ab	4.80	21.52	5.19	18.75	5.55
B.L	7	31.71	5.55	28.55a	4.76	28.44	3.30	23.71a	4.50	19.84	3.86	17.25	4.85
P.L	8	31.95	4.68	28.72a	4.83	27.58	4.89	24.60ab	4.22	21.41	4.60	18.16	4.55
D.M	5	28.30	5.92	25.00a	6.23	23.72	6.43	20.62a	5.45	17.48	4.21	15.37	3.50
L.D.C	6	31.33	6.23	27.19a	4.92	25.63	4.89	22.96a	4.39	18.18	4.94	15.98	4.14

1. Rendzic Leptosols -organogenic; 2. Rendzic Leptosols, haplic; 3. Rendzic Leptosols, chromic luvic; 4. Chromic Luvisol on hard limestones; 5. Terra Rossa
P.M- Parent material; M.L.-Massive limestone; D.L-dolomitic limestone; B.L- bituminous Limestone; P.L-Plate limestone; D.M. Dolomitic Marbles; L.D.C- laminated (plate) dolomiteand calcite

Table 6. Mean values for retention on the horizon (B)rz

Soil type	N	0.33 bars		1 bars		2 bars		6.25 bars		11 bars		15 bars	
		X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
3	5	35.86ab	7.86	32.64a	6.61	31.19a	6.41	27.61a	4.92	23.12a	4.03	19.38ab	2.23
4	14	33.24a	4.41	29.44a	3.83	27.89a	4.07	23.91a	4.08	20.58a	3.60	16.68a	4.69
5	7	40.25b	1.00	38.29b	1.24	36.28b	1.30	33.23b	1.95	28.30b	1.37	23.38b	1.02
P.M	N	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D	X	S.D
M.L	11	38.85b	4.10	36.11	4.48	34.46	4.18	30.59	5.46	26.28	4.62	22.32	3.95
D.L	4	37.52b	8.02	33.72	7.01	32.11	6.99	27.82	5.93	23.67	4.49	18.77	3.46
B.L	4	30.65a	1.87	27.35	1.56	25.73	1.61	22.86	2.75	19.15	1.41	14.67	2.59
P.L	5	31.82a	2.91	28.52	2.35	26.91	2.54	23.83	2.44	20.16	1.50	16.98	3.54
D.M	1	36.98b	/	32.10	/	30.15	/	25.13	/	21.00	/	17.40	/
L.D.C	1	30.20a	/	27.18	/	25.28	/	21.87	/	19.71	/	12.56	/

3. Rendzic Leptosols, chromic luvic; 4. Chromic Luvisol on hard limestones; 5. Terra Rossa;P.M-Parent material; M.L.-Massive limestone; D.L-dolomitic limestone; B.L- bituminous Limestone; P.L-Plate limestone; D.M. Dolomitic Marbles; L.D.C- laminated (plate) dolomiteand calcite

They are dealing with the issue of hydrological constants and the influence of the fires on the retention of Chromic Luvisol on hard limestones around Mediterranean. All profiles of the examined soils were placed on 6 difference pressure regimes (0.33; 1; 2; 6.25; 11; 15 bars). The mean values of moisture, in mass percentages, in Amo and (B)rz horizons are given hereunder in order to have better understanding of the intensity of the moisture retention in the soil types and subtypes. Due to the large content of humus, as well as due to the large content of clay and the high hydrophilicity and the capillary porosity, the soils formed on limestones and dolomites are characterized with high values of water capacities. It can be seen from the Table 5 and 6 that largest retention capacity, of 41.48% in average, under the pressure of 0.33 bars, is found in the humus accumulative horizon (Amo), in the subtype Rendzic Leptosols organogenic. The other subtypes have lower retention in this horizon, i.e. the Rendzic Leptosols, haplic 32.16% in average, and the Rendzic Leptosols, chromic luvic, 31.23% in average, and in the cambic horizon (B)rz at the Rendzic Leptosols, chromic luvic, the retention amounts 35.86% in average.

The average retention values (pressure of 0.33 bars) in the Amo horizon and the cambic horizon (B)rz at Chromic Luvisol on hard limestones amount 33.49%, i.e. 33.24%.

The greater presence of the small pores, the content of humus and clay, also affects the high moisture retention values under pressure of 6.25 bars. The average retention content under pressure of 15 bars in the Amo horizon in (B)rz amounts 17.06%, and 16.68% in average. According to Ćirić (1986) despite the heavy mechanical composition, the good water

permeability of the Chromic Luvisol on hard limestones is due to the favourable structure and the moderate swelling of the soil aggregates. The water infiltration in the soil is slower and it takes more time. The reason for this appearance stems from the higher presence of micro pores within the soil aggregates, and that is why a longer period was necessary to moisturize the soil samples during the analysis of water capacities. This makes the soils dry, especially at a particular constellation of factors (shallow solum, less rainfalls, greater slope field as well as south exposure). Compared with calcocambisols, Terra Rossa has a higher retention capacity in all three tension points (0.33; 6.25. and 15 bars), Chart 3.

The average retention in Terra Rossa under pressure of 0.33 bars in the Amo horizon amounts to 39.05%, and in the (B)rz horizon to 40.25%. Under the pressure of 6.25 bars, the retention in the Amo horizon on average amounts to 29.92%; in the (B)rz horizon to 33.23%, and under pressure of 15 bars in the Amo horizon it amounts to 20.93%; in the (B)rz horizon 23.38% on average. The moisture retention clearly indicates the texture differentiation of the profile: the Amo horizon has the lowest retention and the cambic horizon (B)rz has the highest. Terra Rossa contains high percentage of clay, but unlike in Rendzic Leptosols and Chromic Luvisol on hard limestones, it is joined in stabile structure aggregates under the effect of humus and sesquioxides, due to which the soils show good water permeability, aeration and are warm soils. The Table 7 shows the correlation coefficients between the humus, physical sand and physical clay content and retention in different tension points on both horizons.

Based on the correlation analysis of the Amo horizon, it can be noted that the physical sand has a significant negative correlation with all retention constants ($r = -0.47$), and a significant negative correlation ($r = -0.21$) between the humus and physical sand content has also been noted. From the correlation analysis of the (B)rz horizon it can be noted that the humus has a significant negative correlation with the retention constants ($r= -0.49$), and the physical sand has a small significant negative correlation with retention of 0.33; 6.25 and 11 bars ($r= -0.28$; $r= -0.14$; and $r = -0.04$). Retention curves can be obtained if the tension of soil moisture is constantly measured and for each tension the moisture quantity is measured, expressed in percentage, and if the data obtained are entered in a coordinate system. They reflect the ratio between the attractive forces (tension) and the amount of soil moisture. Matula et al. (2007) emphasize that soil hydraulic characteristics, especially the soil water retention curve, are essential for many agricultural, environmental, and engineering applications. Their measurement is time-consuming and thus costly. The Charts 1, 2 and 3 show retention curves in six different tensions (0.33; 1; 2; 6.25; 11. and 15 bars) for soil testing. From the charts on retention curves of soils formed over limestones and dolomites it can be noted that the soil retention is very high in all three soil types, due to the high content of humus and clay. Chart 1 shows the retention curves for three subtypes of Rendzic Leptosols and it can be noted that the retention curve in the Rendzic Leptosols organogenic subtype is highest which is due to the higher content of humus in this subtype as well as the content

Table 7. Correlation coefficients between the tension points of 0.33; 1; 2; 6.25; 11; 15 bars and humus, physical sand and physical clay content in the Amo and (B)rz horizons

Hor. Amo / Hor. (B)rz	Organic matter	Total sand	Silt+ clay	0.33 bars	1 bars	2 bars	6.25 bars	11 bars	15 bars
Organic matter	1	0.21	-0.21	0.12	0.12	0.18	0.01	0.03	0.16
Total sand	0.01	1	-1	-0.47	-0.46	-0.32	-0.42	-0.41	-0.35
Silt+clay	-0.01	-1	1	0.47	0.46	0.32	0.42	0.41	0.35
0.33 bars	-0.38	-0.28	0.27	1	1	0.90	0.95	0.93	0.89
1 bars	-0.49	0.01	0.15	0.98	1	0.91	0.95	0.93	0.89
2 bars	-0.39	-0.14	0.14	0.97	0.99	1	0.88	0.88	0.84
6.25 bars	-0.44	0.06	0.06	0.90	0.97	0.96	1	0.99	0.93
11 bars	-0.44	-0.04	0.04	0.89	0.96	0.96	0.99	1	0.95
15 bars	-0.39	0.19	0.19	0.73	0.84	0.87	0.88	0.87	1

*The correlations in the upper part of the table refer to the Amo horizon, and in the lower part refer to the (B)rz horizon.

of physical clay. The retention curves in Rendzic Leptosols, haplic and Rendzic Leptosols, chromic luvic is almost parallel in all points of tension. All three curves are almost horizontal with a small decrease in brownised calcomelanosols in tension of 15 bars.

Chart 2 shows the average values of retention curves in Chromic Luvisol on hard limestones. It can be seen that Chromic Luvisol on hard limestones have high retention in all points of tension and do not have a big slope of soil moisture. Also, it can be noted that retention curves with tension of 11 and 15 bars are almost identical. The small difference between retention curves is the result of combined effect from clay, humus and mineral composition of clay.

In the Terra Rossa (Chart 3) retention curved are high, which means that there is high moisture retention (high content of clay, humus). The mineral composition of clay, according to depth of profile, is the same which means that the retention depends on the clay content: the differences in retention between different horizons follow the clay content.

The fall of retention curves from lower to higher tensions is small, which means that a good portion of the water is not held by a great force by the soil particles and is more easily freed from them. The texture differentiation is clearly reflected in the moisture retention: the Amo horizon has the lowest retention, which contains less physical clay, and the cambic horizon (B)rz has higher retention since it contains more physical clay. From the soil retention curves formed upon limestones and dolomites a gradual change in retention forces is noted with the change of moisture without jumps.

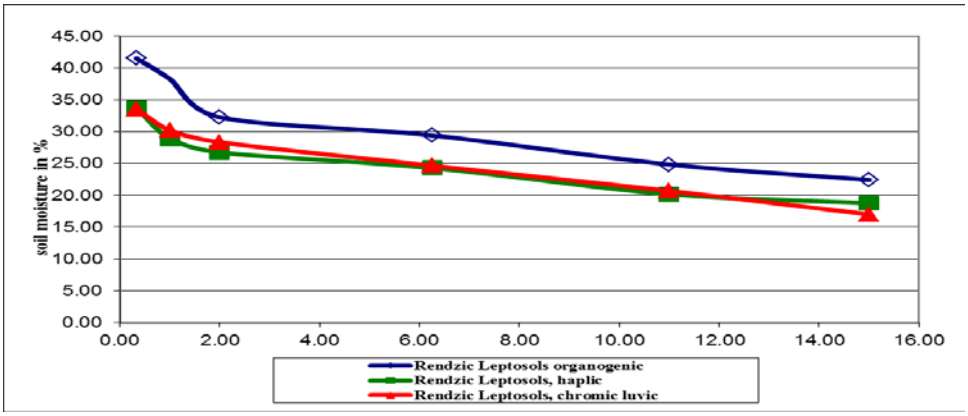

Chart 1. Retention curves of soil moisture in subtypes of Rendzic Leptosols in the Amo horizon

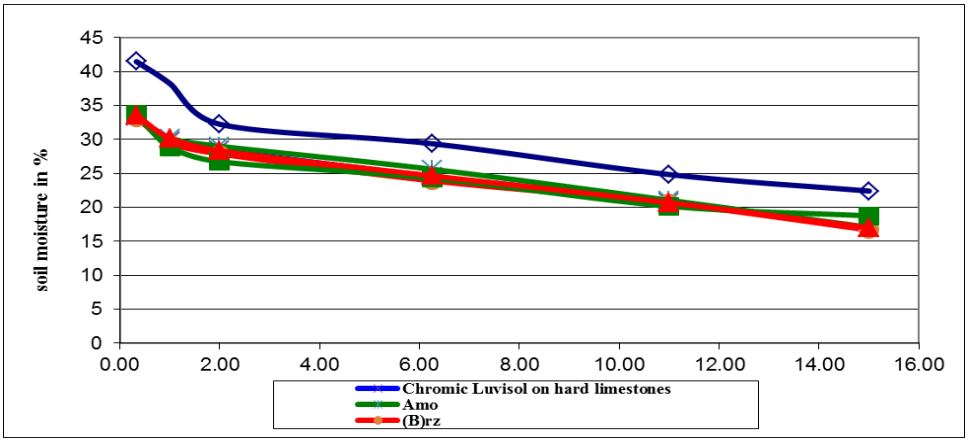

Chart 2. Retention curves of soil moisture in Chromic Luvisol on hard limestones

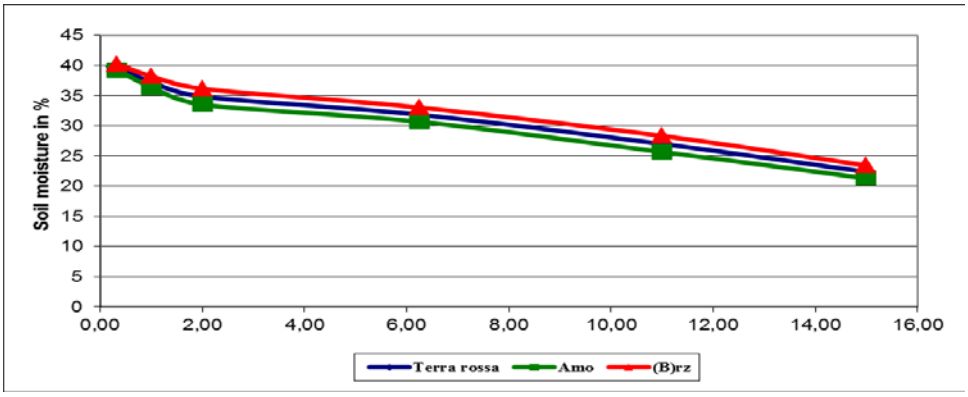

Chart 3. Retention curves of the soil moisture in Terra Rossa

Table 8. Analysis of variances of retention curves of soil moisture for the surface Amo and cambic horizon (B)rz

Hor.	Factors	Df	Mid-squares (Mean Sq)					
			0.33 bars	1 bars	2 bars	6.25 bars	11 bars	15 bars
Amo	Soil types	4	157.48**	162.39***	130.58***	81.68***	59.64*	45.26*
	Parent material	5	56.43	64.01*	38.48	35.32*	31.56	30.49
	Soil type x parent material	9	89.65**	90.80**	74.79**	76.69***	63.66**	61.62**
	error	33	29.31	23.72	19.64	13.26	16.38	15.28
(B)rz	Soil types	2	114.91***	183.15***	164.67***	203.54***	138.85***	105.19**
	Parent material	5	29.05*	20.51	22.57	9.18	8.72	16.08
	Soil type x parent material	2	110.98***	74.44**	73.42**	35.09	24.03	17.20
	error	16	8.71	7.68	8.10	13.75	9.53	12.36

This indicates that the division of soil moisture into various forms is not justified in the retention curve, since the reduction of the water quantity does not have great jumps in different tensions.

The impact of subsoil and soil type on the retention of soil moisture at all points of tension can be seen from the analysis of variance Table 8. It showed

that soil type has a significant impact on the variability of the retention of moisture and at all points of tension and in both horizon. Subsoil similar soil type has a significant impact on retention in Amo horizon and at the points of tension of 1 bar, 6.25 bars, and only subsoil has a significant influence on the horizon (B)rz on the retention of soil moisture at a pressure of 0.33 bar.

CONCLUSIONS

The variance analysis has shown that in both horizons, the soil type has significant effect over the variability of the coarse, silt and total clay content. In the humus accumulative horizon Amo, the soil type also has effect over the physical sand fraction and the physical clay fraction. The parent material in both horizons affects the coarse and silt content variability, and in the Amo horizon it also affects the physical sand and physical clay content. In relation to the effect of the material (Table 4) it can be noted that in the Amo horizon there is the least coarse in the soils formed upon massive limestone (5.79%), value that statistically significantly differs from the soils formed upon dolomitic marbles and laminated (plate) dolomite and calcite (14.52 and 14.79%).

The effect of the remaining substrates does not significantly deviate in relation to the lowest and highest values on the content of this fraction. In the cambic horizon (B)rz, the lowest coarse content is present in massive limestone (1.11%), in dolomitic limestone (1.03%) and in bituminous limestone (0.94%) which significantly statistically differ from dolomitic marbles (8.15%) and plate limestone (4.79%).

There is no statistically significant difference in the effect of the substrates for the coarse sand fraction, but there is a difference for the fine sand fraction. This fraction is the least present in the Amo horizon in soils formed upon massive limestone 28.41% and dolomitic limestone 23.79% compared to dolomitic marbles 49.39%. Unlike the Amo horizon, in the cambic horizon (B)rz there is no statistically significant difference for this fraction. The physical sand fraction has the lowest presence in the Amo horizon in soils formed upon dolomitic limestone (28.71%), which statistically significantly differs from those formed upon dolomitic marbles which have the highest value for this fraction (53.88%). Similarly as for the previous fraction, the physical sand fraction in the cambic horizon (B)rz also has no significant difference in relation to the substrates on which the soils have formed.

For the physical clay fraction (silt + clay) there is only a statistically significant difference in the silt fraction in both horizons. In the Amo horizon, there is the smallest and approximate value in soils formed on dolomitic marbles (15.86%) and laminated (plate) dolomite and calcite (15.93%) and there is the highest average value in soils formed on bituminous limestone (32.86%) and dolomitic limestone (37.76%). The physical clay fraction has the lowest average value in soils formed upon dolomitic marbles (46.12%) and the highest average value in soils formed upon dolomitic limestone (71.29%). A lower statistically significant value is noted in soils formed upon massive limestone (64.83%) and

bituminous limestone (62.06%). The is no statistically significant difference in the cambic horizon (B)rz for this fraction.

Texture of the tested soil is characterized by the predomination of the physical clay and clay separates in the fine earth that strongly influences the soil moisture retention curves.

The amount of moisture that is retained in the soil at the pressure of 0.33 bars is high in all horizons.

The data that were obtained for a wilting point (pressure of 15 bars) are high in all horizons for all the tested sample soil. This is the result of a high content of clay in soil separates.

The results from our research show that in Amo and (B)rz horizon there is a positive correlation between the water retention curves at 0.33; 6.25 and 15 bars and contents of physical clay (clay and silt) and clay. Also there is high negative correlation between the retention curves at 0.33; 6.25 and 15 bars contents of physical sand fraction (coares+fine sand), r = -0.47.

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DOI: 10.17707/AgricultForest.62.4.21

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REMOTE SENSING BASED VEGETATION INDICES FOR ESTIMATING ABOVE GROUND CARBON SEQUESTRATION IN ORCHARDS

SUMMARY

This study aims to apply remote sensing technology to estimate above ground carbon sequestration of orchards with vegetation indices into two forms: 1) Normalized Difference Vegetation Index (NDVI) and 2) Transformed Normalized Difference Vegetation Index (TNDVI). It also aims to explore field data by using the data from a satellite called Landsat 7 ETM+ Path 127 Row 48 that was recorded on 30 January 2015 to adjust the Top of Atmosphere (ToA) reflectance and then determine the percentage of fractional cover, and build the relationship equation between satellite data from Landsat 7 ETM+ and field data collection. The results from NDVI showed the equation $y = 0.2836e^{0.0373x}$ with coefficient of $R^2 = 0.872$. As a result, the calculated amount of above ground carbon was 255.712 tCO_2/rai. Meanwhile, the results from TNDVI showed relationship equation $y = 0.2261e^{0.0388x}$ with the coefficient of $R^2 = 0.877$. As a result, the calculated amount of above ground carbon was 255.400 tCO_2/rai

Keywords: Remote sensing, Vegetation indices, Carbon sequestration, Orchards

INTRODUCTION

Nowadays, the current development of the economy and industry consumes too much natural resources beyond the natural balance, which is a major factor that causes climate change. As a result, the world has faced more natural disasters, which threaten the lives and well-being of mankind. The dramatic increase in the average temperature of the earth's surface is also called “global warming”, which is a part of the world’s climate change caused by various human activities that release greenhouse gases, particularly carbon dioxide, the highest amount of greenhouse gas in the atmosphere. Burning fossil fuels and forestry activities are another activity that has a major role to the change of carbon amount in the atmosphere (Wasun et al., 2010). One of the major reasons that cause climate change is the change of land usage, especially the deforestation. The study found that there is 20 percent of the issue caused by

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

losing carbon that is stored in the wood (Usa et al., 2011). The reason is that forests can absorb and store CO_2 by sequestrating carbon as biomass in various parts of plants, including stem, branches, leaves, and roots. The potential for CO_2 sequestration would depend on the ecological factors including type of forest, forest density, topography and environmental factors. In the assessment, the above ground carbon sequestration of forests is extremely important (Ogawa et al., 1965; Senpaseuth et al., 2009).

Remote sensing with the use of satellite data is considered as a modern technology. It has been used as data to monitor the changes of natural and manmade events in time. Furthermore, it can also be used together with the Geographic Information System effectively (Gomasathit et al., 2011; Laosuwan, et al., 2011; Odindi, et al., 2015). Currently, the remote sensing technology has been developed to help sequestrating above ground carbon because the satellite data can record the reflection of multi-spectral electromagnetic waves, resulting in the ability to estimate the amount of above ground carbon sequestration in the forests quickly and easily through the features of the recorded electromagnetic waves (Lu, et al., 2002; Laosuwan, et al., 2011; Patel, et al., 2007; Schlerf and Alzberger, 2005; Samaniego and Schulz, 2009; Senpaseuth, et al., 2009; Teerawong and Pornchai, 2014). For this study, the purpose is to apply the remote sensing to estimate above ground carbon sequestration of the orchards in Sang Kho sub district, Phu Phan district, Sakon Nakhon Province in northeast Thailand.

MATERIAL AND METHODS

A total orchard areas of 14 local orchard farmers which is 70.10 rai (6.25 rai = 1 hectare) Sang Kho sub district, Phu Phan district, Sakon Nakhon Province in northeast Thailand (Figure 1) has been chosen as the study area for this research.

Figure 1. The study area

This study used data from the satellite named Landsat 7 ETM+ (Table 1) Path 127 Row 48 with recorded data on 30 January 2015 by the satellite data can be downloaded from The US Geological Survey (USGS) through the website http://glovis.usgs.gov/.

Table 1. Landsat 7 ETM+

Band	Wavelength	Characteristics
Band 1	0.450-0.515 µm (Blue-Green)	Ground/plant different, coastal zones
Band 2	0.525-0.605 µm (Green)	Vegetation
Band 3	0.630-0.690 µm (Red)	Differentiate plant species
Band 4	0.775-0.900 µm (Near infrared)	Biomass
Band 5	1.550 - 1.750 µm (Shortwave IR)	Snow/cloud differentiation
Band 6	10.40 - 12.50 µm (Thermal IR)	Thermal
Band 7	2.090 - 2.350 µm (Reflective IR)	Lithology
Band 8	0.520-0.900 µm (Far IR)	Panchromatic

The data preparation before analysis was done by adjusting the Top of Atmosphere (ToA) Reflectance of the satellite data from Landsat 7 ETM+. To ensure the accuracy of information, the preparation was done in two processes, including 1) The process of making convert digital number to radiance values and 2) The process of making convert radiance to ToA reflectance. Also, for the implementation of the two processes, the researchers adopted equation 1 and equation 2 as follows (Senpaseuth, et al., 2009; Usa et al., 2011; Teerawong and Pornchai, 2014).

$$L_\lambda = Grescale * Qcal + Brescale \quad (1)$$

Where;

$$Grescale = \frac{LMAX_\lambda - LMIN_\lambda}{Qcalmax - Qcalmin}$$

$$Brescale = LMIN_\lambda - [LMAX_\lambda - LMIN_\lambda / Qcalmax - Qcalmin] \times Qcalmin$$

Where;

L_λ = Spectral radiance at the sensor's aperture [W/(m^2 sr µm)]

$Qcal$ = Quantized calibrated pixel value [DN]

$Qcalmin$ = Minimum quantized calibrated pixel value corresponding $LMIN_\lambda$

$Qcalmax$ = Maximum quantized calibrated pixel value corresponding $LMAX_\lambda$

$LMIN_\lambda$ = Spectral at sensor radiance that is scaled to $Qcalmin$ [W/(m^{2}sr µm)]

$LMAX_\lambda$ = Spectral at sensor radiance that is scaled to $Qcalmax$ [W/(m^{2}sr µm)]

$Grescale$ = Band specific rescaling gain factor [(W/(m^2 sr µm))/DN]

$Brescale$ = Band specific rescaling bias factor [W/(m^2 sr µm)]

$$\rho_\lambda = \frac{\pi \times L_\lambda \times d^2}{E_{SUN_\lambda} \times \cos\theta_s} \quad (2)$$

Where;

ρ_λ = Unitless planetary reflectance

L_λ = Spectral radiance at sensor's aperture ($Wm^{-2}\,sr^{-1}\mu m^{-1}$)

d = Earth-sun distance in astronomical units

E_{SUN_λ} = Mean solar exoatmospheric irradiances

θ_s = Solar zenith angle

Normalized Difference Vegetation Index (NDVI) is calculated from the ratio between the difference and the sum of the reflection of visible red light and near Infrared objects on the Earth's surface. The results of the calculation are index values between -1 and +1, ground water with NDVI values less than 0, open ground with NDVI values between 0 to 0.1, and plant covered area with NDVI values over 0.1 (Kogan and Sullivan, 1993; Kogan, 1995; Dipanwita et al., 2015). The researchers adopted the equation 3 in order to analyze the NDVI values in this study.

$$NDVI = (NIR - RED)/(NIR + RED) \quad (3)$$

Where;

NIR = Near Infrared Band

RED = Red band

Transformed Normalized Difference Vegetation Index (TNDVI) is measure index of the amount of green biomass and composition of chlorophyll in plants. The value of TNDVI was added by 0.5 to avoid the negative value together with applying the square root with the value (Solaimani et al., 2011; Farooq, 2012; Malini and Somashekar, 2013). The researchers adopted the equation 4 to analyze the TNDVI value in this study.

$$TNDVI = \left((NIR - Red)/(NIR + Red) + 0.5\right) \quad (4)$$

Where;

NIR = Near Infrared Band

RED = Red band

The finding the coefficient of fractional cover: In this process, the researchers would find the coefficient of fractional cover of NDVI and TNDVI by applying equation 5 (Teerawong and Pornchai, 2014; Teerawong and Pornchai, 2016).

$$FC = \frac{(VI - VI_{open})}{(VI_{canopy} - VI_{open})} \times 100 \quad (5)$$

Where;

FC = Tree canopy fractional cover

VI = Vegetation index

VI_{open} = Vegetation index of open areas

VI_{canopy} = Vegetation index of tree canopy

The finding the correlation of statistical data: In this process, the researchers would find the relationship between the amount of above ground carbon sequestration (the dependent variable) and the vegetation indices by using the pixel at the same position with sample plot as shown in Figure 2 (22 sample plots with the size of 20 x 20 meters scattered around the study area). The collection of field data by analyzing correlated coefficient value was to explore the relationship between independent variables and the dependent variables in order to decide the appropriate independent variable for forming the equation of the above ground carbon sequestration estimation.

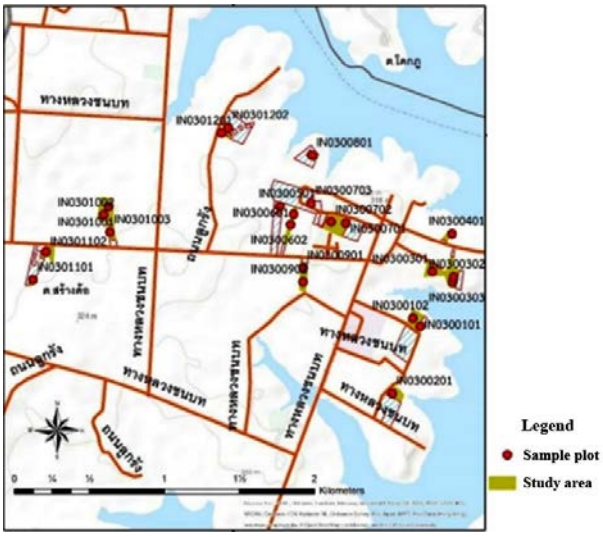

Figure 2. The 22 sample plots with the size of 20 x 20 meters Figure 2. Sampling quadrate of 0.25 m^2 (0.5 m × 0.5 m).

RESULTS AND DISCUSSION

In this study, the results of satellite data from Landsat 7 ETM+ through the adjustment process (ToA) can be presented in Figure 3 and Figure 4.

The analyzing of data from the satellite landsat 7 etm+ was done in two forms, including 1) normalized difference vegetation index (ndvi) and 2) transformed normalized difference vegetation index (tndvi). The coefficient of fractional cover was found by the satellite data from landsat 7 etm+ through the process of normalized difference vegetation index ndvi) and transformed normalized difference vegetation index (tndvi).

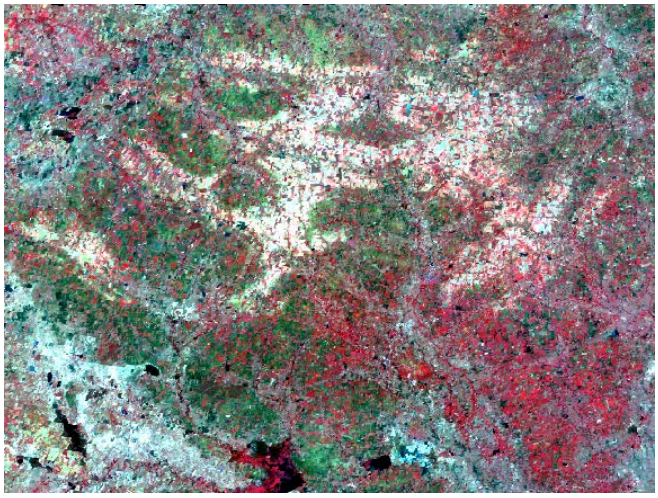

Figure 3. Before ToA

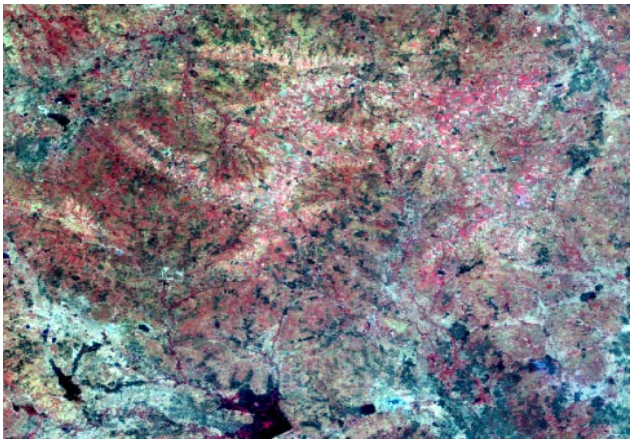

Figure 4. After ToA

To find the relationship of statistical data, the researchers analyzed the satellite data from Landsat 7 ETM+ to find the relationship with the field data in order to form the equation for the above ground carbon sequestration estimation. The results of the study (Figure 5 and Figure 6) from NDVI showed the

relationship equation of $y = 0.2836e^{0.0373x}$ with the defined coefficient of $R^2 = 0.872$. As a result, the estimation of above ground carbon volume was 255.712 tCO_2/rai. On the other hand, the results from TNDVI showed the relationship equation of $y = 0.2261e^{0.0388x}$ with the defined coefficient of $R^2 = 0.877$. As a result, the estimation of above ground carbon volume was 255.400 tCO_2/rai.

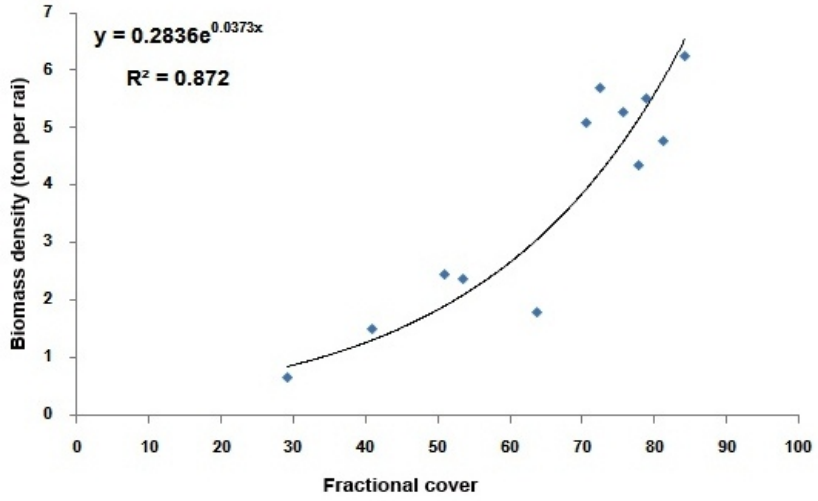

Figure 5. The relationship of statistical data of NDVI

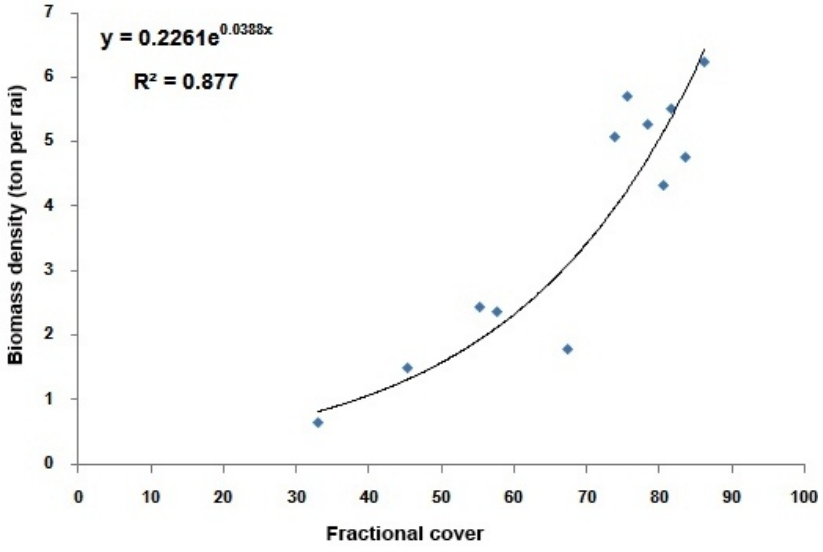

Figure 6. The relationship of statistical data of TNDVI

CONCLUSIONS

This was a tree size used to calculate biomass and carbon sequestration from the field survey in order to identify the relation of the equations. Considering the coefficient of determination, the TNDVI was the most efficient way. The study also found that the result was in the same direction with other research such as Carbon Stock Assessment Using Remote Sensing and Forest Inventory Data in Savannakhet, Lao PDR (Phutchard et al., 2010), Estimating Tree Biomass via Remote Sensing, MSAVI 2, and Fractional Cover Model (Teerawong and Pornchai, 2014) and Mapping Global Forest Above ground Biomass with Spaceborne LiDAR, Optical Imagery, and Forest Inventory Data (Tianyu et al., 2016). In addition, a statistical significance of the above ground carbon sequestration collected from the field survey and analyzed by Landsat 7 ETM+ via Pair Sample T-test of both equations (NDVI, TDVI) was tested. It was found that the two equations yielded the reliability level at 95%. The results can be applied to estimate the above ground carbon sequestration of the orchards in Sang Kho sub district, Phu Phan district, Sakon Nakhon Province in northeast Thailand without having field survey for the entire area. This can reduce cost and time in doing research and obtain up-to-date information that serves urgent needs.

ACKNOWLEDGEMENTS

This research was financially supported by faculty of science, Mahasarakham University. The author would like to thank Miss. Kusuma Arsasana and Miss. Siritorn Dumrongsukit for filed data collection.

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DOI: 10.17707/AgricultForest.62.4.22

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SOME FEATURES OF *Pinus pumila* (Pall.) Reg. AND *Pinus armandii* Franch. SEED FORMATION IN THE CONDITIONS OF INTRODUCTION

SUMMARY

Female cone structure in two species - *Pinus pumila* (Pall.) Reg. and *Pinus armandii* Franch. in the conditions of introduction are presented in this paper. It has been demonstrated that number of scales and fertile/sterile scales index are similar to those in their native areas. At the beginning of the second year development (February) female gametophytes in both species were at free-nuclear stage and only in some *P. armandii* ovules alveolar stage was noticed. It was found out that the main reason for ovules` abortion is lack of pollination. However, ovules` abortion was also noticed at the second year and its reasons need to be studied in details.

Keywords: *Pinus pumila, Pinus armandii,* female cones, fertile scales, ovules, seeds

INTRODUCTION

Pinus L. genus is the largest in the family Pinaceae and includes more than 100 species. They are the most common coniferous tree worldwide and native to all continents and some oceanic islands of the northern hemisphere, chiefly in boreal, temperate, or mountainous tropical regions. In their native areas some species form large forests and used as timber source. From the other hand pines is a very important part of the world ecology. *Pinus* species are introduced all over the world as ornamental and timber trees. In this sphere nut producing pines are of the greatest interest as they are not only good ornamentals but could also provide highly nutritious nuts and some other products.

Growing plants outside their natural range needs some special studies for understanding their adaptive potential and reaction to the new growth conditions. One of the important parameters for evaluation of species' adaptability to the new growing conditions is their reproductive success. But it should be considered that reproductive cycle of most coniferous, and also in some *Pinus* species, is prolonged in time and influenced by number of factors.

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Pinus pumila (Pall.) Reg. and *Pinus armandii* Franch. belong to the group commonly known as "white pines" or "soft pines" and are native to the East Asia. Three variations of *P. armandii* have been recognized within its native area (Wang and Hong, 2004) and some of them have the status of "endanged" taxons. Number of reports describes their genetics and relationships and only some works have studied reproductive cycle in this pine species (Zhang et al., 1992, Wang et al. 2007). There are some reports about reproductive cycle of *P. pumila* in its native area connected with the problem of interspecific hybridization between *P. pumila* and *Pinus sibirica* Du Tour. (Goroshkevich et al., 2008, Vasilyeva, Goroshkevich, 2013). Our investigations were aimed to the understanding the reasons of poor seed production in two introduced pine species - *P. armandii* and *P. pumila*.

MATERIAL AND METHODS

Pinus pumila (Pall.) Reg.

In its native areas is shrub or small tree 2-8 m tall, usually multi-stemmed with creeping branches and gray-brown bark. Needles 5 per bundle, trapeziform in cross section. Seed cones are erect, maturing to pale purple or red-brown, conical-ovoid or ovoid, 3-4.5 × 2.5-3 cm, indehiscent or imperfectly dehiscent at maturity. Seed scales broadly subrhombic or rhombic-obovate. Mature seeds are dark brown, triangular-obovoid; 7-10 × 5-7 mm, wingless, abaxial margin is ridged.

It is distributed in Japan, North Korea, North Mongolia, Eastern Siberia from Yenisey River, some mountain regions of China (at 1000-2300 m elevation) - Heilongjiang, Jilin, Nei Mongol (Flora of China, 2016).

Climatic conditions of its native growth regions.

Winters are long with an average of −31 to −15 °C in January, and summers are short and warm to very warm with an average of 18 to 23 °C in July. The annual average rainfall is 400 to 700 millimetres, concentrated heavily in summer.

We studied *Pinus pumila* plants grown in the O.V.Fomin Botanical Garden (Kyiv) and in the dendropark "Vysokogirny" (Highland) (Gorgany mountain range).

There are three plants of *Pinus pumila* are in the O.V.Fomin Botanical Garden (Kyiv). Two of them were planted in 1985 and the third - in 1982. Each tree has 5–7 trunks, diameter 2-10 cm. The height is 2.5 – 3 m. The start of vegetation varies from 8th to 15th April in different years. Male and female cones annually form on the plants 1985 year planted. In 2013 these trees produced 82 and 86 female cones per tree. Viability of pollen was 71 – 88% in the different years.

In the dendropark "Vysokogirny" *Pinus pumila* plants were brought from different natural habitats and planted in 1970. The start of vegetation was noticed at the end of May. Male and female cones formed annually.

***Pinus armandii* Franch.**

In its native areas is trees to 35 m tall with gray bark fissured into square plates or shed. Crown is conical or cylindric-pyramidal; branchlets are green, gray-green or brown, glaucous. Needles are 5(-7) per bundle, triangular in cross section. Pollen cones are erect or drooping, slender or stout, cylindric or ovoid-ellipsoid. Seed cones pedunculate (peduncle 2-3 cm), green, maturing yellow or brown-yellow, conical-cylindric, dehiscent at maturity, shedding seeds. Seed scales are rhombic-obovate. Seeds are yellow-brown, dark brown, or black, obovoid, 1-1.5 cm × 6-10 mm, wingless or abaxial margin ridged, rarely shortly winged.

It is distributed in China - South Gansu, Central and North-West Guizhou, Hainan, South-West Henan, West Hubei, South Shaanxi, South Shanxi, Sichuan, South-East Xizang, Yunnan at 900-3,500 m; North and Central Taiwan at altitudes of 2,300-3,000 m; North Myanmar.

Climatic conditions of its native growth regions.

Summers are warm to hot and winters are cold to very cold with so large diurnal temperature ranges that maxima often remain above 0°C even in winter. The average temperature is roughly 10 to 20°C, with January temperatures ranging from 1 to 10°C and July temperatures ranging from 17 to 28°C. The highest temperature can reach 41°C, while the lowest temperature can drop to -14.9°C. Total precipitation is between 500 and 1,000 mm, falling mostly between May and October.

In the O.V.Fomin Botanical Garden *Pinus armandii* tree was planted in 1986. Its height is about 6 m, trunk diameter 21 cm. Male and female cons form annually. The start of vegetation was noticed from 9th to 18th April in different years. Pollen viability was 69 - 87%.

Climate of the introduction region.

Kyiv has a humid continental climate with severe winters, no dry season, warm summers and strong seasonality (Köppen-Geiger classification: Dfb). The warmest months are June, July, and August, with mean temperatures of 13.8 to 24.8°C. The coldest are December, January, and February, with mean temperatures of −4.6 to −1.1°C. Snow cover usually lies from mid-November to the end of March, with the frost-free period lasting 180 days on average, but surpassing 200 days in recent years. Total annual precipitation averages 649 mm.

"Vysokogirny" dendropark is located in the zone with minimum air temperature -35.2 °C, maximum + 30.1°C and mean year temperature +4.5 °C. Total annual precipitation averages 1000 mm.

Embryological Methods

P. armandii female cones pollinated in spring 2013 were collected in the plots of O.V. Fomin Botanic Garden in late March 2014 and *P. pumila* female

cones – in the plots of O.V.Fomin Botanic Garden and in the dendropark "Vysokogirny" in late March 2014.

Bracts and ovules were separated and fixed in Karnua solution (96% ethanol : chlorophorm : acetic acid, 6:3:1) and stored in 70% ethanol. Materials for histological studies were dehydrated, infiltrated and embedded in paraffin (Pausheva, 1990). Sections 10-12μm thick was obtained using the rotary microtome. They were mounted and stained by methyl-green and pyronin with alcian blue (Shevchenko et al., 1986; Sklonnaya, 1992). Analyses were carried out with light microscope Jenaval (Carl Zeiss), measurements and microphotograpes were made under the light microscop Axio Scope A.1 (Carl Zeiss) with analyses system Axio Cam ERc5s by the programme Axio Vision rel. 4.8.2.

RESULTS AND DISCUSSION

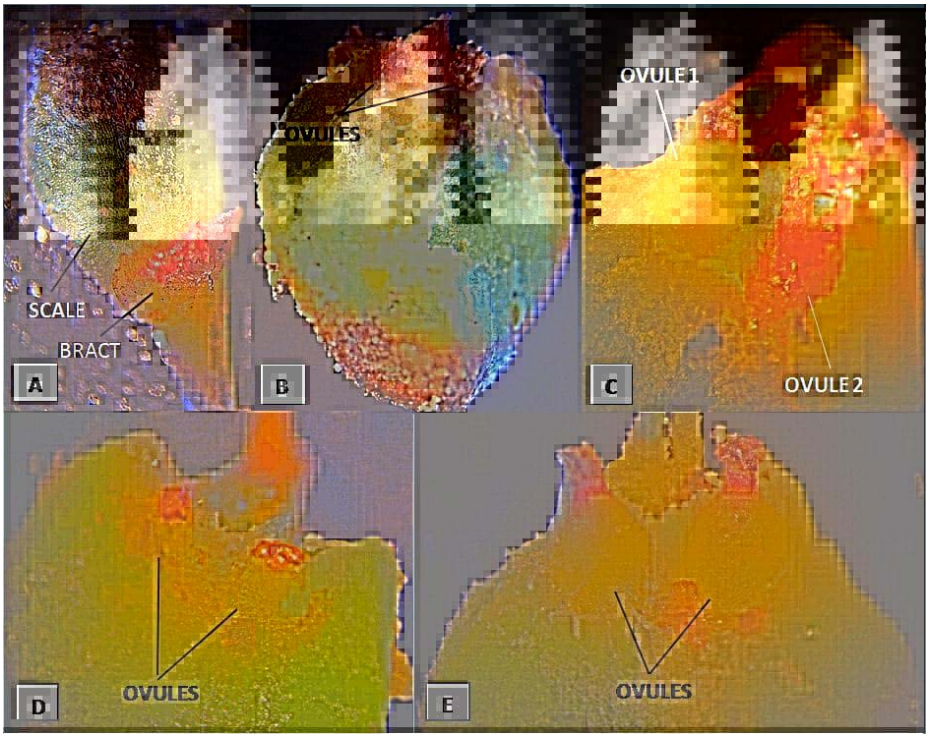
The period between pollination and seed maturity in *P. pumila* and *P. armandii* takes for about 1.5 year. In native growth regions pollination in *P. armandii* occurs in Aprile-May (Flora of China, 2016) and *P. pumila* (growing in the eastern part of West Siberia, 55°47' с.ш., 109°33' в.д.) it occurs in early June (Vasilyeva et al., 2010) while in Kyiv pollination in *P. armandii* occurs in 20-31 May and in *P. pumila* 8-20 May while in Gorgany mountains - in mid-June.After pollination both male and female gametophytes develop inside the ovules and it has been noticed for pine species (*Pinus albicaulis* Eng., *Pinus contorta* Doug.) the early stage of female gametophyte development occur even in nonpollinated ovules (Owens, 2006, Owens et al. 2008). We studied female cones somewhere the middle between pollination and mature seeds. It should be noticed that *P. pumila* female cones collected in Kyiv and in Gorgany were almost at the same development stage. At that time *P. armandii* cones were 21x11 mm (length x diameter) and consisted of the central axis and 60-67 tightly closed cone scales and *P. pumila* cones were 17x8 mm (length x diameter) in Kiiv and 18x10 mm in Gorgany and consisted of the central axis and 43-47 tightly closed cone scales (Table1). Our data for total scales number in *P. pumila* cones corresponds to those in native growth conditions (eastern part of West Siberia) as it has been recorded 42±6 (min 30, max 50) per cone (Vasilieva and Goroshkevich, 2013).

Table 1. *Pinus pumila* and *Pinus armandii* cone analyses

Species	Cone length (mm)	Cone diameter (mm)	Total scales	Sterile scales*	Fertile scales			
					Total	two ovules degenerate	One ovule degenerates + one develops	Two ovules develop
Pinus pumila (Kyiv)	17.5±0.5	8.0	44.5±1.5	12.5±1.5	32.0	5±1	9.5±2.5	8±2
Pinus pumila (Gorgany)	18±1	10.0	47.0	16±1	31±1	3±1	11±1	8±1
Pinus armandii	21±0.33	11±0.57	62±2.33	13±0.33	49±1.85	12±1.54	15±4.25	22±5.03

Note: data presented as mean ± SEM

*- sterile scales include both scales without ovules and scales with rudimentary ovules



Figue 1. Scales in female cone of *Pinus pumila*: A - complex of not fused bract and ovuliferous scale (scale), B – two nonpollinated ovules, C – two pollinated ovules: 1-normally develops, 2 – degenerates, D - two pollinated ovules that stopped their development soon after pollination,
E - two successfully developed ovule

Cones of both species included fertile and sterile scales located only at the bottom of cones (we don`t mind 2-3 fused scales which formed the tip of cones). It has been previously reported that the proportion of sterile and fertile regions of the cones varies among clones of the same species and varies considerably among species of pine (Owens, 2006). Thus in *Pinus albicaulis* Engl. sterile scales present at the top and at the bottom of cones (Owens et al., 2008). In the studied species sterile scales are of two types – scales without ovules which are mostly located at the base of the cone (5-8 per cone in *P. pumila* (Kyiv) and 8-10 (Gorgany) and 5-6 – *P. armandii*) and scales with rudimentary ovules (Fig. 2 A) that formed the region between sterile and fertile scales in the cones of both species (3-9 per cone of *P. pumila* and 6-8 – *P. armandii*). Those rudimentary ovules had small micropylar arms, rudimentary nucellus, and the megaspore development did not occur.Besides, in the upper part of some *P. pumila* cones (just near the tip) complexes of not fused bracts and ovuliferous scales (scale) with rudimentary ovules were noticed (Fig. 1 A).

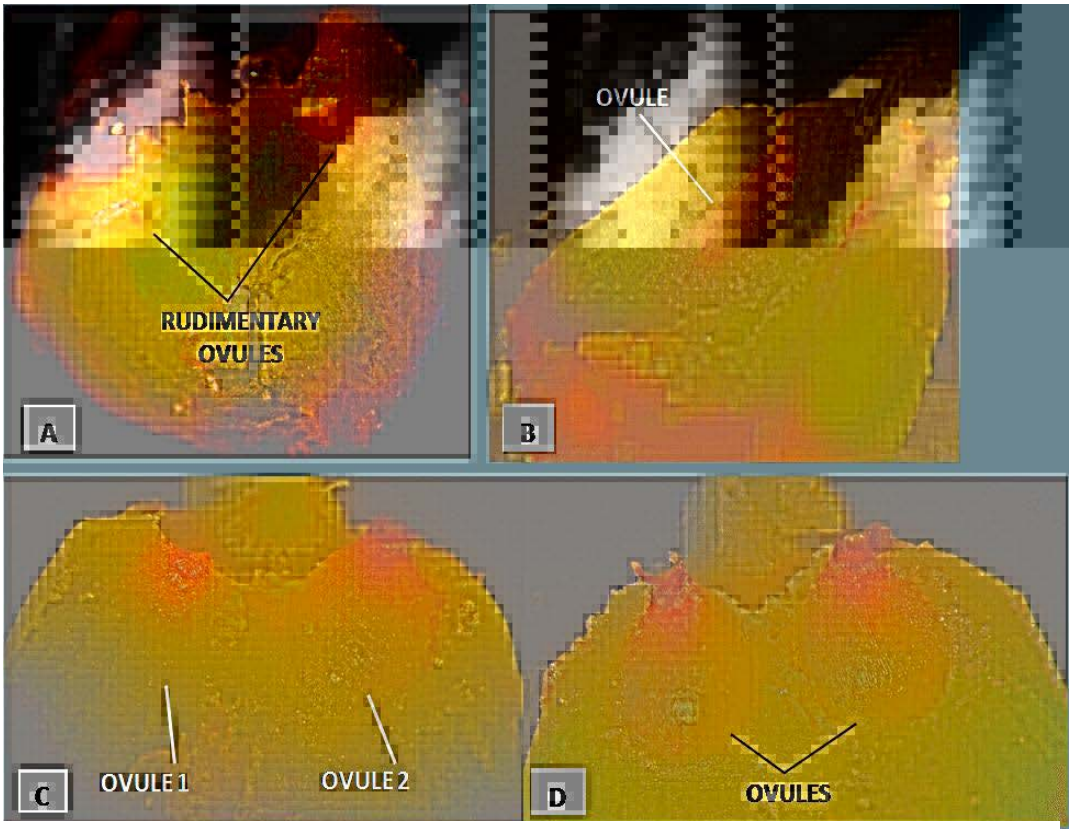


Figure 2. Scales in female cone of *Pinus armandii*: A – two rudimentary ovules, B – nonpollinated ovule, C - two pollinated ovules differed in size: the smaller one degenerated (1) and the other ovule developed (2), D - two successfully developed ovules.

J.N. Owens et al. (2008) recognized eight categories of seeds in mature cones of *Pinus albicaulis* Engelm. The same categories we recognized in mature cones of *Pinus sibirica* (Pohilchenko, Ruguzova, 2014). And in this study we examined *P. pumila* and *P. armandii* cones at the time of their winter dormancy. At this developmental stage ovule categories similar to those in mature cones could be noticed. The first type are fertile scales with two small flat ovules that hadn`t been pollinated and had no further development (23-28% of total fertile scales per cone in *P. pumila* and 21-26% in *P. armandii*) (Fig. 1 B, 2 B)

The second type is scales with two pollinated ovules that stopped their development soon after pollination (7-19% in *P. pumila*) (Fig. 1 D). The third type is scales with two pollinated ovules differed in size – the smaller one degenerated and the other ovule developed (19-31% in *P. pumila* and 13-45% in *P. armandii*) (Fig. 1 C, 2 C). And the last type is scales with two successfully developed ovules (22-40% in *P. pumila* and 34-60% in *P. armandii*) (Fig. 1 D, 2

D). It did not notice any significant differences in scale number and proportion in *P. pumila* cones from Kyiv and Gorgany.

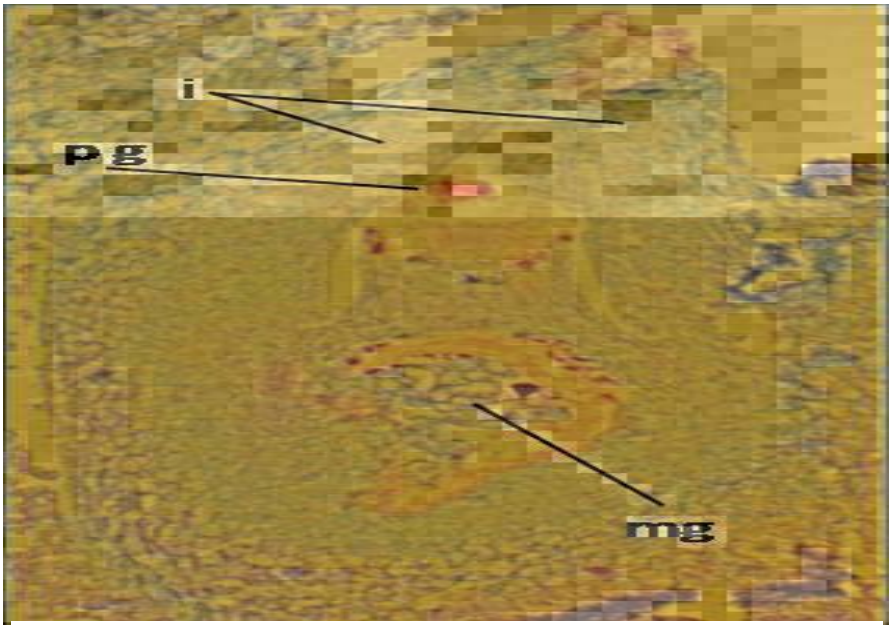


Figure 3. *Pinus pumila* normally developed ovule
(i – integument, mg – megagametophyte, p g – pollen grains)

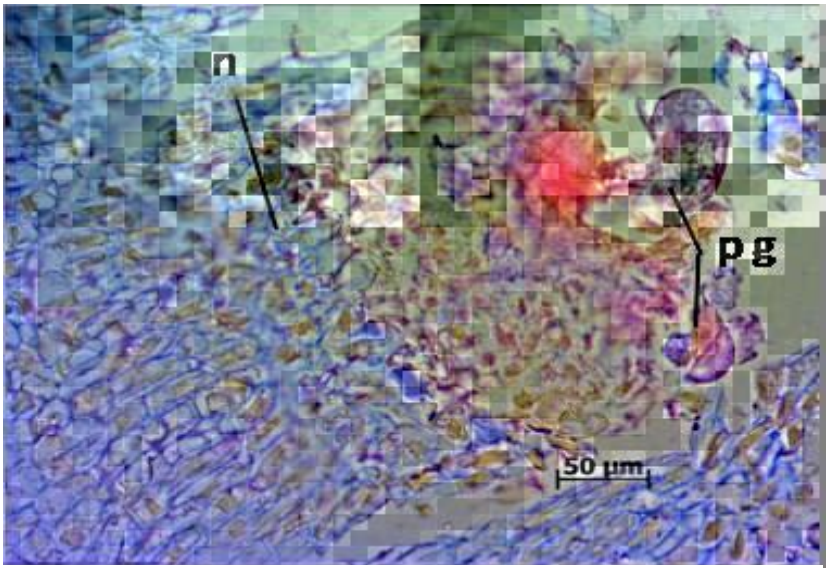


Figure 4. *Pinus armandii* degenerated ovule with growing pollen grains
(n – nucellus, p g – pollen grains)

Under the light microscope developing pollen grains with tubes were noticed in all pollinated ovules they either developed or degenerated (fig 3, 4). Those observations demonstrated that there are some reasons of ovule degeneration besides the lack of pollination.

Our observations correspond to those made for *Pinus lambertiana* Dougl. seed cones pollinated with *Pinus monticola* Dougl. ex D.Don pollen grains (Fernando et al., 2005). The cross between two species resulted in the failure of megagametophytes at the free-nuclear stage that could relate to the presence of the incompatible pollen tube on the nucellus. Since our studied pine trees are the part of dendrological collection and they are neighboring to the other *Pinus* species cross pollination could possibly be one of the reasons for ovules` degeneration during the second year of their development. However, the mechanism of this incompatibility reaction is unknown and it needs further studies. The other possible reason of megagametophyte degeneration at the stage of free nuclear is rusts diseases caused by pathogenic fungi. M.B. Rayachhetry et al. (1995) studied histology of *Pinus maximinoi* cones infected by one of the cone-rust fungus species and observed some ovules contained spongy tissue with degenerating cells in the centre.

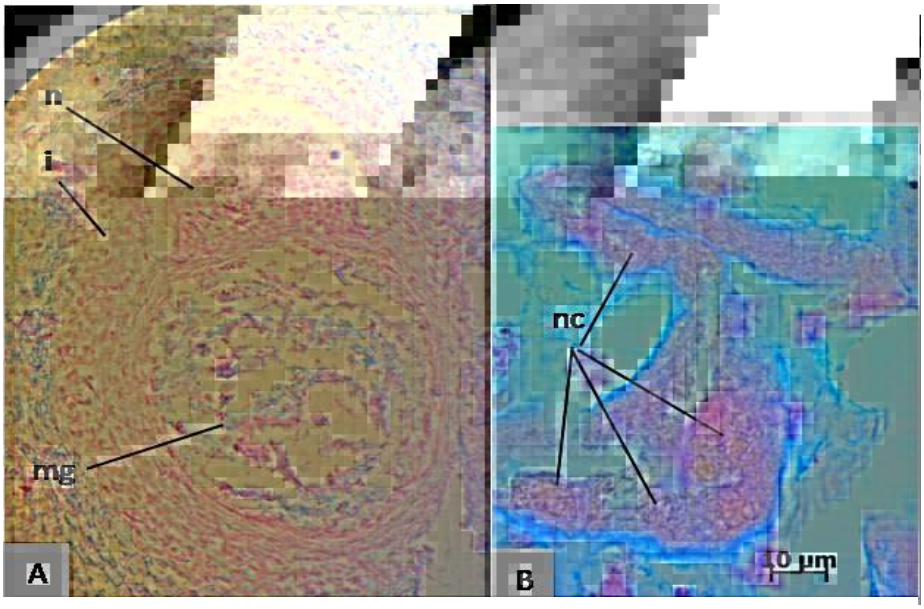


Figure 5. A - Developed ovule in *Pinus armandii*. Megagametophyte at the free nuclear stage.
B – megagametophyte nuclei

For both species in the most of normally developed ovules megagametaphyte was at the stage of free nuclear (fig. 5 A) but in some P. armandii ovules we observed the initiation of alveolar stage. At these stages

ovules had well developed integument the micropillar parts of which have been closed up soon after pollination.

The upper part of nucellus is elongated and on its tip pollen chamber has been formed. Free nuclear megagametophyte located in the basal part of the ovule and attached with tapetum (spongy tissue).

At that time its cell walls have dissolved and tapetum was of plasmodial type. It had dense cytoplasm and large nuclei. In P. armandii mean (±SE) diameter of spongy tissue nuclei was 19.09±1.30 μm and for free nuclei it was 12.59±1.33μm (fig. 5 B).

CONCLUSION

Our data about *P. pumila* and *P. armandii* female cones development correspond with other reports about *Pinus* sp. of different systematic groups (Zhang et al. 2015, Surso, 2015, Lill, 1974). That let us to suppose that these species are well adapted to the new growth conditions. For both studied species the main reason of poor seed formation was lack of pollination. Also, abortion of the pollinated ovules during the second year of their development was observed and its reasons need detailed studies.

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DOI: 10.17707/AgricultForest.62.4.23

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NOTEWORTHY POLYPORES OF PUSHKIN CITY NEAR THE SAINT PETERSBURG (RUSSIA), THE RESERVE OF OLD-GROWTH TREES. 2. *CERIOPORUS VARIUS* AND *C. LEPTOCEPHALUS*

SUMMARY

The paper continues a series focused on noteworthy polypores associated presumably with old-growth broadleaf trees in Pushkin (Tsarskoye Selo) ensemble near St. Petersburg. Some nemoral species complexes are reserved here due to protection of old-growth trees over this area. The species in focus of the present paper are *Cerioporus* (*Polyporus*) *varius* and its sib, *C. leptocephalus* (*Polyporaceae, Agaricomycetes*), both widely distributed in deciduous and mixed forests, but associated mainly with old trees and stumps. These are closely related species differing by morphogenetic features. Five habitats of *C. varius* and four habitats of *C. leptocephalus* were revealed in the Pushkin city. All these are documented, and an enlarged morphological and ecological description of both species is given. The rare form *C. varius* f. *circumpurpurascens* was epitypified and re-combined in the genus *Cerioporus*.

Keywords: *Polyporaceae*, polypores, polyporoid fungi, parks, old-growing trees, Tsarskoye Selo park ensemble

INTRODUCTION

The paper continues a series devoted to polypores attached to old-growth leaf trees protected in the Pushkin city (known also as Tsarskoye Selo, Russia), where urban sites and old parks form a peculiar mosaic and levels of technogenic pressure are sufficiently reduced (Zmitrovich, 2016). The presence of old trees, especially of broad-leaf ones over this area makes favorable conditions for development of some polypore species which are less presented in neighboring managed forests. The main purpose of this series is enlarged description of ecological polymorphism of noteworthy polypores species found in Pushkin city (Tsarskoye Selo) territory and representation of habitats of these organisms.

Since XVIII century, the species in question have been described as *Boletus leptocephalus* (Jacquin, 1778) and *B. varius* (Persoon, 1796). After E. M. Fries study (1821) and till the most recent times, both species have been regarded into *Polyporus* genus, however molecular phylogenetic studies show their

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

position within an independent genus *Cerioporus* (Zmitrovich & Kovalenko, 2016).

In XX century, a tendency to merging of two species appeared (Bondartsev, 1953; Gilbertson & Ryvarden, 1987; Bondartseva, 1998), although Overholts (1953) was sure that *Polyporus varius* differed from *P. elegans* (latter synonym of *P. leptocephalus*). Such a view is obvious from original descriptions and drawings of these taxa. As it is seen from Fig. 1, the original drawing of *Boletus leptocephalus* with a minute clear pileus and a pronounced subcentral stalk is contrasted to Bolton's (1788) drawing of *B. lateralis* which was selected by Persoon (1796) as an illustration of *B. varius* – a clustered polypore with a lateral stem and an undulating colored margin.

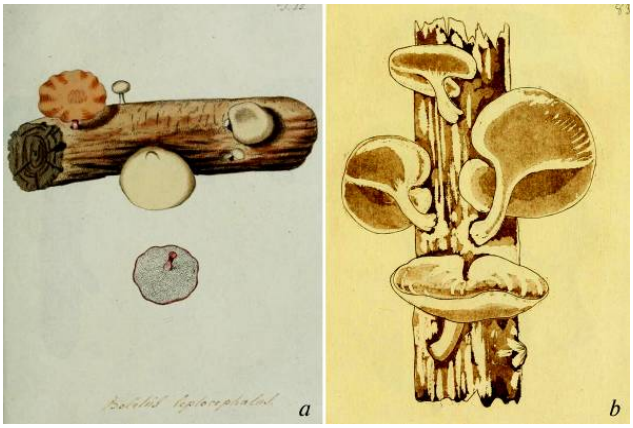

Figure 1. Original drawing of *Boletus leptocephalus* by Jacquin (1778) with a minute clear pileus and a pronounced subcentral stalk (*a*), and Bolton's (1788) drawing of *B. lateralis* which was selected by Persoon (1796) as a figure of *B. varius* (*b*).

Due to highly similar nature of basidiome texture, surfaces structure and micromorphology in both taxa, their differences can be interpreted as having of ecotypic, but not species nature, but we prefer keeping both species separately, similarly to another member of the complex, *C. choseniae* (Zmitrovich et al., 2014), at least until an exhaustive molecular testing. The current experience on another species pair in the genus *Cerioporus*, namely *Cerioporus squamosus*/*C. rangiferinus* (Zmitrovich et al., 2017), shows that morphogenetic differences connected to the reaction to light of aerial mycelium can lead to a lineages divergence. *C. leptocephalus* as well as *C. rangiferinus* has an extended displaced stipe, covered with a black multilayered crust, its cap is not extensively developed. On the contrary, *C. varius* has an expanded cap and a modest (often reduced) stipe with a not expressed crust − even if it is covered by dark pruina.

Both *C. varius* and *C. leptocephalus* have a cosmopolitan, but mainly Holarctic distribution, however occupy rather diverse niches. *C. varius* is associated mainly with standing old trees, where it often grows as a pathogenic

saprotroph, whereas *C. leptocephalus* (=*Polyporus elegans*, =*P. nummularius*) is associated with old stumps and fallen branches (Bondartsev, 1953; Bondartseva et al., 2014). A global substrate range, known for *C. varius* with inclusion of *C. leptocephalus* only, contains such tree genera as *Acer*, *Aesculus*, *Alnus*, *Betula*, *Buxus*, *Castanea*, *Corylus*, *Crataegus*, *Eucalyptus*, *Fagus*, *Frangula*, *Fraxinus*, *Juglans*, *Malus*, *Ostrya*, *Populus*, *Prunus*, *Pyrus*, *Rhamnus*, *Ribes*, *Robinia*, *Rubus*, *Quercus*, *Salix*, *Sambucus*, *Sorbus*, *Tilia* and *Ulmus* (Ryvarden & Melo, 2014). In Denmark, *Cerioporus varius* is protected having a «Least concern» (LC) status (The Danish…, 2016). Over the Pushkin area these interesting polypore species have a certain distribution.

MATERIAL AND METHODS

Territory studied

Tsarskoye Selo (Pushkin) city occupied hilly landscape on border of the Prinevsky Lowland and the Izhora Plateau in 15 kilometers to the south of St. Petersburg on approx. 59.75°N and 30.36°E crosshairs. The highest areas lie here about 100 m above the sea level. Park zone of Pushkin (Tsarskoye Selo) ensemble occupies the area of 704 hectares (Zmitrovich, 2016).

Material collection

A total of thirty km^2 of protected territory were under the monitoring since 2012. Old-growth leaf-trees phytosphere was examined on the occurence of the polypore species. The fruitbodies were fixed on photo or collected in the case of necessity of study their morphology. In the laboratory, –20 °C freezing of material followed by drying were carried out. The material is kept in mycological herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE).

Material elaboration

The macroscopic descriptions were based on a study of fresh and dried specimens. Microscopic preparations were mounted from dried material in Melzer's solution, 10% ammoniacal Congo Red and 5% aqueous solution of KOH, using a LOMO Micmed-6 light microscope. The hyphal system was revealed and described using the updated technique (Zmitrovich et al., 2009). For the spore size measure were used 30 spores of every sample. For preparations were used distilled water and Melzer's solution. The taxonomic position of the species was based on recent molecular studies (Zmitrovich, Kovalenko, 2015). The higher taxa are given according to Hibbett et al. (2014).

RESULTS AND DISCUSSION

Agaricomycetes

Polyporales

Polyporaceae

Cerioporus leptocephalus (Jacq. : Fr.) Zmitr. et Kovalenko, Int. J. Medicinal Mushrooms 18(1): 33, 2016. – Bas.: *Boletus leptocephalus* Jacq., Miscell. Austriac 1: 142, 1778 ex Fr., Syst. Mycol. 1: 349, 1821 [ut *Polyporus*

leptocephalus]. ≡ *Coltricia leptocephala* (Jacq. : Fr.) Gray, Nat. Arr. British Pl. 1: 645, 1821. ≡ *Polyporellus leptocephalus* (Jacq. : Fr.) P. Karst., Medd. Soc. Fauna Fl. Fennica 5: 38, 1879. ≡ *Leucoporus leptocephalus* (Jacq. : Fr.) Quél., Enchir.: 166, 1886. = *Boletus elegans* Bull., Herb. France 2: 76, 1782. ≡ *Polyporellus elegans* (Bull.) P. Karst., Medd. Soc. Fauna Fl. Fennica 5: 37, 1879. ≡ *Melanopus elegans* (Bull.) Pat., Essai: 80, 1900. = *Boletus nummularius* Bull., Herb. France 3: tab. 124, 1883. ≡ *Coltricia nummularia* (Bull.) Gray, Nat. Arr. British Pl. 1: 644, 1821. ≡ *Polyporus nummularius* (Bull.) Fr., Observ. Mycol. 1: 123, 1815. = *Boletus nigripes* With, Arr. British Pl. 4: 316, 1796. = *Polyporus elegans* Fr., Epicr.: 440, 1838.

Icon.: Jacqiun (1778: fig. 12 ut *Boletus leptocephalus*), Sowerby (1797: tab. 89 ut *B. nummularius*).

Figure 2. *Cerioporus leptocephalus* found on *Tilia cordata* stump on the square near Pushkin administration with *T. cordata* and *Quercus robur* (59°43’23”N, 30°25’02”E).

Basidiomata annual, solitary, differentiated into single or branched stipe and rather minute pilei. Pilei (0.8)1–3(6) cm in diam., 0.1–0.5 cm thick at the base, spathulate, concave, convex or plane, in sublateral forms their length exceeds a width. Upper surface even, covered with ivory to stramineous cuticle of fine radially undergrown-fibrillose texture. Margin isochromatic, initially straight, then incurved, as a rule not undulating, but sometimes sinuous in outline, especially near the base. Stipe up to 10 cm long and -5 mm in cross-section, elongated and deeply rooted into substrate, in some cases twice-branched, sometimes undulating, more or less isodiametric, central or eccentric, covered with brown-black to radical black crust. Hymenophore as a single tube layer 0.5–3.2 mm thick well differentiated from the context; pore surface initially white, then isabelline to cinnamon, in medial states cream or honey yellow; pores 3–5 per mm, roundish, rather thick-walled, isodiametric in large areas. The

context 0.1–2 mm thick, homogeneous, dense, cream to clay-yellow. The consistency is tough in fresh, cretaceous at drying (Fig. 2).

Hyphal system dimitic with skeleto-binding hyphae. Generative hyphae 1.5–5 μm in diam., with prominent clamp connections, regularly branched, inamyloid, hyaline. Skeleto-binding hyphae 1.5–6.5 μm in diam., as a rule with inflated axial element and dendritic appendages, slightly thick-walled to subsolid, hyaline or yellowish. Caulocystidia 20–55 × 7–10 μm, clavate-cylindric, thick-walled to subsolid, yellowish-brown, arranged into 2–4-layered palisade which forms a stipe crust. Basidia 18–30 × 5–9 μm, clavate, with a basal clamp connection, 4-spored. Basidiospores 8.5–9.5 × 2.8–3.9 μm, fusoid, with a minute apiculus, hyaline, thin-walled, inamyloid, acyanophilous.

Substrates and ecology. *Cerioporus leptocephalus* grows on stumps and fallen twigs of trees and shrubs causing a white rot. Basidiomata exist during a single season (Jule–October), but in warm winters they can stay as bodies with decolorated pilei and coffee-colored hymenophore.

The most typical habitats of this fungus are shrubs thickets within broadleaf stands, with abundant wood debris (Fig. 3).

Figure 3. Typical habitat of *Cerioporus leptocephalus* in the Pushkin city.

Distribution. EUROPE: United Kingdom, Norway, Sweden, Finland, France, Germany, Austria, Poland, Czech Republic, Switzerland, Montenegro, Russia; OCEANIA: New Guinea; AUSTRALIA: New Zealand; NORTH AMERICA: USA, Canada; SOUTH AMERICA: Brazil (Global.., 2016).

Table 1. Records of *Cerioporus leptocephalus* on the Pushkin city (Tsarskoye Selo) territory

N	Coordinates	Locality, habitat	Substrate	Specimen
1.	59°43’36”N, 30°22’02”E	Fermsky Park, mixed old stand with *Acer platanoides*, *Fraxinus excelsior*, *Malus sylvestris*, *Ribes* spp.	*Ribes rubrum* twigs	*Cerioporus leptocephalus* f. *nummularius* (LE 287637)
2.	59°43’23”N, 30°25’02”E	The square near Pushkin administration with *Tilia cordata* and *Quercus robur*	*Tilia cordata* stumps	typical form –
3.	59°42’53”N, 30°25’21”E	Clinic of Eberman park with *Tilia cordata*, *Padus avium*, *Frangula alnus*, *Sorbaria sorbifolia*	*Padus avium* and *Sorbaria sorbifolia* stumps, *Tilia cordata* fallen twigs	typical form –
4.	59°44’41”N, 30°25’42”E	Alexey Tolstoy Boulevard, intra-quarter gardening with *Ulmus glabra*, *Acer platanoides*, *Tilia cordata*, *Salix fragilis*, *S. alba*	*Tilia cordata* stump, *Ulmus glabra* twigs	typical form –

The distribution of this species over Pushkin (Tsarskoye Selo) territory is rather scarce. Only 4 habitats of *C. leptocephalus* are revealed (Table 1; Fig. 4). All these are presented by shady micro-sites covered by non-cut shrubs.

Morphological variability.

In the field, *C. leptocephalus* can be recognized due to its minute basidiomata with ivory to clay minute pilei on black-colored long isodiametric stipes. The pileus outline can be roundish, but in some cases it is spathulate or shell-like. Upperside is characterized by fine fibrillose internal structure and homogeneous light coloration. In old exemplars, upper surface staying clay-buff to pale and so fibrillose structure is invisible. The stipe in this species is furnished by a black-colored crust reaching a hymenophore area.

There are two main forms of the species – *C. leptocephalus* f. *leptocephalus*, having an elongated (up to 5–6 cm) pileus and an eccentric stipe and *Cerioporus leptocephalus* f. *nummularius* (Bull.) Bondartseva, Zmitr. et Sidelnikova comb. et stat. nov. (Bas.: *Boletus nummularius* Bull., Herb. France 3: tab. 124, 1883; MB 818684), characterized by a small (up to 3 cm in diam.) roundish (*nummularius* = coin-like) pilei on subcentral stems. Both forms are presented in the area studied (Table 1).

Figure 4. Pushkin city quarter plan with indications of habitats of *Cerioporus leptocephalus* (red circles). The numbers are corresponded to those in Table 1.

Cerioporus varius (Pers. : Fr.) Zmitr. et Kovalenko, Int. J. Medicinal Mushrooms 18(1): 33, 2016. – Bas.: *Boletus varius* Pers., Observ. Mycol. 1: 85, 1796 ex Fr., Syst. Mycol. 1: 352, 1821 [ut *Polyporus varius*]. ≡ *Polyporellus varius* (Pers. : Fr.) P. Karst., Medd. Soc. Fauna Fl. Fennica 5: 37, 1879. ≡ *Melanopus varius* (Pers. : Fr.) Pat., Hymen. Eur.: 137, 1887. = *Boletus calceolus* Bull., Herb. France 8: tab. 360, 1786. = *B. lateralis* Bolton, Hist. fung. Halifax 2: 83, 1788. = *B. ramulosum* J. F. Gmelin, Syst. Nat. 2(2): 1435, 1792. = *Polyporus petalodes* Fr., Epicr.: 444, 1838. = *P. blanchetianus* Berk. et Mont., Ann. Sci. Nat. Bot. 11: 238, 1849. = *P. boltonii* Rostk. in Sturm, Deutschl. Fl. 7: 47, 1848. = *P. leprodes* Rostk. in Sturm, Deutschl. Fl. 7: 33, 1848. = *P. gintlianus* Velen., České Houby 4–5: 687, 1922.

Icon.: Bolton (1788: fig. 83 ut *Boletus lateralis*), Bulliard (1796: pl. 360 ut *B. calceolus*), Niemelä (2005: fig. 227 ut *Polyporus leptocephalus*), Bernicchia (2010: p. 748 ut *P. varius*), Melo & Ryvarden (2014: fig. 283 ut *P. varius*).

Basidiomata annual, clustered, rarely solitary, differentiated into wide pileus and eccentric to lateral narrowing stem. Pilei 3–8(10) cm wide, 0.3–0.8 cm thick at the base, dimidiate, snell-like or infundibuliform, the pileus width as a rule exceeds its length. Upper surface glabrous to matt, covered with cream to

clay or pale-ochraceous cuticle of radially-fibrillose internal of external texture; in some cases can be radially ridged. Margin colored – purple-brown or cinnamon-brown, initially straight, then incurved, as a rule undulating or sinuous. Stipe up to 3 cm long and -8 mm in cross-section, rather short, straight, more or less wedge-shaped, eccentric or lateral, naked or covered with brownish to brownish-black pruina, but without a thick crust formation. Hymenophore as a single tube layer, 0.8–5.5 mm thick, well differentiated from the context; pore surface initially white, then honey-yellow to clay-buff; pores 4–8 per mm, roundish, thick- to thin-walled, isodiametric in large areas and rather sinuous near the base. The context 0.3–5 mm thick, homogeneous, dense, cream to buff. The consistency is tough in fresh, suberose-coriaceous to cretaceous at drying (Fig. 5).

Figure 5. *Cerioporus varius* found on *Tilia cordata* notch in the vicinities of Saint-Petersburg State Agrarian University, *T. cordata* stands (59°43’51”N, 30°23’25”E).

Hyphal system dimitic with skeleto-binding hyphae. Generative hyphae 1.5–5.5 μm in diam., with prominent clamp connections, regularly branched, inamyloid, hyaline. Skeleto-binding hyphae 1.5–7.0 μm in diam., as a rule with inflated axial element and dendritic appendages, slightly thick-walled to subsolid, hyaline or yellowish. Caulocystidia 15–55 × 5–10 μm, clavate-cylindric, thick-walled to subsolid, yellowish-brown, lying into 1-layered loose palisade which forms a stipe pruina. Basidia 18–35 × 5.5–9.5 μm, clavate, with a basal clamp connection, 4-spored. Basidiospores 8.5–11.5 × 2.8–3.9 μm, fusoid, with a minute apiculus, hyaline (often multiguttulate), thin-walled, inamyloid, acyanophilous.

Substrates and ecology. *Cerioporus varius* grows on living or dead trees, stumps and fallen logs, causing a white rot. The typical habitats of this fungus are hollows and cracks of living trees, and *C. varius* has a certain pathogenic importance. Basidiomata exist during a single season (Jule–November), and, owing to their pliable context, rather quickly destroying by insects.

The most typical habitats of this fungus are old broadleaf stands, where it infests a hollows and cracks of the trees (Fig. 6).

Figure 6. Typical habitat of *Cerioporus varius* in the Pushkin city.

Distribution. EUROPE: United Kingdom, Denmark, Norway, Sweden, Finland, Estonia, Latvia, Lithuania, Belgium, France, Germany, Austria, Poland, Czech Republic, Switzerland, Italy, Montenegro, Bulgaria, Romania, Ukraine, Belarus, Russia; ASIA: Russia, China, Taiwan, Japan; AUSTRALIA: Australia, New Zealand; NORTH AMERICA: USA, Canada; SOUTH AMERICA: Brazil, Chile (Global.., 2016).

Table 2. Records of *Cerioporus varius* on the Pushkin city (Tsarskoye Selo) territory

N	Coordinates	Locality, habitat	Substrate	Specimen
1.	59°43’36”N, 30°22’12”E	Fermsky Park, mixed old stand with *Fraxinus excelsior*, *Tilia platyphyllos*, *Malus* spp.	*Fraxinus excelsior* fallen log	*Cerioporus varius* f. *circumpurpurascens* (LE 287633).
2.	59°43’51”N, 30°23’25”E	Vicinities of Saint-Petersburg State Agrarian University, *Tilia cordata* stands	Living *Tilia cordata* notch	typical form –
3.	59°44’12”N, 30°24’24”E	Great Architecture Massive, *Tilia cordata* stands	Living *Tilia cordata* notch	typical form –
4.	59°42’09”N, 30°24’08”E	Novaya str., public garden	Living *Aesculus hyppocastanum* notch	typical form –
5.	59°42’45”N, 30°23’29”E	Catherine Garden, Cascade ponds	Living *Tilia cordata* notch	typical form –

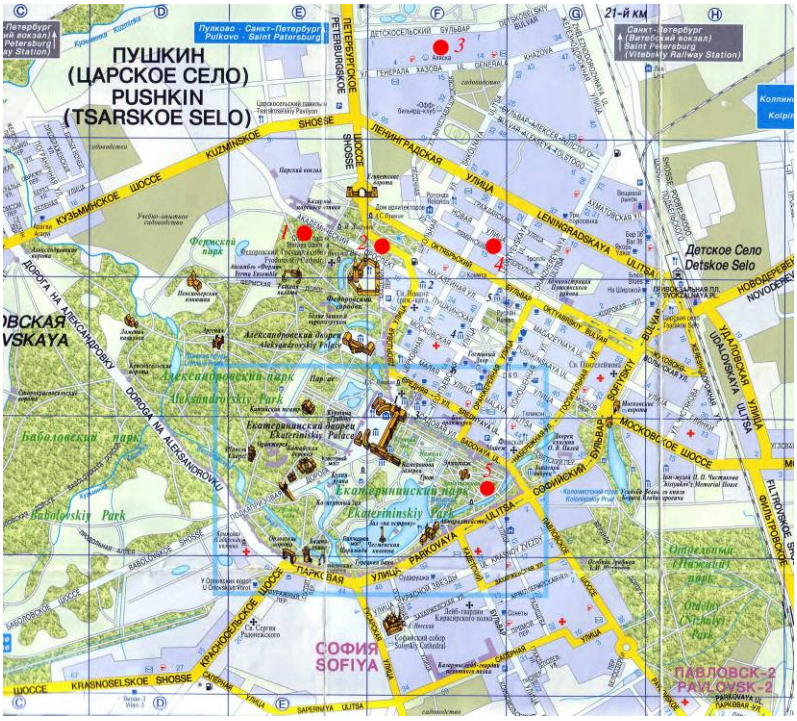

Figure 7. Pushkin city quarter plan with indications of habitats of *Cerioporus varius* (red circles). The numbers are corresponded to those in Table 2.

The distribution of this species over the Pushkin (Tsarskoye Selo) territory is scarce, too. Only 5 habitats of *C. varius* are revealed (Table 2; Fig. 7). All these are presented by broadleaf stands, where it is attached to notches and fallen logs of the trees.

Morphological variability

C. varius can be easily recognized due to its medium-sized minute basidiomes (basidiome clusters) with clay or pale ochraceous pilei on rather short wedge-shaped stipes. The margin upperside is easily colored in brownish or even purplish shades. Upperside is clay-colored, but can be reaching an ochraceous tints or decolorating to ivory-white colors. Something ridged surface is highly characteristic. The stipe in this species is rather short and a black crust is not characteristic for this species, but brown or blackish pruina can be oserved near stipe base.

There were described several forms among which, besides a type form, are most reliably recognizable the *C. varius* f. *undulatolobatus* (Bourdot et Galizn) Bondartseva, Zmitr. et Sidelnikova comb. nova (Bas.: *Melanopus varius* f. *undulatolobatus* Bourdot et Galzin, Bull. trimest. Soc. mycol. France 41(1): 111, 1925; MB 818685), distinguished by an undulating margin and not presented on the Pushkin area, and *C. varius* f. *circumpurpurascens*, which was found in the Pushkin city and is epitypified in the present report.

Cerioporus varius f. *circumpurpurascens* (Pilát) Bondartseva, Zmitr. et Sidelnikova comb. nova. – Bas.: *Polyporellus varius* f. *circumpurpurascens* Pilát, Atlas Champ. L'Europe III 1: 109, 1937 (MB 818686).

Protologue: «Pileus minute, having an eccentric stipe, in a middle part faintly ochraceous, reddish-brown at the margin» (Pilát, 1937).

Figure 8. *Cerioporus varius* f. *circumpurpurascens* (LE 287603): *a* – upperside of basidiome, *b* – pores near the margin. Scale bar: *a* – 1 cm, *b* – 1 mm.

Epitype: Russia, St. Petersburg, Pushkin city (Tsarskoye Selo), Fermsky Park, mixed old stand with *Fraxinus excelsior*, *Tilia platyphyllos*, *Malus* spp., on *Fraxinus excelsior* fallen log, 19.08.2016, coll. I. V. Zmitrovich (LE 287603).

Material description: Basidiome annual, solitary, differentiated into wide pileus and eccentric stem. Pileus 4.5 cm wide and 0.5 cm thick at the base, conchate (almost infundibuliform). Upper surface glabrous, covered with clay cuticle, of radially-fibrillose internal texture, even. Margin of castaneous-purplish-brown coloration (*k4*/*n4* according to Bondartsev, 1954), more or less even in outline. Stipe 1.7 cm long and -4 mm in cross-section, rather short, straight, lateral, covered with brownish-black pruina. Hymenophore as a single tube layer, 4.5 mm thick, well differentiated from the context; pore surface initially clay-buff; pores 4–5 per mm, roundish, thick-walled, isodiametric The context -0.4 mm thick, homogeneous, dense, cream-colored. (Fig. 8).

The microstructure varies as in a neutral type. Basidiospores 8.9–10.5 × 3.0–3.5 μm.

CONCLUSION

Two species, recorded in Pushkin city, *C. varius* and *C. leptocephalus* represent quite distinguishable component of polypore biota. The first species is mainly associated to old broadleaf stands, where infests a notches of living or fresh fallen trees. The second one is mainly associated to shrub understory of broadleaf massifs, where infests minor stumps and fallen branches of shrubs and

trees. The differences between *C. varius* and *C. leptocephalus* lye rather in morphogenetic field and are comparable to another species pair, *C. squamosus*/*C. rangiferinus*: the second ones represent an arrhythmic phenotypes characterized by developed stipes and minute pilei. Until precise molecular testing, we prefer to consider both items as independent species adapted to specific micro-environment of temperate leaf forests.

ACKNOWLEDGEMENTS

The authors are very grateful to Prof. Lydia G. Perevedentseva for important notes on the manuscript. The work of M. A. Bondartseva and I. V. Zmitrovich was carried out in canvas of the program of the Russian Academy of Sciences «Biodiversity of nature systems. Biological resources of the Russia: an estimation and fundamental bases of monitoring», the nomination «Systematics and phylogeny of important groups of the Fungi in connection to the problem of fungal communities formation» and «Check-list of aphyllophoraceous fungi of European Russia».

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DOI: 10.17707/AgricultForest.62.4.24

Soghra KIANI and Kayvan AGAHI [1]

CORRELATION AND PATH COEFFICIENT ANALYSIS OF MORPHOLOGICAL TRAITS AFFECTING GRAIN SHAPE IN RICE GENOTYPES (*ORYZA SATIVA* L.)

SUMMARY

This work was undertaken to look into the interrelationships among morphological traits and rice grain shape. For this purpose, a set of 25 rice genotypes was sown and subjected to a farm survey, based on the standard evaluation system for rice. Correlation coefficient analysis showed that the grain shape was positively correlated with grain length, panicle length, plant height and the number of tillers while, there were statistically significant and negative correlations between grain shape with maturity date, number of grains per panicle, grain breadth, 100-grain weight and flag leaf width. Sequential path analysis revealed that grain breadth, grain length and number of grains per panicle, as first-order variables, was responsible for about 98% of the variation in grain shape. Also, 100-grain weight, maturity date, number of tillers and flag leaf width were determined as second-order predictors.

Amongst second-order predictors, 100-grain weight was a noteworthy trait regarding its high direct and indirect effects on grain breadth and grain length. Study of multicollinearity measures revealed that sequentializing of predictor variables reduced problems due to multicollinearity leading to a better understanding of the interrelationships among the various traits and their relative contribution. Also, the bootstrap analysis indicated that all direct effects were significant.

The results suggested that grain breadth, grain length and number of grains per panicle, as first-order predictor variables had the highest direct effect on grain shape and could be used as a selection criterion to improve rice grain shape. Also, 100-grain weight, maturity date, number of tillers and flag leaf width, as second-order predictor variables affect the rice grain shape indirectly through their effects on first-order predictors. The authors recommend for the use of sequential equation modeling to conduct a proper sequential path analysis.

Keywords:conventional path analysis, sequential path analysis, stepwise multiple linear regression, structural equation modeling

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

INTRODUCTION

Rice appearance is a character considered as one of the main quality attributes by consumers therefore, measuring and understanding factors influencing rice grain appearance is a great challenge for industries and breeders in meeting consumer preferences (Haider et al., 2014). In genetics, grain shape has been widely accepted as a complex trait controlled by multiple genes with small effects (Yin et al., 2015). Thus, understanding the complexities governing the relationships among traits leads to increased selection gain in breeding programs. In this regard, correlation coefficients, multiple linear regression and path analysis are some of the common statistical methods. Determination of correlation coefficients is an important statistical procedure to examine the relationship between traits. However, increase of the number of independent variables controlling a particular dependent variable can lead to increased interdependence. In such situation, correlations may be insufficient to explain the associations in a way that will enable breeders to decide on a direct or indirect selection strategy (Ofori 1996; Sabaghnia et al., 2010).

Path coefficient analysis is a statistical tool that partitions correlation coefficients into direct effects (showing how a 1 unit change in the predictor variable will affect response variable) and indirect effects (association of one predictor variable with response variable mediated through other predictor variables in the model). It is obvious that decomposition of the effects depends on the model intended to describe the causal relationships among variables (modeling). Therefore, modeling is an important step towards path analysis.

Among path analyses, we can distinguish two types of models: (i) simple models, in which all traits except for the dependent one are set up at the same ontological level, which makes for their being treated as co-related; and (ii) complex models, in which traits are set up at different levels, and relations (that is, co-relations or cause-and-effect relationships) between them are to reflect possible biological relations. Complex models are referred to as se¬quential models reflecting sequential development of crop traits and, thus, sequentiality of cause-and-effect associations among them (Kozak and Azevedo 2014). Although traits are set up in some sequential order in the model, the methodology of this setting is based upon stepwise vari¬able selection in regression analysis and analysis of the total contribu¬tion of the traits to the variation of the dependent vari¬able (Mohammadi et al., 2003). In a regression analysis however, collinearity (or multicollinearity) is the undesirable situation where the correlations among the independent variables are strong. Tolerance is a statistic used to determine how much the independent variables are linearly related to one another (multicollinear). Tolerance is the proportion of a variable's variance not accounted for by other independent variables in the model. A variable with very low tolerance contributes little information to a model, and can cause computational problems. VIF, or the variance inflation factor is the reciprocal of the tolerance. As the variance inflation factor increases, so does the variance of

the regression coefficient, making it an unstable estimate. Large VIF values are an indicator of multicollinearity (IBM Released 2010).

The objective of this paper was to address the nature of the relationships between some prominent morphological traits and rice grain shape. The outcome could be helpful to develop selection strategies in genetic breeding programs aimed at improving marketability or cooking quality of rice.

MATERIAL AND METHODS

Plant materials and studied traits

Field experiment was carried out at the Tonekabon Rice Research Station located at 36°51'N, 50°46'E and -22 meter below the sea level. A set of twenty five rice genotypes was prepared from the Iran Rice Research Institute, Rasht including: Alfa, AliKazemi, Anbarboo, Binam, Century Patna231, Dom Siah, Gharib, Gharib Seyah Rayhani, Hassani, Hassan Saraei, Hassan Saraei Atashagah, Hassan Saraei Pichide Ghalaf, IR28, IR58, Lido, Line213, Line304, Line305, Mussa Tarom, Plano, Salari, Sanaderia, Sange Jo, Strella, and Zenith. After a primary growth in the nursery, the derived seedlings were transplanted to the main field, according to a squared lattice experimental design with two replications.

The mean of temperature and average annual rainfall of the station were 15oC and 1100 mm, respectively. Each plot with an average area of 9m2 comprised of four rows spaced 25cm apart.

To collect data, rice plants were randomly selected and measured. Studied traits were: days to heading (DH); days to maturity (DM); plant height (PH); number of tillers (NT); panicle length (PL); flag leaf length (FL); flag leaf width (FW); length of the uppermost inter-node (LU); grain length (GL); grain breadth (GB); hundred grain weight (GW); number of grains per panicle (GP). Grain shape (GS) was calculated as the ratio of GL to GB. All the traits were measured on the basis of the standard evaluation system for rice published by the International Rice Research Institute (IRRI 1996).

Statistical analysis

Normality of the distribution of the data was evaluated using skewness and kurtosis parameters, related to each trait, using AMOS 22 (AMOS 2013) statistical software. Simple correlation coefficients were calculated for all possible comparisons using Pearson correlation coefficient.

Stepwise multiple linear regression analysis was performed in order to determine the best sequential model representing causal relationship between predictor variables and rice grain shape. In fact, predictor variables were organized into first- and second-order paths. After identification of the model, direct and indirect effects of the predictor variables on response variable were computed using structural equation modeling (SEM) using AMOS 22 (AMOS 2013) statistical package. Correlation and regression analysis were carried out using IBM Statistics 19 (IBM Statistics 2010. To estimate the standard error of path coefficients, bootstrap analysis was performed using AMOS 22 (AMOS 2013) statistical package.

RESULTS AND DISCUSSION

Simple correlations

Simple correlations and path coefficient analysis were studied to determine the interrelations governing direct and indirect grain shape components. The simple correlation coefficients (Table 1) showed there were significant correlations between grain shape and all of the measured traits except for FL, UL and DH. The correlation between grain shape and other traits was of great importance for us, because the main purpose of this study was to identify traits affecting the grain shape. As shown in Table 1, traits GL, PL, PH and NT were positively and significantly correlated with GS. There were statistically significant and negative correlations between GS and some other rice characters such as DM, GP, GB, GW and FW (Table 1).

Table 1. Pairwise Pearson correlation coefficients between 13 traits of 25 rice genotypes

Trait [a]	DH	DM	GP	GL	GB	UL	PL	PH	GW	NT	FW	FL
DM	0.72**											
GP	0.17	0.22										
GL	0.19	-0.34*	-0.46**									
GB	-0.16	0.24	0.50**	-0.70**								
UL	0.22	0.22	-0.03	0.01	-0.01							
PL	0.45**	0.15	-0.41**	0.42**	-0.47**	0.55**						
PH	0.30*	0.06	-0.17	0.39**	-0.40**	0.78**	0.75**					
GW	-0.18	-0.20	0.25	-0.06	0.57**	-0.06	-0.35*	-0.24				
NT	0.35*	0.24	-0.41**	0.12	-0.42**	-0.15	0.32*	-0.01	-0.37**			
FW	0.17	0.35*	0.75**	-0.48**	0.49**	0.05	-0.37**	-0.15	0.16	-0.26		
FL	0.36**	0.35*	0.19	-0.22	0.18	0.38**	0.41**	0.31*	0.003	0.21	0.34*	
GS	0.18	-0.32*	-0.46**	0.87**	-0.94**	0.01	0.48**	0.45**	-0.41**	0.29*	-0.49**	-0.20

* and ** mean that correlation is significant at the 0.05 and 0.01 level (2-tailed), respectively

[a] The symbol of traits consist of: DH, days to heading; DM, days to maturity; GP, the number of grains per panicle; GL, grain length; GB, grain breadth; UL, the length of the uppermost inter-node; PL, panicle length; PH, plant height; GW, 100-grain weight; NT, the number of tillers; FW, flag leaf width; FL, flag leaf length; GS, grain shape.

Considering that, grain length and grain breadth were the two important components of grain shape therefore, the relationship between grain length and grain breadth with other rice traits was also important. In this study, GL was significantly positively correlated with FW and FL while, it was significantly negatively correlated with DM and GP (Table 1). Significant positive correlations were observed between grain breadth with GP and FW while, the correlation between grain breadth with PL and NT was significantly negative (Table 1).

Conventional and sequential path analysis

To determine the relative importance of the traits affecting rice grain shape, the relevant correlation coefficients were separated into direct and indirect effects using conventional path analysis.

Table 2**.** Direct (the bold numbers on the diagonal) and indirect effects of first-order predictor variables on grain shape of 25 rice genotypes plus two common measures of collinearity (tolerance, Tol and variance inflation factor, VIF) in conventional path analysis

Trait[a]	DH	DM	GP	GL	GB	UL	PL	PH	GW	NT	FW	FL	Tol	VIF
DH	**-0.043**	-0.031	-0.007	-0.008	0.007	-0.009	-0.019	-0.013	0.008	-0.015	-0.007	-0.015	0.07	15.25
DM	-0.006	**-0.008**	-0.002	0.003	-0.002	-0.002	-0.001	-0.001	0.002	-0.002	-0.003	-0.003	0.11	8.89
GP	0.016	0.021	**0.097**	-0.044	0.048	-0.003	-0.040	-0.017	0.024	-0.040	0.073	0.019	0.11	8.86
GL	0.088	-0.158	-0.210	**0.460**	-0.320	0.004	0.191	0.178	-0.029	0.056	-0.220	-0.103	0.09	10.96
GB	0.097	-0.146	-0.307	0.427	**-0.614**	0.004	0.289	0.243	-0.352	0.255	-0.302	-0.113	0.10	9.98
UL	-0.009	-0.009	0.001	0.000	0.000	**-0.043**	-0.024	-0.033	0.003	0.006	-0.002	-0.016	0.14	7.27
PL	-0.001	0.000	0.001	-0.001	0.001	-0.001	**-0.001**	-0.001	0.000	0.000	0.001	-0.001	0.09	11.55
PH	0.021	0.004	-0.012	0.027	-0.027	0.053	0.051	**0.069**	-0.016	-0.001	-0.010	0.021	0.10	9.69
GW	0.008	0.009	-0.011	0.003	-0.026	0.003	0.016	0.011	**-0.045**	0.016	-0.007	0.000	0.22	4.63
NT	0.001	0.000	-0.001	0.000	-0.001	0.000	0.001	0.000	-0.001	**0.002**	0.000	0.000	0.16	6.16
FW	-0.003	-0.007	-0.015	0.010	-0.010	-0.001	0.007	0.003	-0.003	0.005	**-0.020**	-0.007	0.10	9.85
FL	0.006	0.006	0.003	-0.004	0.003	0.006	0.007	0.005	0.000	0.003	0.006	**0.016**	0.24	4.23

Dependent Variable: GS, Residual effect=0.124

[a]The symbol of traits consist of: DH, days to heading; DM, days to maturity; GP, the number of grains per panicle; GL, grain length; GB, grain breadth; UL, the length of the uppermost inter-node; PL, panicle length; PH, plant height; GW, 100-grain weight; NT, the number of tillers; FW, flag leaf width; FL, flag leaf length; GS, grain shape.

The results concerning direct and indirect effects of the studied traits on rice grain shape using conventional path analysis, where all traits were considered as first-order variables, with grain shape as the response variable (Model I), are presented in Table 2. Also, the results of two measures of collinearity analysis (Tolerance and Variance Inflation Factor) are presented in this Table.

As shown in Table 2, according to the conventional path analysis and collinearity analysis, the collinearity measure (VIF) was almost high for some traits such as DH (VIF = 15.25), PL (VIF = 11.55) and GL (VIF = 10.98). Also, is some cases the VIFs were close to 10 (GB, VIF = 0.98; PH, VIF = 9.69; FW, VIF = 9.85) suggesting the potential existence of multicollinearity.Results of the sequential path analysis are shown in Table 3, where predictor traits, as grouped into first- and second-order variables, and grain shape as the response variable (Model II). Compared with the model I, grouping predictor variables by means of stepwise multiple linear regression resulted in decreased VIF values for all predictors (Table 3). Thus, compared with model I, application of sequential path analysis facilitated detection of the actual contribution of predictors with minor confounding effects. Likewise, some researchers such as Mohammadi et al., (2003) reported that, sequential path procedure reduces collinearity problems leading to a better understanding of the interrelationships among the various traits and their relative contribution.

Normally, researchers are interested in testing the significance of statistics obtained in various statistical analyses. To perform such tests, standard error of the statistic is needed.

Table 3**.** Direct effects of predictor variables on the grain shape of 25 rice genotypes along with two common measures of collinearity values (tolerance and variance inflation factor, VIF) for predictor variables in conventional path analysis (CPA, all predictor variables as first-order variables) and sequential path analysis (SPA, predictors grouped into first- and second-order variables)

Predictor variables	Response variable	Direct Effect			Tolerance			VIF	
		SPA	CPA		SPA	CPA		SPA	CPA
GB	GS	-0.66	-0.61		0.48	0.10		2.10	9.98
GL		0.43	0.46		0.50	0.09		1.99	10.96
GP		0.07	0.10		0.73	0.11		1.37	8.86
GL	GB	-0.21	-0.43		0.83	0.12		1.20	8.42
GW		0.53	0.46		0.85	0.38		1.18	2.64
NT		-0.28	-0.25		0.81	0.20		1.24	5.08
DM		0.33	0.27		0.79	0.12		1.27	8.43
GB	GL	-0.84	-0.47		0.67	0.13		1.49	7.66
GW		0.42	0.26		0.67	0.23		1.49	4.36
FW	GP	0.69	0.39		0.93	0.13		1.07	7.96
NT		-0.23	-0.33		0.93	0.18		1.07	5.58

The symbol of traits consist of: DM, days to maturity; FW, flag leaf width; GB, grain breadth; GL, grain length; GP, the number of grains per panicle; ; GS, grain shape; GW, 100-grain weight; NT, the number of tillers.

Bootstrap analysis, as a re-sampling technique, provides an estimation of the standard error of any statistic. Results of the bootstrap analysis for direct effects are presented in Table 4. The low standard error and bias for all the path coefficients indicated the robustness of the model II in comparison with the model I.

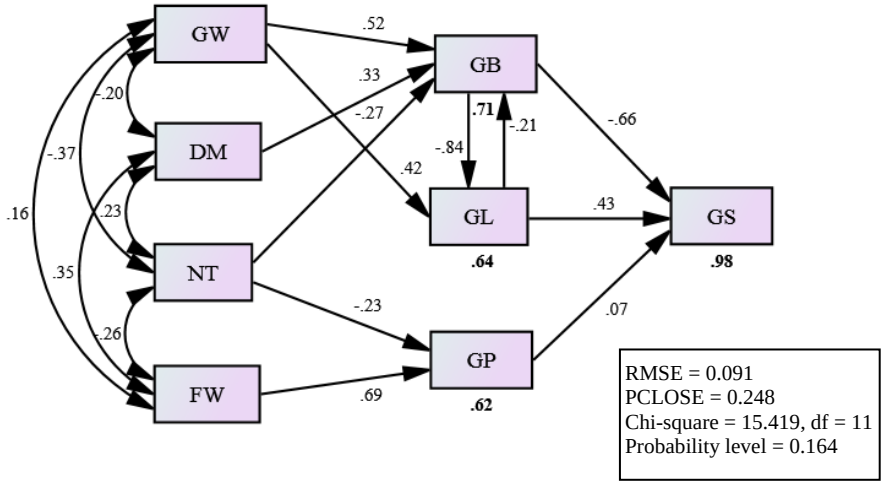
Table 4. Estimation of standard error values of path coefficients using bootstrap analysis with 200 bootstrap samples

Predictor variables	Response variable	Adj. R^2	Direct Effect		Bootstrap		
					Mean	Bias	SE
GB	GS	0.98	-0.662		-0.666	-0.004	0.029
GL			0.430		0.429	-0.001	0.026
GP			0.071		0.066	-0.005	0.037
DM	GB	0.71	0.333		0.333	0.000	0.079
GL			-0.214		-0.213	0.001	0.069
GW			0.525		0.526	0.001	0.081
NT			-0.275		-0.275	0.000	0.067
GB	GL	0.64	-0.840		-0.84	0.000	0.119
GW			0.418		0.425	0.007	0.129
FW	GP	0.62	0.694		0.692	-0.002	0.075
NT			-0.232		-0.243	-0.011	0.100

The symbol of traits consist of: DM, days to maturity; FW, flag leaf width; GB, grain breadth; GL, grain length; GP, the number of grains per panicle; ; GS, grain shape; GW, 100-grain weight; NT, the number of tillers. Calculation were carried out by usin AMOS statistical pakage (AMOS 2013).

Graphical presentation of the results facilitates understanding of the complex relationship among the various variables and their contributions. In this regard, the diagram of sequential path analysis drawn by using AMOS statistical package helps in better understanding of the complexity of relationships between

variables (Figure 1). The Root Mean Square Error of Approximation (RMSEA) fit statistic for the model was 0.091.



The diagram was drawn using AMOS statistical package (AMOS 2013). The adjusted R square values have been indicated by bold numbers below each of the dependent variables.
The symbol of traits consist of: DM, days to maturity; FW, flag leaf width; GB, grain breadth; GL, grain length; GP, the number of grains per panicle; ; GS, grain shape; GW, 100-grain weight; NT, the number of tillers.

Figure 1. Causal model diagram illustrating the interrelationships among various traits contributing to rice grain shape.

The PCLOSE represents the *p* value for testing the null hypothesis that the population RMSEA is no greater than 0.05 indicating a close fit of the model in relation to the degrees of freedom. Also, the Chi-square (CMIN statistic) value was equal to 15.42. The *p* value was 0.164 revealing a proper goodness of fit for the model.

Estimation of the adjusted coefficient of determination (Adj. R^2) revealed that GB, GL and GP as first-order variables accounted for about 98% of the variation in grain shape (Figure 1 and Table 4). Also, the results of sequential path analysis, when the second-order variables were used as predictors, and the first-order variables as response variables, indicated that GW, DM positively and GL, NT negatively influenced the GB and accounted for 71% of the observed variation. Moreover, the GW positively and the GB negatively influenced GL and accounted for 64% of the observed variation while, FW positively and NT negatively influenced the GP and accounted for 62% of the observed variation (Figure 1 and Table 4). Direct and indirect effects of first- and second-order predictor variables on response variables, including GB, GL, GP and GS have been presented in Table 5. According to this table, the indirect effects of DM and NT on GS were low while, indirect effects of FW, GW, GL and GB on GS were

high. Amongst second-order predictors, GW was a noteworthy trait with regard to its high direct and indirect effects on GB and GL. Also, NT had a negative direct effect on GP while, FW showed a relatively high positive direct effect on GP. As shown in Table 5, in this study, one of the remarkable advantages of using SEM was that the indirect effects resulting from second-order predictors on GS were also estimated.

Table 5. Direct (d) and indirect (i) effects for the predictor variables in sequential path analysis.

Response variable		predictor variables						
		FW	DM	NT	GW	GL	GB	GP
GL	d	-	-	-	0.418	-	-0.84	-
	i	-	-0.064	0.033	-0.933	0.219	-0.322	-
GB	d	-	0.333	-0.275	0.525	-0.214	-	-
	i	-	0.008	-0.004	0.007	-0.027	0.219	-
GP	d	0.694	-	-0.232	-	-	-	-
	i	-	-	-	-	-	-	-
GS	d	-	-	-	-	0.43	-0.662	0.071
	i	0.217	-0.071	0.035	-0.692	0.243	-0.931	-

The symbol of traits consist of: DM, days to maturity; FW, flag leaf width; GB, grain breadth; GL, grain length; GP, the number of grains per panicle; ; GS, grain shape; GW, 100-grain weight; NT, the number of tillers. Calculation were carried out by usin AMOS statistical pakage (AMOS 2013).

Identifying and analysis of associations between quantitative traits is important because, it can be used as a resource for designing selection-based breeding programs in the future. Most of the correlations obtained in this study were consistent with other studies. For example, likewise, significant positive correlations ($P < 0.01$) have been reported by Rabiei et al., (2004) between: GL and GS, GB and GW, GS and PH, DH and DM, DH and PL, PH and PL. Moreover, they reported significant negative correlations ($P < 0.01$) between: GL and GB, GB and GS, GB and PH, GB and PL which were totally in agreement with the results of the present work. Furthermore, in contrast to our results, they reported a significant positive correlation between PL and GP. Similar to the results of current work, a significant positive correlation between PH and PL was found by Hossain et al., (2015) and Kishore et al., (2015) while, Rai et al., (2015) reported it significant, but negative. According to our observations and in agreement with Hossain et al., (2015), the correlation between GW and GP was significant and positive while, Kishore et al., (2015) reported it significantly negative. A significant positive correlation between GW and GL was reported by Haider et al., (2014) and Kumar (2015) while, like current study, Rafii et al., (2014) found no such correlation. Similar to the results of this study, Hossain et al., (2015) found a significant negative correlation between NT and GP. They also reported a positive correlation between NT and DM while was in contrast to our observations. It seems that the discrepancies between the reports, regarding correlation coefficients between traits, are largely caused by working with

different populations, regarding the fact that diverse populations also have a different genetic structure. In this study, some relatively strong correlations were recorded such as FW-GP, DH-DM and PH-UL .Strong correlations are likely due to pleiotropy or linkage. QTL mapping is a method to answer whether linkage or pleiotropic effect is the responsible for the occurrence of a strong correlation (Chen and Lübberstedt 2010).

Previous researches have indicated that path analysis provides more information on the interrelationships between traits than correlation coefficients (Kozak and Kang 2006; Sabaghnia et al., 2010). Path analysis provides a framework for better understanding of how plant morphological components affect a final target trait in field crops through determination of the most important factors that directly and/or indirectly affect the final target trait.

In path coefficient analysis the amount that a trait contributes to yield is influenced by the different traits through different paths. Imprecise assessment of a trait's contribution through incorrect pathways may misdirect breeding attempts, thus limiting the efficiency in selecting favorable cultivars (Agrama 1996; Sabaghnia et al., 2010). Also the conventional approach for path analysis might result in a multicollinearity of variables, particularly when the correlations among some of the variables are high (Mohammadi et al., 2003). On the other hand, yield and shape-related traits are complicatedly interrelated, often leading to high multicollinearity. Thus, in this study, sequential path analysis was used to avoid the problems due to conventional path analysis and multicollinearity of variables. Such a strategy has been used before by researchers (Mohammadi et al., 2003; Sabaghnia et al., 2010).

Most of the studies on the path coefficient analysis in rice have been conducted to analyze yield and yield components. Rabiei et al., (2004) performed sequential path analysis to identify direct and indirect effects of traits affecting GS. Based on their results, GB, GL and PH were determined as first-order predictor variables whereas, GE, PL and GP were as second-order predictors. In the present study however, GP included in the path model as first-order variable rather than PH whereas, GW, DM, NT and FW were considered as second-order predictors. In this study, the adj. R^2 value calculated for each dependent variable was more than that of reported by (Rabiei et al., (2004)).

As mentioned by Kozak and Azevedo (2014), path analysis is an integral part of structural equation modeling. Despite of this, some researchers use standardized stepwise multiple linear regression to organize the variables into different order paths to determine causal relationships. Subsequently, they draw the path diagram manually by using common software such as MS office Word. In fact, to construct the path diagram, they integrate information obtained from each separate regression analysis while the standardized regression coefficients are used as the path coefficients. Such a method of demonstration of information may be accompanied with error because of the following reasons:

- By employing such a method, we assume that all of the model variables are perfectly measured (i.e. no measurement error). Hence, the ignored errors will downward bias of parameter estimates and leads to non-sign
- Normally, the model is structural when several paths are analyzed simultaneously. Therefore, inter-relationships among variables in the model would be complex. For example, in a sequential path diagram (similar to Figure 1) the first-order variables play two fully featured roles simultaneously as: (i) each of them acts as a response variable for the second-order predictors and (ii) they play the role of predictor variables for the main response variable. We don't see such a complexities in separate regression analyses.
- It is natural that there may be reciprocal effects between first-order predictor variables (see Figure 1) while, stepwise regression analysis can't estimate such effects because they belong to separate models and stepwise regression analysis can't integrate them into one model.

Therefore, it is always useful to switch to a full SEM using special software designed for this purpose such as AMOS. Although the process through which AMOS calculates the path coefficients is similar to a standardized regression, however, when we make the model sequential, (i.e. when we have both first- and second-order predictor variables in the model at the same time) the AMOS calculates path coefficients using a more complicated model. Hence, the outcome can't be equal as compared to the separate standardized regression coefficients.

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DOI: 10.17707/AgricultForest.62.4.25

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Enver KENDAL, Erol ORAL [1]

IDENTIFYING OF RELATIONSHIP BETWEEN TRAITS AND GRAIN YIELD IN SPRING BARLEY BY GGE BIPLOT ANALYSIS

SUMMARY

Grain yield is very important and complicated trait in spring barley breeding and rely upon combination varied plant traits (agronomic and quality). For this reason, a real barley breeding program necessaries an appropriate mentality of the relationships between grain yield and traits. The aim of study was evaluate the relationship of grain yield and other traits of 25 spring barley genotypes in one location during two years by GGE Biplot analysis. The experiments were performed according to a complete randomized block design with three replications. Factors (G, GE, and GEI) were found to be highly significant ($P < 0.01$) for grain yield. GGE Biplot indicated that three group were occurred among traits, first group (thousand grain weight, protein content, crude cellulose and cold damage), second group (hectoliter weight, lodging, plant height and heading time), third group (grain yield and seed humidity). Moreover, the study showed that negative correlation was found between grain yield and traits without seed humidity. The results of AMMI model and GGE Biplot indicated that G12, G13, G16 and G18 is proper for grain yield, G2, G6, G19 and G1 desirable origin for quality and other agronomic traits to select for advance stage and use in barley breeding program.

Keywords: Spring Barley; GGE biplot; Yield; Traits, Correlation

INTRODUCTION

The considerable variation in crop circumstances, because of climatic conditions and different soil constituents, cause large annual variations in yield performance of crops. This is mainly because of low heritability of yield as a typical quantitative trait. Thus, grain yield could be affected by not only genotype, but also by environment as well by and genotype × environment interaction (Mortavazian et al 2014).

Barley (*Hordeum vulgare* L.) is the second important cereal crop of Turkey and accounts for about 25% of the total cereal production (SAP 2010). In South-Eastern Anatolia, barley has been cultivated for many years and has a significant role. It is also grown mainly on rainfall conditions, but genotype × environment interaction (GEI) restricts the progress in yield improvement under

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

rainfed and unpredictable climatic conditions. Thus, experimental research needs to be carried out over multiple environment trials in order to identify and analyse the major factors that are responsible for genotype adaptation and final selection (Kilic 2014).

The yield of each variety in any environment is a sum of environment (E) main effect, genotype (G) main effect and genotype by environment interaction (GE or GEI) (Farshadfar et al 2013). Farmers need varieties that show high performance in terms of yield and other essential agronomic traits.

Modern barley breeding is largely directed towards the development of genotypes characterized with increased yield potential, wide adaptation and high responses to agronomic inputs (Przuli et al 2014). Some agronomic and technological traits such as lodging (LOG), plant height (PH), thousand-kernel weight (TKW), hectoliter mass (HM) and grain protein content (GPC) have significant influence on barley grain yield and quality.

Different statistical analysis, such as correlation, path coefficient and principal component analysis (PCA) can be used to reveal associations between yield and other agronomic traits. The impact of AMMI and GGE Biplot methods has been clearly showed by different researchers using multi-environment. This methods; provide the correlative size and significant effects of GEI and its interaction (Asfaw et al 2009), This method enables better understanding of genotypes performance over several environments, and selection of stable and high yielding genotypes (Mirosavlievic et al 2014), Also it is important for testing promising lines under across environments to estimate stability and performance (Hagos and Abay, 2013), and thus, it is useful for breeders and supporting breeding program decisions. The cultivars which are used in South-eastern Anatolia Region are different depending on sub- regions, as three main sub-regions have different conditions to cultivate barley cultivars. So it is very important to identify cultivars for specific sub-region (Kendal 2016).

The major objective of study reveal adaptation of barley genotypes using AMMI and GGE Biplot analysis to estimate the importance of GE interaction on yield, define the correlations among traits and estimate performance of genotypes and recommend lines to release in breeding program.

MATERIAL AND METHODS

The experimental material comprising twenty lines, three national and two regional varieties (Table 1) were evaluated in different growing season (Fig. 1).

The experiment was conducted in a randomized block design with four replications at two rainfall-growing seasons (during 2004-05-2005-06). The seeding rate was used 450 seeds m-2. Plot size was 7.2 m-2 (1.2 × 6 m) consisting of 6 rows spaced 20 cm apart. Sowing was done by winter stagier drill.

The fertilization rates for all plots were used 60 kg N ha-1 and 60 kg P ha-1 with sowing time and 60 kg N ha-1 was applied to plots at the early stem elongation. Harvest was done using Hege 140 harvester up on 6 m2. The climate data of growing seasons showed in Fig. 1.

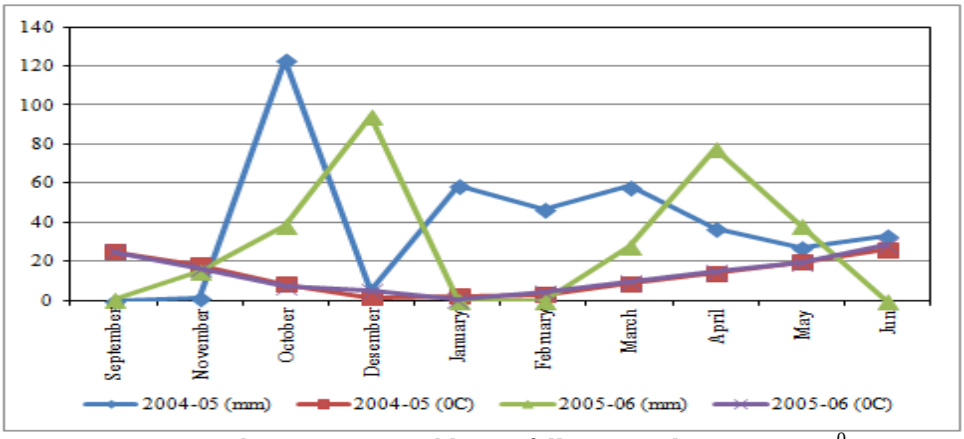

Fig.1. The average monthly rainfall(mm) and temperature(^{0}C).

Table 1. The information's about genotypes, used in experiment.

Genotype	Cultivar and /Pedigree of Province line
G1	GOB/HUMAI10/3/MPYT169.1Y/LAUREL//… CBSS95M00804T-F-1M-2Y-3M-0Y
G2	GOB/HUMAI10/3/MPYT169.1Y/LAUREL//... CBSS95M00804T-F-1M-5Y-1M-0Y
G3	FRESA CMB94A.917-D-4M-1Y-2M-2Y-1M-0Y
G4	FRESA CMB94A.917-D-4M-1Y-2M-2Y-2M-0Y
G5	1.1.1.ŞAHİN-91
G6	CEN-B/2*CALI92//ROBUST/3/LIGNEE640/DS/… ICBSS95M00871T-A-4M-1Y-1M-0Y
G7	GOB/HUMAI10/3/MPYT169.1Y/LAUREL//OLM… CBSS95M00804T-F-1M-10Y-1M-0Y
G8	CARDO/QUIBENRAS/3/ROBUST//GLORIA-B… ICBSS96WM00273T-C-1M-1Y-2M-0Y
G9	LBIRAN/UNA80//LIGNEE640/3/BBSC/4/CH… ICBSS97Y00828T-D-4Y-1M-0Y
G10	1.1.2.SUR-93
G11	LENT/BLLU//PINON ICBSS97M00698T-C-2M-1Y-0M
G12	CEN-B/2*CALI92//MINN DESC1/3/CABUYA ICBSS96M00727T-L-1M-1Y-1M-0Y
G13	TOCTE/QUINA/3/RHODES/CI14100//LIGNEE5…CBSS96M00842T-U-3M-2Y-1M-0Y
G14	GLORIA-BAR/COPAL//SEN/3/BBSC/… CBSS97Y00874T-D-3Y-1M-0Y
G15	1.1.3.TOKAK-157
G16	CABUYA/4/GLORIABAR/COPAL//BEN.4D/3/S… CBSS97Y00819T-D-2Y-1M-0Y
G17	WI2269/Espe/3/WI2291/Bgs//Hml-02 ICB97-0152-0AP-13AP-0AP
G18	Kv//Alger/Ceres.362-1-1/3/WI2269/4/Sara ICB93-0727-F7SSD-92AP-0AP
G19	Kv//Alger/Ceres.362-1-1/3/WI2269/4/Sara ICB93-0727-F7SSD-98AP-0AP
G20	1.1.4.VAMIKHOCA
G21	Sara/4/H.spont.96-3/3/Roho//Alger/Ceres362-1-1 ICB93-0698-F7SSD-43AP-0AP
G22	Hashma/4/Baca's'/3/Ac253//CI08887/CI05761 ICB97-0238-0AP-5AP-0AP
G23	Hashma/Kataf-01 ICB97-0239-0AP-7AP-0AP
G24	Line3229C26/3/Moroc975//WI2291/CI01387/6/Aths/… ICB97-1345-0AP-5AP-0AP
G25	1.1.5.AKHİSAR

Statistical analysis: The data grain yields of twelve (25) genotypes in two growing seasons were evaluated by AMMI analysis (Gauch 1988). The AMMI and GGE biplots were used to identify the mega- environments and superior genotypes for grain yield and other traits. All statistical analyses were performed using GenStat Release 14.1 (Copyright 2011, VSN International Ltd.) and GGE biplot software programs.

The data were graphically analyzed for interpreting GE interaction using the GGE biplot software (Yan and Thinker, 2006). GGE biplot methodology is composed of the biplot concept (Gabriel 1971) and GGE concept (Yan et al 2000). The graphs generated based on (1) The AMMI 1 model showing genotype x environment means, (2) Mega environments “which-won-where" pattern to identify the best genotypes in each season, (3) The relationship genotype by trait, (4) “which-won-where" pattern to identify the best genotypes for traits, (5) Ranking genotypes based on traits by mean and stability, (6) Comparison of genotypes based on traits by ideal genotype.

RESULTS AND DISCUSSION

The results of AMMI Analyses in grain yield: The variance of AMMI analysis showed that as $p<0.01$, the factors (genotype and environment) had significant effect on barley grain yield of twenty five genotypes tested in two growing seasons, while GEI was not significant (Table 2 and 3).

Table 2 The variance of AMMI analysis on grain yield of barley

Source of Variance	DF	Sum of square	Mean of squares	F Ratio	F_prob
Total	149	120248993	807040	*	*
Treatments	49	79415660	1620728	3.97	0.00000
Genotypes	24	52259398	2177475	5.34	0.00000
Environments	1	12103134	12103134	28.73	0.00000
Block	4	1685176	421294	1.03	0.39426
Interactions	24	15053128	627214	1.54	0.07387
IPCA	24	15053128	627214	1.54	0.07387
Residuals	0	0	*	*	*
Error	96	39148157	407793	*	*

df, degrees of freedom; **, $p<0.01$; G, Genotypes; E, Environments.

The high addition of environment effects showed that there were important differences between growing season for grain yield. The results of AMMI analysis showed similar results of Kendal and Dogan(2015) and Yan and Rajcan (2002), reported that the environment effect had the highest effect than other factors on barley and soybean grain yield respectively.

The results of Environment, Genotype and G x E effects obtained from this study illustrated similar results of the studies described above and the effect of environment >genotypes > GEI.

The existence interaction of grain yield displayed by GGE biplot, especially when the interaction portioned between two-interaction principal component axis (PCA) (Table 2). This status of GGE biplot made it establish and the biplot calculate effects of genotype and environment. The results of mean square of the interaction axis PCA 1 was significant ($p<0.01$), while PCA 2 was not significant. (Kendal 2015, Kilic 2014). Results of GGE biplot analysis also indicated that the PCA 1 axis accounted 78.58%, PCA2 accounted for 21.42% (Fig. 3). GGE biplot showed existence interactions of G x E, so it was portioned between first and second IPCA (Interaction Principal Component Axes).

The barley grain yield variation is depending on genotypic and environment factors as shown Table 1 and Fig 1. Gauch & Zobel (1997), Kendal and Dogan(2015), suggested that the AMMI model is the most accurate a model because it can predict using the first two IPCAs.

The closer the IPCAs scores to zero are meaning that genotypes are the most stable across their environments. Actually, these biplots is removed two types, model of AMMI 1 and model of GGE biplot (Carbonell et al 2004). In AMMI 1, the genotype and environments means are plotted on coordinate, the IPCA scores of same genotypes and environments, which are on the ordinate.

For interpretation of AMMI, size and signal scores of the IPCA1 were observed, score near to zero were typical of genotypes and environments, which contribute little to the interaction that is they are stable (Tarakanovas and Ruzgas 2006).

The AMMI model showing genotype x environment means of grain yield: In the AMMI model, x-axis represents the genotypes and environment main effect and y axis represents the effects of interaction (Fig. 2). The environment and genotypes indicated much more variability in both main effect and interaction. According to AMMI, majority of genotypes (G4, G9, G10, G12, G13, G14, G16, G18, G24, G25) showed good performance, because of they took place above on axis (mean yield). It is believed that these genotypes were high yielding and desirable.

On the other hand, G1, G2, G3, G15, G6, G7, G21 demonstrated low performance, due to they located under on axis (mean yield). So, these cultivars and environments, which located under on axis (mean yield) were low yielding and undesirable. Moreover, 2005-06 growing season had both high yield potential and positive IPCA1 scores; it means that this growing season is desirable, because of high rainfall. While G16 and G25 had highest grain yield amongst genotypes, G12, G16 was very stable with low and positive IPCA scores (Table 4). According to Mirosavlievic et al (2014), the genotypes have small IPCA1 values are more stable, Becker & Leon (1988) the basic static concept of stability shows minimal variance of stable genotype across different environments. Therefore, G17 can be recommended to all environments, while G8 and G23 for high yield potential environments (special environment). Similar outputs were recorded by Mohammadi et al (2013), in barley.

Table 3. The average yield performance of genotypes at two growing seasons (kg ha^{-1})

Genotype	2004-05		2005-06		Mean		IPCAg[1]
1	4417	aj	6283	cg	5350	HK	-1.940.078
2	5172	aj	4914	Ij	5043	IK	1.234.731
3	5731	af	5544	Ej	5638	DI	1.126.829
4	5608	ag	5972	Cı	5790	DH	305.114
5	5517	ah	5603	Ej	5475	EJ	973.276
6	4942	aj	5433	Fj	5272	HK	-138.945
7	4661	aj	5817	Cı	5239	HK	-877.658
8	6081	ad	7667	A	6874	AB	-1.520.920
9	5336	aj	6225	cg	5781	DH	-479.251
10	6017	ae	5664	Dj	5840	DH	1.375.833
11	6297	ac	7792	A	7044	A	-1.383.968
12	5747	af	6506	Cf	6126	CG	-284.197
13	5689	af	6636	Be	6163	BF	-566.402
14	5525	ah	6217	cg	5871	DH	-184.595
15	4608	aj	4686	J	4647	K	732.572
16	5672	af	6919	Ac	6296	BD	-1.014.611
17	5106	aj	6228	cg	5667	DI	-827.857
18	6094	ad	6267	cg	6181	BE	591.469
19	4517	aj	5064	Hj	4790	JK	0.31209
20	6647	a	6733	Ad	6690	AC	720.121
21	5053	aj	5483	Fj	5268	HK	205.512
22	5369	aj	5514	Fj	5442	FJ	632.970
23	5433	aı	5378	Gj	5406	GJ	931.775
24	5300	aj	6531	Cf	5915	DH	-989.710
25	6381	ab	6047	Ch	6214	BD	1.346.783
Mean	5477	B	6044	A			
CV(%)	10.0		11.2				
LSD	97.0**		111.9**				

Mega environments “which-won-where" pattern to identify the best genotypes in each environment: Dividing the target environment into meaningful mega-environments and deploying different cultivars for different mega-environments is the only way to utilize positive GE and avoid negative GE and the sole purpose for genotype by environment interaction analysis (Yan et al 2000).

A mega-environment is defined as a group of environments that consistently share the same best cultivar(s) (Yan and Rajcan 2002). This definition explain the following biplot based on the multi-environment trials (MET) data of barley yield illustrates two points: 1) A mega-environment may have more than one winning genotypes (sector 2), and 2) even if there exists a

universal winner (G12, G14), it is still possible, and beneficial, to divide the target environments into meaningful mega-environments (Fig. 3).

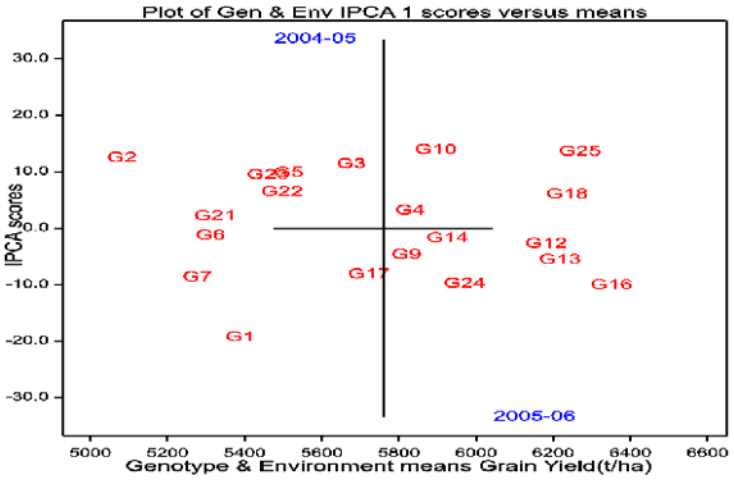

Fig. 2. The AMMI 1 model showing grain yield (kg/ha-1) of genotypes and growing seasons.

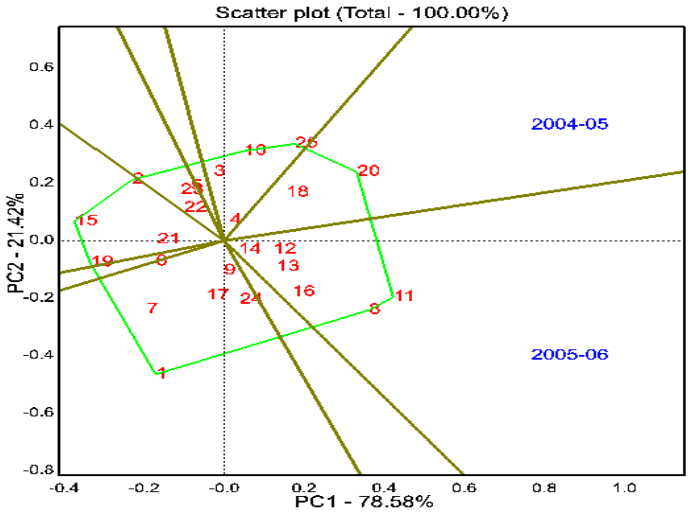

Fig. 3. Which genotype performed better in which environment for grain yield of barley

Mainly, these eight lines divide the biplot into eight sectors. The growing seasons located in different sectors; this means that the growing seasons had different ecological conditions. On the other hand, first sector consist of G11, G6 G23, G25 and G23 was high yielding and represented of vertex the 2005-06 growing season. The second sector consists of 2004-05 season with G18, G20, took places of vertex of this sector. The other sector just consists of some

genotypes, which are not related with study growing season. Consequently, G11 had high yielding at 2005-06 growing season; while G20 for 2004-05. The result of this study showed that G11 is suitable to recommend to high potential growing season, while G20 for low potential growing season. Solonechnyi et al (2015), reported that there is a strong correlations between growing seasons, which located in same sector. Sarkar et al (2014), the large variation due to location indicated strong influence of environments and existence of mega-environment among trial conducting locations, this suggests the usefulness of GGE biplot technique for identifying mega- environments among barley growing locations. Mortazavian et al (2014), reported that the GGE biplot graphic analysis complements the AMMI biplot stratification, defining mega-environments and the cultivars that optimize performance in such mega-environments.

The GGE Biplot Analysis of genotypes by traits: In this analysis, the results of traits(Table 4) were examined by GGE Biplot analysis using different figures. The biplot of the principal component analysis illustrates relationships between the studied barley traits and genotypes at five environments (Figure 4-Figure 7). First PCA explained 29.85% of total variation, while second PCA explained 22.29%. Together, both axes accounted for 52.14% of the total variation in the data. According to the biplot figures, the relations between genotype and traits by traits were examined. The GGE Biplot showed that the breeders could select best genotypes for all traits and specific genotype for specific trait in breeding program.

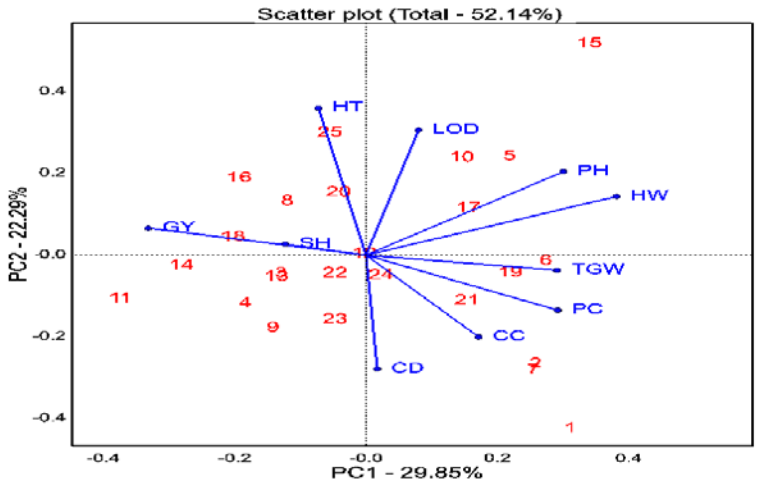

Fig. 4. The relationship genotype by trait in five environments

Therefore, the results of traits showed that there is high variation among genotypes. According to results, G11, G14 and G18 are desirable for GY, G6 and G19 are desirable for quality, so they can be recommended for release. On the other hands, all standarts(G5, G10, G15, G20 and G25) and G8 ,G16 had been late maturity time, very tall and lodging scores, so these genotypes is not

recommend for growing season. The genotypes which located near to the biplot center(G20) have less contribution to G or GE, while genotypes having longer vectors show the most contribution of G and/or GE (Yan and Tinker 2006). When the angle between two genotypes is >90°, then this two genotype are different as genetic. So, G11 with the longest vectors is the best genotypes for grain yield, while G6 for TGW and PC. The genotypes are far from center of Biplot graphs, are specific genotypes(G1, G15 and G11) for specific trait.

Table 4. The traits value of average of 25 genotypes across 5 environment.

Genotypes	Grain yield (kg/ha^{-1})	Heading time (date)	Plant height (cm)	Thousand grain weigh (g)	Hectoliter weight (g/l)	Protein content (%)	Crude cellulose (%)	Seed humidity (%)	Lodging (%)	Cold damage (Scor.1-9)
1	5350	104	98	47.5	73	12.3	6.05	8.4	0	6
2	5043	103	95	49.6	72	12.7	6.10	8.5	20	7
3	5638	110	88	43.1	65	11.1	5.45	8.6	30	5
4	5790	110	85	41.0	63	11.6	5.55	8.6	30	6
5	5560	118	103	47.6	75	12.4	5.95	8.7	30	3
6	5188	113	105	40.3	73	12.7	5.35	2.8	20	7
7	5239	104	95	50.6	73	12.9	5.85	8.3	0	6
8	6874	118	100	39.9	70	11.6	5.45	8.6	20	7
9	5781	120	95	34.5	65	12.1	5.60	8.4	0	7
10	5840	118	105	44.5	75	11.9	5.55	8.5	20	4
11	7044	112	88	35.0	61	11.6	5.55	8.5	0	5
12	6126	113	98	42.8	70	12.2	5.00	8.6	2	5
13	6163	110	95	40.0	68	11.0	5.60	8.5	0	5
14	5871	120	88	39.3	63	10.2	5.70	8.7	0	2
15	4647	117	110	42.3	76	12.5	5.60	8.6	80	0
16	6296	112	95	36.1	66	11.1	5.45	8.7	60	4
17	5667	110	103	43.8	73	12.2	5.75	8.8	30	3
18	6181	113	95	37.8	66	11.1	4.75	8.6	0	4
19	4790	105	110	38.4	74	11.8	6.05	8.8	0	4
20	6690	111	105	42.1	74	10.7	5.15	8.8	0	3
21	5268	108	103	40.1	71	12.6	6.15	8.5	0	5
22	5442	110	93	38.1	65	12.8	5.35	8.5	0	1
23	5406	107	93	38.1	65	12.4	6.10	8.9	0	3
24	5915	109	103	38.8	71	12.0	5.60	8.8	0	5
25	6214	113	98	44.0	71	10.3	6.10	8.6	50	1
Mean	5457	111	96	39.7	63.2	12.3	4.9	10.7	28.5	3.9

There are negative correlation between two traits, are opposite to each other(GY-TGW, HT-CD) on graph and the angles of vector is >90°. Therefore, there is major contribution of trait to traits; because of they have opposite direction, so they can make up different genetic contribution (Jalata 2011).

"Which-won-where" pattern to identify the best genotypes for traits:The GGE biplot visualize the correlation amongs tratits and grouping them also to visualize the interaction patterns between genotypes and traits (Yan and Tinker 2006). Visualization of the "which won where" pattern of MET data is necessary for studying the possible existence of different more traits in the target traits (Yan et al. 2000). Fig. 5 represents a polygon view of barley genotype MET data in this investigation. In this biplot, a polygon was formed by connecting the vertex genotypes with straight lines and the rest of the genotypes placed within

the polygon. The partitioning of GT interaction divided into three group. The traits(TGW,PC, CC and CD)took place in first group and related with G6, G19, G21, G2 and G7; HT, LOD, PH and HW in second group and related with G5, G10 and G25; GY and SH in thirth group and related with G13 and G14.Other genotypes did not related with any group of tratis. On the other hand, there were correlation amongs tratis which took places in same group. it means that the genotypes, have late heading time, are very tall and have lodging features as well as. The vertex genotypes in this study were G1, G15 and G11. These genotypes were the best for special traits or the poorest genotypes some for all of traits because they were farthest from the origin of the biplot(Yan and Kang 2003).

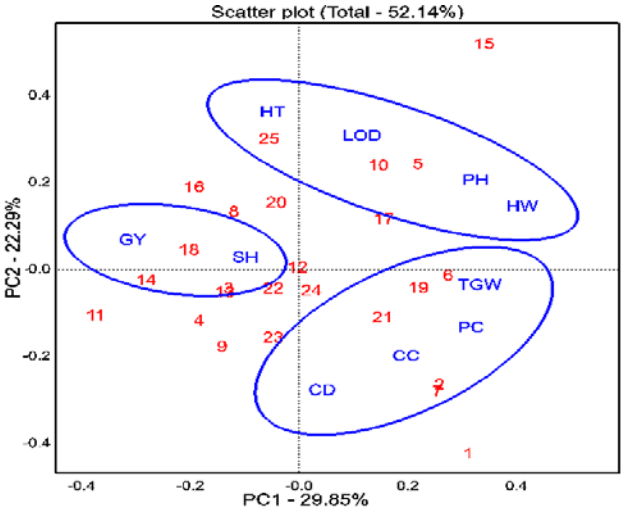

Fig. 5. Gge biplot showing the performance of each cultivar at each environment.

Ranking genotypes based on traits mean and instability: the genotype has both high traits mean and high stability is called a favorable genotype. It should have both high mean performance and high stability for all traits (fig. 6). The center of the concentric circles (ideal) is a point on the aea(“absolutely stable”) in the positive direction and has a vector length equal to the longest vectors of the traits on the positive side of aea (“highest mean performance”). Therefore, genotypes located closer to the stabile line and has high mean values of traits are meaning that it is more favorable than others (yan and tinker, 2006; farshadfar et al 2013.thus, g17 is located center of aea (“absolutely stable”), but; g5, g6 and g15 took place of near center of aea and high mean of traits. So these genotypes are favorable than others. According to fig.5, the g15, g5 and g6 are low stable and more favorable, while g17 are “stable” and favorable, because this genotype has high mean value of traits. From this example, we can recommend g17 and g5 to study for more traits. On the other hand, some genotypes (g11, g14, g4, g9, g18, g13, g16, g23, g8 and g22) were unfavorable, because these genotypes had low mean values of traits.

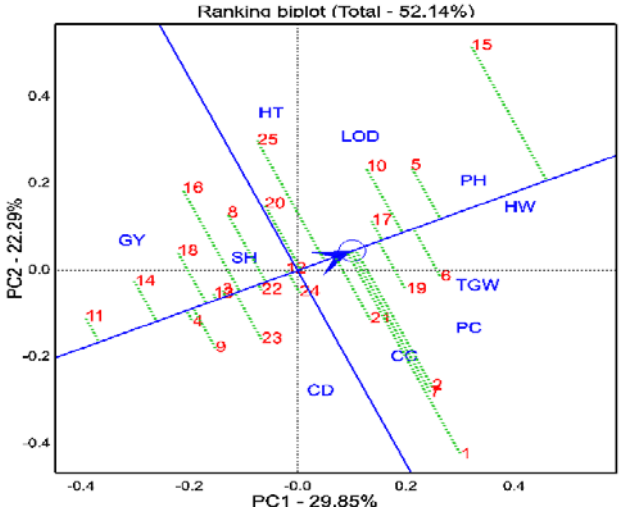

Fig. 6. Ranking genotypes based on traits mean and instability

Comparison of genotypes based on traits by ideal genotype: the genotype has both high traits mean and high stability is called a ideal genotype (fig. 7).

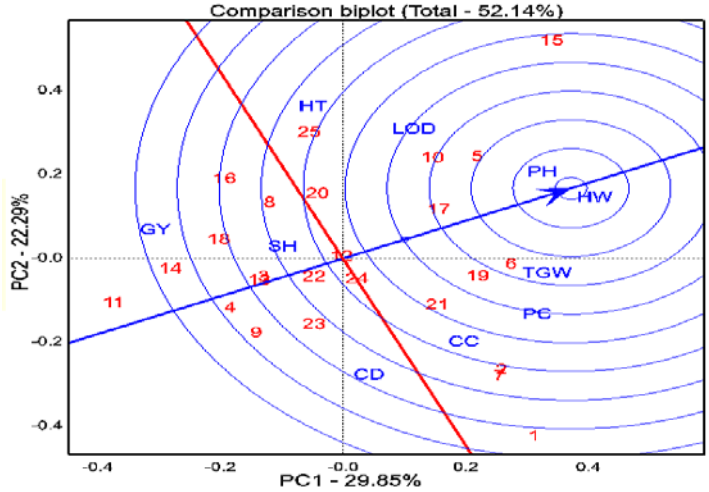

Fig. 7. The relationship genotype by trait in two seasons

The center of the concentric circles is a point on the aea (“absolutely ideal”) in the positive direction and has a vector length equal to the longest vectors of the traits on the positive side of AEA (“highest mean performance”). So, genotypes located closer to the ideal circle are meaning that it is ideal genotype than others (Yan and Tinker, 2006). In the study, any genotype was not located center of AEA (“absolutely stable”), but; G5, G6 and G17 took place of near center of AEA. So these genotypes are ideal than other genotypes.

Consequently, G6 and G17 are close to ideal genotype, so, these genotypes can be recommended for release in terms of all traits.

On the other hand, more genotypes located far from ideal genotype and, these genotypes (g11, g14, g4, g9, g18, g13, g16, g23, g8 and g22) are not ideal genotypes to study and release. The researchers reported that the biplot show excellent discriminating to select genotypes for all traits and to recommendation for release (sayar and han, 2015).

CONCLUSIONS

The GGE Biplot and AMMI results indicated that yield performance of barley genotypes were highly influenced by growing season conditions (rainfall, hot stress). The genotype G12, G13, G16 and G18 demonstrated best performance among genotypes tested growing seasons, while G2 had least grain yield and adaptability. Therefore, the G16 was desirable in terms of high mean yield and stability; this means that the study provided an indication of the genetic progress. According to the results, the specific genotypes were appropriate for specific traits (G6, G19 and G21 for quality, G14 and G18 for GY, G2 and G7 resistance to cold damage). The AMMI method and GGE Biplot analysis permitted a meaningful and useful summary of GE interaction data and assisted in examining the natural relationships and variations in genotype performance across test growing seasons. As a result indicated that that G12 and G16 are suitable to recommend for release and G6, G19 and G21 valuable source for quality to use in barley breeding program.

ABBREVITATIONS

AMMI, Additive main effects and multiplicative interaction; AEA, average-environment axis; AEC, average-environment coordination; GE, genotype by environment interaction; GGE, G + GE; MET, multi-environment trials; PC, principal component; PCA, principal component analysis; E, environment; G, genotype; TGW, thousand grain yield; HW, hectoliter weight; PC, protein content; SA, sieving above; LS, lower sieving.

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Marta VUKOTIĆ LAZAR
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ORGANIZED PROVISION OF GREENERY IN BELGRADE IN ORDER TO UPGRADE THE QUALITY OF LIFE WITHIN THE CITY

SUMMARY

This work is the outcome of the long-time experience in urban planning and architecture of Belgrade and consequently accomplished researches concerning the beginnings and development of the organized provision of Belgrade's greenery.

Urban planning in Europe takes a new course in the 19th century, conditioned with the sudden transformations in the economic and social structures of cities, impelling accelerated development of industries and transportation. Characteristics of urban planning of that time are reconstructions of the old city entities (reconstructions of the existent cities) and their sprawl (planning of the new cities), permanently marked the architecture of Serbia of that period. Induced by the abrupt and uncontrolled urbanization, the second half of the 19th century was characterized by the increased perception concerning the importance of environmental preservation and increased need to develop city greenery, parks, sports and children playgrounds, also of the development of forest parks and weekend getaways. Besides, induced with the modern planning from the end of the 19th century, urban legislation - subject matter of the research - has been developing and improving ever since. In that sense, along the lines of this Work, the attention was directed not only towards the changes taking place in the design of the space by interaction of natural and human factors - significant for understanding of human communities and organization of social life in general - but towards the lives of the pioneers of this profession, public activists and 'hygienists', as Maksimović (1974) called them – towards their tireless missionary work and persistence in their struggle for the revival of the old and in building of the new, healthier cities - Emilijan Josimović (Stara Moldava, 1823 – Soko Banja, 1897), first Serbian urban planner and Aleksandar Krstić (Kragujevac, 1902 – Beograd, 1980), horticulture engineer and the first Serbian garden designer. The first worked in the second half of the 19th century, and the second between the two world wars and after the Second World War. Time limit of the research period is between the years 1867 and 1941.

Keywords: urban planning, horticulture, sustainable development, heritage, Emilijan Josimović, Aleksandar Krstić

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

INTRODUCTION

As a contribution to the studying of the beginnings in the organized provision of greenery, the intention of the Work is to stimulate the past development research in this field, Also to contribute to the clarification of life and work of certain authors. because their opus is the inexhaustible source of knowledge, significant not only for preservation of cultural heritage, but for the development of different elements of the urban space in general and for the most up to date question - sustainable development (Stovel: 2003; Jokileto: 2014; Jokileto: 2015; Nikolic: 2015; Vujcic et al.: 2016).

Its intention is to open numerous questions and to show how very important is the studying of a city past as well as of the history of the urban planning ideas and initiatives, within the context of the pioneers' opus, including their progressive tendencies in the history of the urban planning thoughts and practice, initiators of the organized care about greenery in these spaces. The Work presents and analyzes the First Urban Plan of Belgrade from 1867, with special emphasis on the green belt of Belgrade - Emilijan Josimović, the first Serbian urban planner in the second half of the 19th century, as well as the work of Aleksandar Krstić, horticulture engineer and the first Serbian garden designer, important not only for the period between two wars, but for the period after the Second World War till the end of 1970's (the work is dealing with his opus between the wars).

A comprehensive published material - books, texts, studies and newspaper articles, as well as cartographic and planning documentation – has been collected and analyzed - direct testimony about the researched periods of these two pioneers of contemporary urban planning theory and professional achievements. Their work was viewed within context of the new standards in planning and construction, as well as environmental regulations, demands unification of the complete constructions law. With their big knowledge and experience, these two tireless professionals left the permanent mark not only in Belgrade, but everywhere in the ex Yugoslav space, where their work later had numerous and fruitful followers.

The goal of the research was not exclusively making chronology of events within the given marginal years of the researched period, though also clarification and comprehension of connections and relations the events from the past are transferring, not only within the given limits but beyond, up till today. Professional-scientific sustainability of the research for this paper reflects in consideration of the first urban plan in Serbia, Emilijan Josimović (Stara Moldava, 1823 – Soko Banja, 1897) in the context of Belgrade's green belt, and activity of Aleksandar Krstić (Kragujevac, 1902 – Beograd, 1980) in relation to the lessons of this 19th century plan, during many years of his work in design, reconstruction and realization of the great number of Belgrade's parks, squares and alleys. Modifications in shaping the space as the elements of social changes, also the creative work of the pioneers of Serbian urban planning and organized greenery of Belgrade care were also elaborated. The special review was given

concerning their books, studies and texts, published in newspapers and periodicals, impossible to be avoided while dealing with the history of urban planning and horticulture in these spaces.

Induced by modern planning up till the end of 19th century, urban legislation has been developing and improving, also the subject matter of this paper.

MATERIAL AND METHODS

In function of the integral consideration of the subject matter and achievement of the given goal of the research, several scientific methods were used systematically as the benchmarks for the research procedure used to check out proposed hypothesis. Historiographical method was applied, harmonized with the subject matter and goals of the research. The focus of the work has been the collection, classification, systematization and analysis of the archives' materials (plans, old photographs, drawings, documents, correspondence, etc), also of the published materials (laws, bylaws, regulations, registers, etc.), as well as published periodicals and literature from the period within the given time of the research, though from the earlier period as well.

The research (collecting of materials and data) was carried out in museums, libraries, archives, specialized institutions, urban planning and geodetic institutes, etc. A comparative analysis was applied in order to compare activities of the authors, pioneers of the organized care about the greenery of Belgrade, with special emphasis on Emilijan Josimović and Aleksandra Krstić, aiming to make objective conclusions and correct valorization of their contributions to the development of urban planning in Belgrade, with special review of their contribution to the organized care of the greenery of Belgrade.

By using the method of critical analysis, the checking out and founding of the given hypothesis and results of the research has been carried out. As an ultimate result of the research and analysis, the conclusions about the work of Emilijan Josimović and Aleksandar Krstić has been made, the first in the second half of the 19th century and the second in the 20th century, about their work, place and part in the development of Belgrade. The significant knowledge is that each of the authors implemented the elements of personal attitude and creativity in his own projects, not for a moment violating the precisely defined rules of constructions, with a stronger legal foundation in the defined period. All the things done made a more complete picture about their significance and their pioneer work in the beginnings of the organized development of the Belgrade's greenery, and the intention of work is to present these results.

RESULTS AND DISCUSSION

Judging by today's matrix of the city with few preserved public building from the period of Turkish reign (period of Otoman Impery), it is not easy to conclude that Belgrade was a significant settlement of the ottoman empire, with population of almost 100,000 and with characteristics of the eastern town (mosques, caravanserais, bezistans, amams, etc). Buildings from that period were

mostly demolished during Austro-Turk wars in 17th and 18th century, the others dilapidated because of the friable construction material, and those lasted up till the 19th century were demolished after the liberation of the cities in Serbia (Đurić – Zamolo: 1977). The lack of plans for the first half of the 19th century is illustrated with the fact that only less than twenty plans is known in that period in comparison with more than hundred plans in the last decade of the century. Among these scarce plans 'measuring of the alleys' from the beginning of the 19th century, a special place is reserved for the five fragmentary sketches, connected with the choice of certain sites, land use and new streets layouts, signed all by Frantz Janke, the Slovak (places and years of birth and death unknown; who lived in Belgrade from 1834 – 1843) "pravitelstveni indžinir" of the prince Miloš Obrenović, also the designer of the various significant buildings in Belgrade at the time, constructions were the messengers of novelties from the west, indicating all together intensive planning activity in that period (Đurić – Zamolo: 1981). From that period is also the precious cartographic document "Turkish plan of the city and the borough in the trench" from 1863, kept in the national library of Serbia, the plan containing 172 different structures and localities in Belgrade, as well as all the registered houses in the city with the ethnicity of the owners - residents. It was politically covered, precisely with the special use to show ethnicity and ownership in different parcels for the borough within the trench (Golubović: 2006).

In that period great merit for the development of greenery in Belgrade and its surroundings belong to Atanasije Nikolić (Bački Brestovac, 1803 - Beograd 1882), the associate and the man of confidence of the prince Mihailo Obrenović and Ilija Garašanin, lyceum professor and a distinguished engineer. He has been lecturing agriculture from 1840, and founded in 1853 agricultural (zemljodelsku) school in Topčider aimed to improve it. Up till 1859 when it ceased to exist, more than 200 students graduated this school. In that place Nikolić created the experimental estate and planted Topčiderski Park. He initiated the edition "Poljoprivredni list" and wrote manuals to be used in the lecturing of Zemljodelska school (crop farming, grape growing, fruit growing, forestry, etc.). He elaborated concept designs for the Topčider park, Košutnjak and fruit nursery garden in Topčider (flooded fen in 1850), and started planting of numerous alleys along the streets of Belgrade, decorating Terazije with multiple lines of chestnut trees and in the streets Kneza Miloša, Abadžiska and Ministarska with poplar trees, existing in Topčider road even today (Šolaja and Magdić: 1994; Šolaja : 1995).

Adopting of the legal and later planning regulations - despite tough economic and social conditions - will influence not only the development of urban planning and construction, though also the overall cultural life in Serbia. Towns will gradually acquire planning regulated structures, dominated by prince' residences - konak, courts, private houses, mehana, schools, quarantines, etc. A set of law and bylaws was adopted in 1860' regulating urban planning and settlements. Two laws adopted 1866 are the most significant: "law on

settlements“ classifying urban type settlement as towns and small towns - varošice and the „law on expropriation of the private real estate for public use, with compensation, one of the first such type laws in Europe (Roter – Blagojević: 1998). Founded on these two laws in september 1867 the state council considered ”draft law about regulation of the Belgrade town“, suggesting establishing of a commission to adopt “new regulation plan of Belgrade town“, the aim was to stop previous practice of partial regulation of the city and to establish legal conditions for the implementation of planning regulation in Belgrade. The law could not be adopted due to financial and technical reasons.

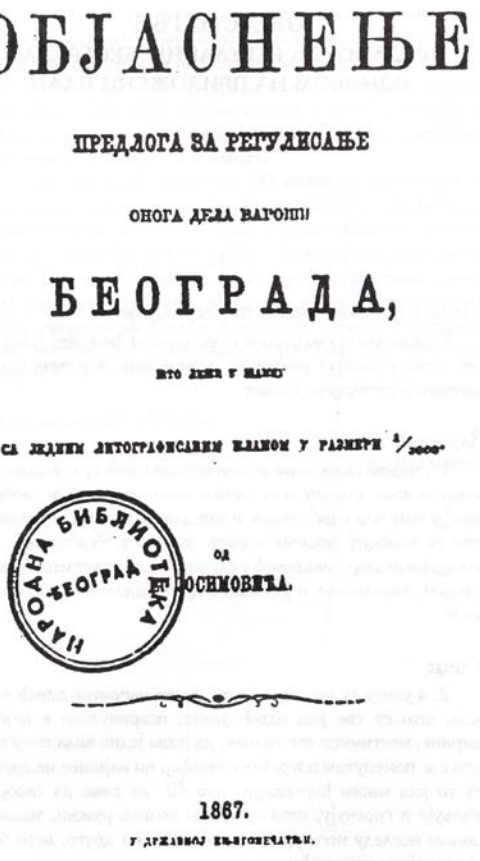
The period between 1867 and the beginning of the First World War is exceptionally important for the growth and development of Belgrade. All the street networks, city blocks, squares and parks of the nowadays central parts of Belgrade - with their good and bad characteristics - are coming from this period. The beginning of the period is marked by the final surrender of Belgrade under Serbian rule and elaboration of the first urban plan of the borough in the trench. When the urban planning regulation is in question, significant were modifications of the “law on settlements“ from 1866, among them of exceptional importance was the obligation that all the towns and small towns must elaborate their regulation and terrain leveling plans. One article was supposed to define the settlement limits – city territory where construction rules are applied, as well as municipality obligations about communal-infrastructure provision within the territory (Roter – Blagojević: 1998).

In solving out the issues of future development of the old borough in the trench, the main role – legalized on the appointment of the state council and construction ministry – was given to emilijan josimović, professor of mathematics, geodesy and architecture in the gunnery school, lyceum and university professor and the first Serbian urban planner. “Plan of the old Belgrade (part within the trench) as it is now and as it was regulated“ from 1867, was elaborated on demand of the prince Mihailo Obrenović and it is the most significant Serbian document for the regulation of Belgrade borough in the 19th century. In urban planning sense, it follows modern European tendencies and trends, deserving for Josimović the honor of the first Serbian urban planner.

Although Josimović’ work was connected to Belgrade, it has wider significance, because it became the first example for the reconstruction of the inherited towns in Serbia. His book following this plan titled “the explanation of the suggestion for regulation of this part of the city of Belgrade within the trench with one lithographed plan, the scale 1:3000“ (the plan in the end of the book as an appendix), where Josimović wrote and published his understanding of the problem and methods of the reconstruction – the first theoretic urban planning work in Serbia. (figure 1). The importance of Josimović’s work for the later development of the urban planning in Serbia is seen in the fact that in the same 1867 the project of “The law on regulation of Belgrade town“ was presented to the state council, accomplishing the influence of his work on the creation of the first urban planning law in Serbia.

Figure 1. Emilijan Josimović, Old Belgrade (The Area in the Moat) as it is today and as it was regulated, from 1867.

For the reconstruction of the old town, he used the integral analysis of structure – the existent situation - mainly consisting of clearing out of all the drawbacks, elaborated in the chapter of the book titled "City drawbacks – objects of regulation". The procedure applied, in words of Branko Maksimović (1967) only a long time later would become obligatory part or urban planning elaborations and practice. This renown urban planner was suggesting even then making connection of the forest-park belt by the Danube - 250 meters wide - with the Kalemegdan park, and consequently with the Sava riverside. Pointing out natural and geographic position of Belgrade, he thought that the city on the banks of the Sava and Danube, two waterways, must get the key around the town, adjusted to the river transport, as well as the basin dock besides the Danube, main waterway of Europe, in his words.


ОБЈАСНЕЊЕ

ПРЕДЛОГА ЗА РЕГУЛИСАЊЕ

ОНОГА ДЕЛА ВАРОШИ

БЕОГРАДА,

СА ЛЕДЕЊИМ ЛИТОГРАФИСАНИМ ПЛАНОМ У РАЗМЕРИ 1/3000.

1867.

У ДРЖАВНОЈ КЊИГОПЕЧАТЊИ.

Насловна страна „Објашњења..." из 1967. године

Figure 2. Titlepage of the book Justification of the Proposal for regulating the Area of Belgrade in the Moat with the aid of a lithographed plan, scaled at 1 : 3000.

He was also suggesting sanitization of the Danube riverside area - occasionally flooded - considering that the space should be forested with a special kind of trees, succeeding in the wetland. Together with josif pančić, he marked out the two hectares space in that area for the location of the botanical garden.

When the lack of the city greenery was in question, he openly criticized careless treatment of the authorities, giving even credit to turks that they'd paid more attention to it, being taken in that time less as an objective professional opinion, and more as an insult (Terzić: 1994). He was suggesting refilling and

leveling of the trench spreading around the old town and that the old Belgrade would be later encircled from the Sava to Danube with venci, planted alleys, with separate pathways for pedestrians, cart and riding transport. Suggested seven garlands ("sedam venaca") were spreading two and half kilometers long, today left only three of them: Obilićev, Topličin and Kosančićev venac. The green belt around the old town had in his opinion double importance, first, the city would get belts of parks of around 50.000 square meters, and second, such a belt would preserve historical contour of the Belgrade trench, partially preserved even today in the broken directions of the three preserved city garlands and towards the Danube with the line of Skadarska street. Josimović also suggested that Kalemegdan should be developed as the city park, as well as the space of Velika pijaca (Big market – Students square nowadays), and from the six suggested smaller parks in Belgrade, only two were realized - in Topličin venac and in front of the house of the army of Serbia. His visionary and the most courageous suggestion was the tunnel under Kalemegdan what would enable convenient and fast connection between the Sava key and the Danube port (Maksimović: 1967).

Although neither many of his ideas nor the street network of the borough in the trench were not completely realized as he imagined them in 1867, the essence of the Josimović solution was preserved and witnesses about the first and strong steps towards modernization and europeanization of Belgrade.

Up till the end of the first world war

Construction works in Belgrade were in accordance with regulation plan and in the limited construction territory. After the war, destroyed and demolished city became the capital of the kingdom of Serb, Croats and Slovenes (SCS), and later kingdom of Yugoslavia (1929). First years after the liberation were characterized by clearing the terrain, rehabilitation and construction works, and in the same time the city was facing uncontrolled population increase. The fact that all the state institutions and administration of the new kingdom were concentrated in Belgrade as the capital, the abrupt population growth was highly expected. General urban plan of Belgrade adopted after the international open competition in 1924, and then the other known as the "Obradović' regulation plan of Belgrade" from 1927, were incapable to follow growth and development of Belgrade. While the plans were adjusting to the various demands and circumstances, the city was shaping spontaneously, regardless of the plans, according to the wishes of its inhabitants. So called "informal construction" became one of the immediate problems. Belgrade started to sprawl towards its hills, where the land was cheap, though without infrastructure – streets pavement, lighting, sewerage, etc. Despite the big and numerous obstacles, during the period 49 settlements of various types were founded in: Karaburma, Pašino Brdo, Dušanovac, Voždovac, Banjičko Selo, Topčidersko Brdo, Mihajlovac, etc. (Ćorović: 2009). In order to stop and prevent "informal constructions" during 1923 – 1929 period, preparations for the design of various regulation plans were carried out: Topčider, Košutnjak, Bulbuder, Laudanov Šanac, Pašino Brdo, Voždovac, Čukarica and Marinkova Bara, as well as the outskirts of Belgrade. It

was also the period of elaboration of the general urban plan and of solving out numerous issues in the city by using the urgent procedure. Those were the years when the concept of living known as "Garden city" according to the idea of Howard Ebenezer was promoted in the capital for the first time, as well as the idea of modern architecture and its socioeconomic significance. In the suburban parts of Belgrade "colonies" and "koteži" started to grow (both terms are synonyms for the improvement of the quality of housing and life in general), and the first settlement founded in Belgrade according to the principle of "Garden city" was "Kotež neimar" (1924 – 1930).

In the period between two world wars

From the very start the authorities adopted the policy that the issue of the parks development (public and private) was in the close feedback with the regulation, growth and development of the city. A modern urban regulation, concerning health care and aesthetics, is impossible to plan without sufficient city greenery (Maksimović: 1930). Even then, „housing hygienic" got a new and important dimension. Belgrade municipality - via the "department for parks and forestation" – worked a great deal to promote this issue. Greenery ceased to be treated only as a part of communal activities, but with its role for embellishment, recovery and upgrading of settlements, more precisely for life in general. Unoccupied space was treated as "fresh air reservoirs" planned to be accessible and available to the citizens in any moment, and only a small number of the public parks areas was - for security reasons - planned to be closed by night (glass gardens, botanical garden, zoo, etc.).

Construction law (1931) and Construction rulebook (1935), regulate relation between horizontal projection constructed and the total area part of a lot (index of occupancy). The rulebook definitely determined that proportion for the particular zones, what was of vital significance for individuals, owners, also for communal, hygienic and esthetic issues in the large sites of the very important and expensive city construction land properties. Municipal authorities was taking care of the communal equipment of every square meter in its territory, leaning primarily on law enforcement, and also using propaganda, appealing on civic consciousness and other similar methods. The shortage of parks, children playgrounds and sports grounds has been for years a grave city problem. Visionary concepts of Emilijan Josimović about the pars belt around the old Belgrade town were left unrealized, though after the first world war municipal authorities made the decision to make all the necessary steps to realize numerous ideas concerning the city greenery, not leaving them aside anymore.

Natural position of the main promenade in Kalemegdan, encouraged the authorities to initiate its extension, in order to develop a new terrace in the lower level, connected by the staircase with it, giving Kalemegdan park a good connection with the fortress (1928-1930). "concept design of the sava promenade with big staircase in Kalemegdan" is the work of Aleksandar Krstić (Kragujevac, 1902 – Beograd, 1980), horticulture engineer and the first Serbian garden

designer and longtime head of the department of parks and reforestation in the Belgrade municipality, who was later the chief manager of the city greenery and economy. In the period when he worked in the department of parks and foresting, as well as after the Second World War, for almost twenty years Aleksandar Krstić was designing, reconstructing and developing most of Belgrade parks, squares and alleys (the area around Serbian national assembly, Kalemegdan, Topčider park, Voždovac park, etc.). He stays in the similar positions after the war (directorate general for spas of NR Serbia, park service; head of the biological-technical service in the institute of hygiene of NR Serbia, and from 1953 he was professor in academy of applied art. His significant works from the period are numerous spas in Serbia, memorial cemetery of the liberators of Belgrade, development of national parks Lovćen and Durmitor in Montenegro, etc.). As the first garden designer, he was the member of association of applied artists and designers of Serbia and was the founder of association of garden experts of Yugoslavia. He spent almost fifty year of th3 200th century working and struggling for his profession. (Milanović: 2006). Air pollution in the second part of the 20th century is multiply increase as the consequence of the equal increase in population of Belgrade, emphasizing significance of greenery for the national health (Vujčić et al 2016.)

Design of the main project with all the decorative details krstić confided to his associate, Đorđe Pavlović Kovaljevski (1888 Jelisavetgrad, Rusija) Russian emigrant and the leading urban planner of the municipality responsible for the general urban plan of Belgrade (Jovanović: 1996). Before the design of "sketch for the new promenade in Kalemegdan (1928)" Kovaljevski had previous experience in the park design during elaboration of "situation plan for the building student dormitory king aleksandar i within Belgrade university (student dormitory "Lola" nowadays) and national library in the park of Ćirilo and Metodije, 1926, scale 1:500. The dormitory as well as the park was realized and today they are still in function.

Never digressing from the basic idea of Krstić, Kovaljevski elaborated and figuratively shaped this sketch, scale 1:100. During realization of the representative tree-part staircase with two platforms, he is adding to it semicircle enlargements to uplift monumentality of the composition. His project was almost completely realized in 1928.

Upper town in Kalemegdan was opened for the visitors in 1931, the works in the Little Kalemegdan were carried on and the works in the space between the church Ružica and Little Kalemegdan - conceded to the municipality by the army- were initiated. After the demolition of the barracks in 1939, within the city block where the officers' club and the theatre building "Manjež" were situated, the works on the construction of park started, what was partial realization of the suggestion from the general plan 1923, about the opera building within the park space in that particular site. After the First World War the land of topčider court economy and Košutnjak was divided. The biggest par of land with regulated parks and forests was taken over by the Topčiderski park administration, and the

other smaller part was ceded to the palace complex in dedinje. In August 1924, the Ministry of agriculture and water management announced the "open international competition for the design of the general plan for regulation of Topčiderski park and Košutnjak". The competition was closed in 1925.That is how this 35 hectares turf space was transformed into the Belgrade's resort. It is connected with Topčiderski park with the old road, as well as with the designed alley, realized in the same time when Dedinje Boulevard was finished and planted with poplar trees. After the building of the "royal palace" and the "new palace" dedinje and senjak have got „regulation plan", transforming after a while from suburban area with summer houses, to the space of residential type, planned and used for the elite housing. Today's complex occupies around 130 hectares and the park space covered with rare plants brought from America and Asia, occupies one third of the total area. Around 70 hectares of space, mostly towards the Topčiderska river, is preserved as the forest area (Ivanović: 1993) (fig. 3).

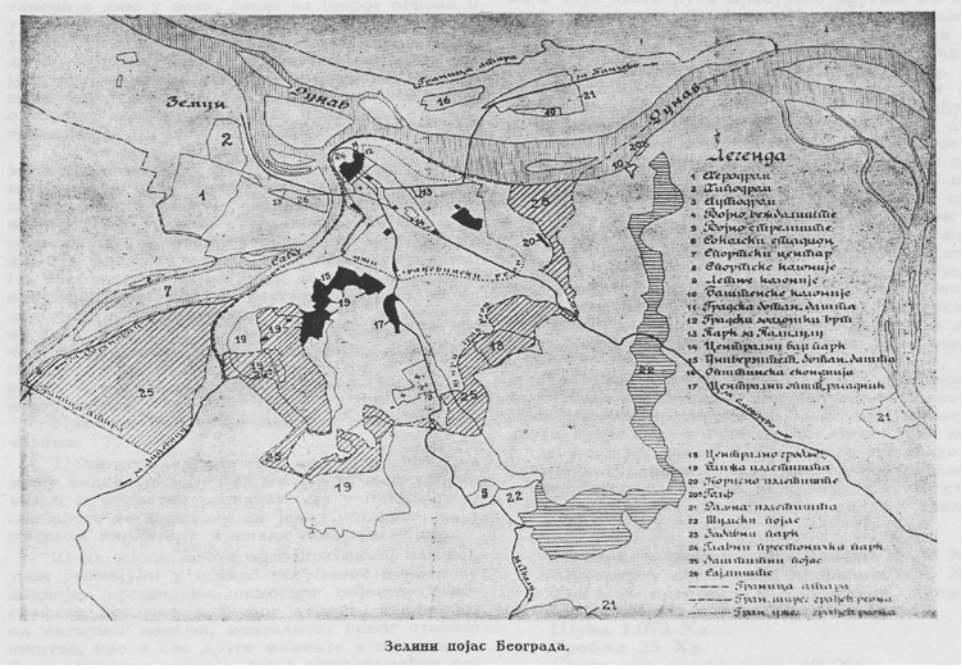


Figure 3. Aleksandar Krstić, Planning Problem of Belgrade and its surroundings. Afforestation and beautification. (Appendix to the text published Aleksandra Krstic in BON no. 10/1936. p.710).

Forestation of Belgrade begins in a greater extent in 1933, when the jurisdiction for these affairs was transferred to the Belgrade municipality - "Department for parks and forestation" headed by Aleksandar Krstić. Up till then, these affairs were in the state jurisdiction via "committee for forestation and regulation of the Belgrade's vicinity" (Krstić: 1934). There was no difference and no priorities in forestation of all the parts of Belgrade: on the Danube, in

Topčider hill, around public housing, Prokop, Mijić imanje, Marinkova forest, at the power plant in banjica, pionir, around public housing in bulbuder, close to elementary schools, at the butchery, etc (Krstić: 1934). Between new constructed parks in 1930s are: "Park prestolonaslednika Petra", park "Manjež", park at "Gospodarska mehana", park in the place of "Velika pijaca", then the park at the new power plant, park in Čubura, Zvezdara, etc. Fourteen squares were also accomplished in the same period and in different parts of Belgrade (at "Tri ključa", under Dalmatinska street, squares in Palmotićeva street, square Starine novaka street, in Senjak etc.), and also 38 yards of various institutions were finished, with the priority given to schools and children care institutions (school yards, gardens, children playgrounds). Street alleys and gardens were also extensively planted. (Krstić: 1934). Till the beginning of the Second World War, Belgrade had a great number of the open markets (Zeleni venac, Kalenića guvno, Cvetni trg, Smederevski đeram, Bajlonijeva, Palilulska etc.) And it was planned to build a couple of new ones around the city.

Belgrade zoo was opened in 1936 - on the initiative and at the expense of the mayor vlada ilića, engaged during his term of office (1935-1939) with the erection of the big Belgrade's bridges, beautiful constructions, solving out social problems, building of workers' accommodation, health care institutions and children shelters, schools, with filling of the bogs and swamps in the left bank of the sava (beginnings of new Belgrade), foresting and parks building. It was Ilić' wish that the first manager of Belgrade's zoo became aleksandar Krstić - his position till 1941. In that time, the Zoo was twice as big as it is today (figure 4).

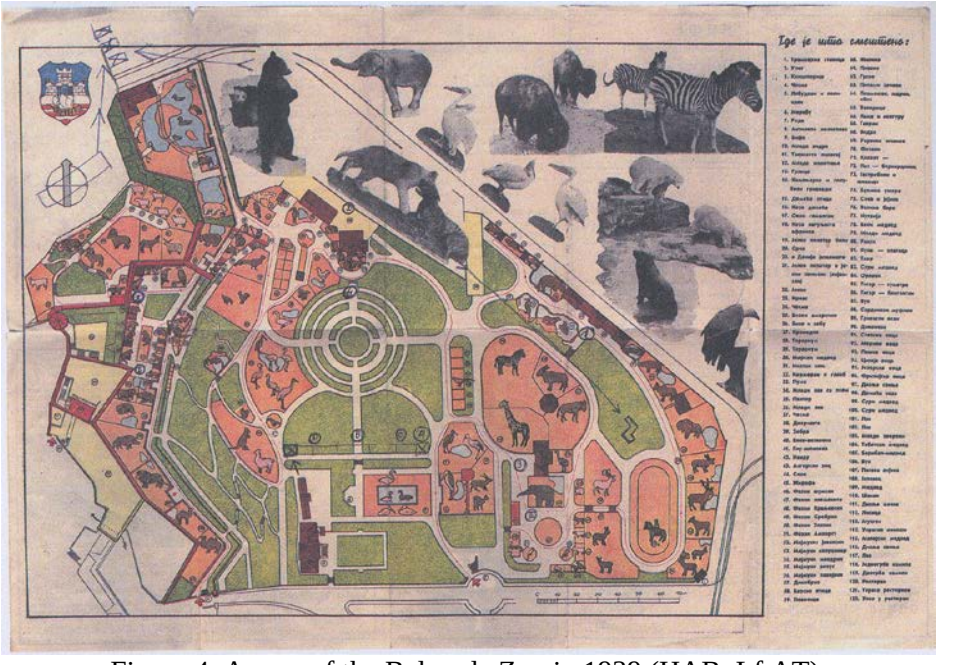


Figure 4. A map of the Belgrade Zoo in 1939 (HAB, Lf.AT).

CONCLUSIONS

Founded on the presented data, it is obvious that the planning development of the greenery in Belgrade got its full swing by the end of the 19th century, making a partial shift from the right course with the appearance of illegal constructions till the beginning and shortly after the First World War. The discipline in planning established between two wars had as a result renewed significance of greenery, expressed by the design of the developed green spaces - essential even today - of Senjak, Dedinje and Topčider. This trend is preserved after the Second World War by maintaining and developing greenery as the obligatory component of all the urban plans.

This example confirms the fact that the planning of green spaces and planning approach to this segment gives as a result better quality of life in the cities and higher value of space in general.

ACKNOWLEDGMENTS

The work is dedicated to Emilijan Josimović, in the occasion of 150 years anniversary frtom the first urban plan in Belgrade elaborated in 1867 - the jubilee (1867 – 2017) to be celebrated the next year.

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CHARACTERISATION OF WHEAT MUTAGEN DEPRESSION AFTER GAMMA-RAYS IRRADIATED

SUMMARY

Here we report cytogenetic, plant growth and development characteristics of mutation induction variability of the new wheat varieties and some relationships between means of cytogenetic characteristics and different doses and types of mutagens. The strategy of investigation combined the identification of genotypes carrying specific low-sensitive to mutagen factor using cytological and morphometrical analyzes screening of mutagen treated wheat populations with the approach of comparing different varieties by breeding methods to reveal its connections and differences, specific sensitive to mutagens effects on cell and plant level. The main purposes of investigations in this area were determination of the mutagen-polluted area suitability for agriculture; determination more suitable varieties for planting on mutagen-polluted area or as a object for mutation breeding.

Keywords: mutagen depression, winter wheat, gamma-rays, chromosomal aberrations

INTRODUCTION

There are three reasons for the study of plant mutant generation M1 after mutagenic effect. The first one is the determination of the mutagen-polluted area suitability for agriculture. More than 70% of soil in Ukraine used for agriculture is constantly exposed to chemical and physical mutagens. The second reason is the fact that the amount of material, obtained from the first generation, limits the opportunities of mutation breeding programmes (especially using the lethal doses). Wheat is the top food crop in Ukraine as well as in the whole world and the biggest part of grain is obtained primarily from winter wheat. The total area for winter wheat cultivation in Ukraine covers 6.8 mln. ha with actual productivity of 24 mln. tons and average capacity of 2.8 t/ha. The third reason is the use of obtained M1 population to extract valuable mutant strains in further generations. Mutagenesis reduces plant growth and other crop yield structure components, increases the pollen sterility and cuts the germination and survival abilities of plants by means of chemical agents and gamma rays; sometimes the great part of population is killed with the critical doses (Solanki, Sharma, 2000). Depression increases with the increase of dose (Yilmaz, Erkan, 2006).

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

But sometimes we observe stimulating effect (in case of low doses) or absence of depression (at medium concentrations of some chemical mutagens) (Subudhi et al, 1992). In first generations for wheat as cultivar composed from three genomes we observed only some dominant. Positive desirable mutant will be selected and be incorporated in future breeding programs (Ali Sakin et al, 2005).

Mutagenic effects of chemicals or ionizing radiation have been assessed by analysis of chromosomal aberrations (Rakhmatullina and Sanamyan, 2007). 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).

Mutated plants typically show reduced fertility, mainly caused by chromosomal rearrangements and genomic mutations during meiosis. 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 (Hossain and Alam, 2001; Huaili et al, 2005; Shu et al, 2011).

Analysis of chromosomal aberrations after mutagen action of any kind of mutagen by meto-anaphases method is one of the most precision methods which we can be used for determine fact of mutagen action on plants, identify nature of mutagen factor (Lifang et al; 2001, Adlera et al, 2004; Özel et al, 2015; Ukai, 2006, Waugha. 2006). Usually, analysis are widely used as for radionuclide's pollution of environment and its level (Korogodina et al, 1998), danger of this pollution (Geraskin et al, 2002) as for determine optimal doses of radiation and chemical agents in breeding work with plant material (Ahloowalia et al, 2004; Nazarenko, 2015). However, this compound exhibits high mutagenic, clastogenic and recombinational activity in plants, frequently stronger than that of the most powerful alkylating agents (Grant and Owens, 2001). A correlation between "clastogenic adaptation" expressed as a reduction of chromatid type aberrations, micro-nuclei and aneuploid cells, and the "clastogenic adaptation" has been shown (Bignold, 2009). We have next reasons for the study of chromosomes changes after mutagenic treatment: the determination of the mutagen-polluted area suitability for agriculture, correlation between aberrations and visible mutations in next generations (for mutation breeding purposes) and for identifying fact of mutagen action and its nature. Advantages of the method are promptness, objectivity of the results, the reliability and the ability to assess the impact of integrated wide variety of mutagens by nature (van Harten, 1998; Karthika and Subba, 2006; Albokari, 2014).

The main purposes of investigations in this area were determination of the mutagen-polluted area suitability for agriculture; determination more suitable varieties for planting on mutagen-polluted area or as a object for mutation breeding.

MATERIAL AND METHODS

Dried seeds (approx. 14% moisture content) 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 under vernalizaion has been used as mutagen factor) of winter wheat (Triticum aestivum L.) were subjected to 100,150, 200, 250 Gy gamma irradiation. Each treatment was comprised of 1000 wheat seeds. These doses are optimal for the breeding process that has been repeatedly established earlier (Ahloowalia et al, 2004; Nazarenko, 2015). Non-treated varieties were used as a check.

Treated seeds were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, to raise the M1 population.

The untreated seeds of mother varieties (parental line/variety) were also planted after every ten rows as control for comparison with the M1 population. M1 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 M1 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.

The seeds used in this study were of the M0 generation. After mutagen treatment dry seeds were germinated in Petri dishes under 24 – 72 hours (depends on presoaking and mutagen action), temperature +250C. Afterwards 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 2^0C (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 600C). Anaphase of cell division was observed by light microscope JNAVAL. No less than 800 cells in proper phases of mitosis were observed in each variant (Lifang et al, 2001; Rank et al, 2002, Natarajan, 2005).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by ANOVA, the grouping by the nature of mutagens was performed by cluster analysis (Euclidian distance) (Klekka, 1989).

Used the standard tools of the program Statistica 8.0 for cluster analysis (Multivariate Exploratory Techniques, cluster analysis, single linkage, Euclidian distance), factor analysis (Statistics 8.0, ANOVA module).

RESULTS AND DISCUSSION

Analysis of grows and development of plants.

In M1 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 – 3). 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. Plant survival ability ranges from 55 (Voloshkova) to 5.3% (Sonechko) at 250 Gy, while it ranged from 98 to 92% under untreated control.

As for the impact of gamma rays on the germination and survival abilities, it is the usual effect in plants for most crops previously observed by many researchers in wheat as well.

However, we can see that some varieties were statistically more successful in survivals ability.

Table 1. Main parameters of grown of winter wheat plants at M1 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
gamma-rays, 100 Gy.	79±0,76*	76±1,01*	75±1,07*	70±1,11*
gamma-rays, 150 Gy	69±1,09*	66±1,13*	71±1,15*	66±1,18*
gamma-rays, 200 Gy	58±1,48*	54±1,71*	47±1,24*	44±1,43*
gamma-rays, 250 Gy	38±1,26*	36±1,34*	37±0,83*	35±1,10*
Variety	Voloshkova		Sonechko	
Check	92±0,57	87±0,93	94±0,94	89±0,98
gamma-rays, 100 Gy.	73±0,76*	69±1,01*	65±0,57*	62±0,93*
gamma-rays, 150 Gy	64±1,09*	60±1,13*	43±0,57*	40±0,93*
gamma-rays, 200 Gy	55±1,26*	52±1,34*	31±1,14*	29±1,72*
gamma-rays, 250 Gy	55±1,48	51±1,71	5,6±1,07*	5,3±1,39*
Variety	Favoritka		Hurtovina	
Check	98±0,57	91±0,93	92±0,94	84±0,98
gamma-rays, 100 Gy.	82±0,76*	76±1,01*	73±1,07*	67±1,11*
gamma-rays, 150 Gy	58±1,09*	54±1,13*	52±1,15*	48±1,18*
gamma-rays, 200 Gy	49±1,26*	45±1,34*	55±0,83*	50±1,10*
gamma-rays, 250 Gy	39±1,48*	36±1,71*	36±1,24*	33±1,43*
Variety	Lasunya		Line 418	
Check	98±0,57	94±0,93	93±0,94	92±0,98
gamma-rays, 100 Gy.	54±0,76*	52±1,01*	74±1,07*	67±1,11*
gamma-rays, 150 Gy	48±1,09*	46±1,13*	70±1,15*	55±1,18*
gamma-rays, 200 Gy	42±1,26*	41±1,34*	48±1,24*	36±1,43*
gamma-rays, 250 Gy	37±1,48*	35±1,71*	39±0,83*	35±1,10

* - difference is statistically significance from check at $P_{0.05}$

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
gamma-rays, 100 Gy	91,2*	82,9*	81,3*	84,5*	79,9*	82,3*	84,8*	89,1*
gamma-rays, 150 Gy	82,7*	74,6*	74,5*	70,9*	64,7*	67,8*	71,2*	81,6*
gamma-rays, 200 Gy	71,2*	69,8*	69,2*	64,5*	50,7*	59,9*	61,3*	73,4*
gamma-rays, 250 Gy	64,6*	52,5*	61,6*	42,3*	42,5*	47,9*	43,8*	66,1*

* - difference is statistically significance from check at $P_{0.05}$

In general, the correlation between the dose value and survival abilities of plants is at the level of -0.9 for gamma rays. Sonechko was extremely sensitive to gamma rays. Plants of all varieties showed higher level of depression being processed with highest dose of gamma rays.

Correlation between the dose value and pollen fertility was -0.9. As we can see, the highest level of this indicator was observed after the mutagenic effect on Kolos Mironivschini seeds. Frequency of pollen sterility was on the highest level after gamma irradiation, primarily, in the varieties obtained by processing with gamma rays. Only these varieties showed extremely low fertility at a dose of 250. Pollen sterility is the more reliable parameter for monitoring depressive consequences compared to germination and survival rates. The Table 2 shows that resistance to mutagenic effect directly depends on the genotype of processed material.

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. But only three (plant height, grain weight per spike and 1000 grain weight) showed statistically difference level of mutagen depression under any dose action. Regarding the plant height, correlation between the dose and the indicator constituted -0.89, (high invert correlation). This parameter decreases if the dose increases. However, the differences between versions can be statistically unreliable. Gradual decrease in height is a tendency. We have not observed any differences between the varieties. The indicator of grain weight per spike was more informative, weight was falling statistically valid with every increase in dose. Here we have the same situation with the varietal specificity by depression as in the previous case. Sonechko responded to mutagenic effect in the worst manner. The correlation coefficient

was -0.92. The thousand grain weight is the most informative indicator. Depression value at each dose is clear and statistically valid. The correlation coefficient was -0.96.

Table3. Correlation between gamma-rays dose 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
Dose	-0.89	-0.34	0.14	0.12	-0.69	-0.92	-0.76	-0.96

Chromosomal aberrations analysis.

The results of our investigation are represented at table 4 (total number of observing mitosis, number of division cells with chromosomal aberrations, general frequency of aberrations). Standard error (± SE) values of the treated populations are at tables too.

As we can see from table 4 frequencies of aberrations were changed from 7,06 % (Favoritka, gamma-rays 200 Gy) to 47,5 % (Voloshkova, gamma-rays 200 Gr.) percent from total number of mitosis. All the variables are statistically significantly different from each other and from the check.

Higher frequency of aberrations in any cases characteristic for varieties obtained by breeding without using any mutagens (line 418, Kolos Mironovschiny, Voloshkova) and we can predict more rate of visible mutations (regarding previous investigations).The higher frequency of aberrations has been obtained by used 200 Gy dose. The high aberration rate was observed in Voloshkova. This variety was unstable in check too. According to the results of cluster analysis (was generated by number of chromosomal aberrations) it was found a clear determine between the method of variety obtaining and the nature of the mutagenic factor.

Identified four different groups of varieties – by using gamma rays Favoritka, Lasunya, Hurtovyna, by the action of chemical mutagens Kalinova, Sonechko and obtained using recombinant breeding – Kolos Mironovschiny, line 418, and is entirely separate – variety Voloshkova (termomutagenesis). Thus, this method of grouping finally confirmed the conclusion that the effect of mutagenic factor is largely determined sensibility for mutagen action if these factors have used for obtained initial material. After spectra of chromosomal aberrations had been investigated next types were identified: chromosomal bridges and double-bridges, fragments of chromosomes and double-fragments, micronucleus, lagging chromosomes.

Cases with two or more types of aberrations in one cell and fragments-bridges ratio were calculated separately. Quantity of any type of chromosomal aberrations was increased with dose increased (correlation coefficients 0,8 – 0,9).

Table 4. Frequency of chromosomal aberrations in M_1 generation of winterwheat varieties

Variable	Mitosis, number	Chromosomal aberrations		Mitosis, number	Chromosomal aberrations	
		n.	%		n.	%
	Favoritka			418		
Control	984	19	1,93±0,31	962	11	1,14±0,11
Gamma-rays, 100 Gy.	1006	71	7,06±0,74*	992	161	16,23±1,14*
Gamma-rays, 150 Gy	1004	139	13,85±1,09*	1056	245	23,20±1,19*
Gamma-rays, 200 Gy	943	230	24,43±1,53*	747	228	30,52±1,57*
Gamma-rays, 250 Gy	466	126	27,06±1,48*	586	247	42,15±1,89*
	Lasunya			Hurtovina		
Control	1056	15	1,42±0,19	1034	12	1,16±0,11
Gamma-rays, 100 Gy.	979	88	8,99±0,78*	1012	100	9,88±0,89*
Gamma-rays, 150 Gy	1012	158	15,62±1,0*6	981	147	14,99±1,03*
Gamma-rays, 200 Gy	810	198	24,45±1,53*	1011	228	22,56±1,45*
Gamma-rays, 250 Gy	399	98	24,56±1,54	742	193	26,01±1,63*
	Sonechko			Voloshkova		
Control	1026	8	0,78±0,04	1003	31	3,09±0,34
Gamma-rays, 100 Gy.	1010	194	19,20±1,14*	1000	213	21,30±1,24*
Gamma-rays, 150 Gy	1003	288	28,70±1,31*	1007	332	32,97±1,39*
Gamma-rays, 200 Gy	888	342	38,51±1,85*	560	266	47,5±1,98*
Gamma-rays, 250 Gy	411	190	46,23±2,04*	478	198	41,43±1,81*
	Kalinova			Kolos Mironivschini		
Control	1047	9	0,86±0,11	909	10	1,10±0,13
Gamma-rays, 100 Gy.	1000	192	19,20±1,14*	1019	179	17,56±1,04*
Gamma-rays, 150 Gy	937	269	28,70±1,31*	890	215	24,16±1,23*
Gamma-rays, 200 Gy	817	315	38,51±1,85*	738	243	32,93±1,65*
Gamma-rays, 250 Gy	459	212	46,19±2,04*	510	196	38,43±1,84*

* – difference statistically significant on $P_{0,01}$

In our past investigations gamma-rays induced more bridges than fragments (fragments-bridges ratio lower than 1). After chemical mutagens more fragments have been observed (fragments-bridges ratio more than 1) (Nazarenko, 2015). We will be able to use this parameter for identify nature of unknown mutagen if it's right for other kinds of chemical mutagens. To sum it up, some authors wrote about prevalence of bridges over fragments after gamma-rays, some researchers mentioned more rates of fragments than bridges after some chemical mutagens (EMS, DMS), especially double fragments (Natarajan, 2005; Shu et al, 2011), but no one said about dominance of bridges regarding to gamma-rays. Secondly, we don't know other works about genotype-mutagen interaction on cell level

depends on breeding methods of varieties obtaining. In our previous works we only mentioned about higher depression at M_1 generation of plants when parameters of growth and development were investigated in case of mutation winter wheat variety Smuglyanka (Nazarenko, 2015). Thirdly, some aspects of mutagen-genotype interaction are well-known, but not on so deep level as changes in chromosomal aberrations rates, only as decreasing of some types of visible mutations such as dwarfness and earliness (Shu et al, 2011).

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. Thus, the greatest depression among all varieties was observed in Sonechko under all parameters (except thousand grains weight). Kolos Mironivschini was the most resistant to mutagenic effect. Varieties obtained by action of mutagenesis show specificity in demonstration of mutagenic depression based on the some indicators of crop yield structure.

Mutation varieties were less sensitive to same chemical mutagens. We can predict less number of mutations when these varieties would be used for mutation breeding purposes. The higher rates of chromosomal aberrations are typical for varieties obtained by used field hybridization without any mutagen treatment or when initial material for breeding has been changed by low temperature action (variety Voloshkova). Comparing between bridges and fragments is a reliable mean for identification of mutagen nature (chemical or gamma-rays). In first case more fragments have been induction, in second – bridges.

We recommended crossbreeding varieties (such as Kolos Mironovschiny) either for planting on mutagen pollution areas or as object for mutation breeding.

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DOI: 10.17707/AgricultForest.62.4.28

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SOCIO-ECONOMIC DEVELOPMENT REQUIREMENTS FOR AGROTOURISM IN MONTENEGRO

SUMMARY

Rural areas are consisted of areas around city settlements inhibited by rural population, whose main activity is agriculture. Rural tourism is developing in areas where natural environment is preserved and where local community with its culture and cultural heritage exists. Agrotourism is a form of rural tourism, which includes the stay of tourists in rural areas and gives them the opportunity to actively participate in the life and work on the farm. Montenegro is abundant in areas of exceptional value that represent real environmental oases. Such regions are featured by traditional culture and diversity of the cultural heritage. On the other hand, rural areas are affected by the process of "discharge" and "dying" of villages. The process of industrialization after World War II caused depopulation and deagrarisation. These are the most significant socio-economic changes in Montenegro, which have left a negative impact on rural development.

Economic purpose of agroturism is reflected in the activation of the agricultural population, and in linking agricultural production with tourism. Its role is also important in order to decrease the population migrations from the countryside. Human factor plays an important role regarding the development of agrotourism activities. Human resources imply knowledge, experience, creativity, innovation and ability of individuals focussed on the advancement of society, and in their need to constantly invest (Đerčan, Bubalo Živković and Lukić, 2010 a). Agrotourism is an opportunity for overcoming regional differences in Montenegro and revitalization of villages in the northern part of Montenegro.

The paper analyzes the social economic conditions for development of agroturism in Montenegro. The focus is on the northern part of Montenegro on the ground of the existence of natural resources on the one hand and the lack of socio-economic conditions on the other. Development of agroturism is observed from the standpoint of improving the quality of life and improvement of agricultural production on family farms. The popularity and demand of tourists to stay in conditions of untouched nature provides an opportunity for rural areas in the north of Montenegro to adequately valorise their natural resources. In 2009, Author Ružić, points out that the rural forms of tourism valorise all those values

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

that rural areas provide to the modern man, such as return to the natural values, agricultural environments, depressurisation from the stresses of urban areas and etc.

Key words: rural areas, agroturism, socio-economic prerequisites, regional development

INTRODUCTION

Agrotourism in Europe has begun to develop before the end of the 20th century. Review of domestic and foreign literature reveals a number of different definitions of agrotourism. Terms agritourism, tourism on farms, tourism and related ones are often used interchangeably with agroturism and with each other (Barbieri and Mshengu, 2008; Roberts and Hall, 2001; Wall, 2000). In 2006, Marques, highlighted a specific aspect of rural tourism by which the house must be integrated into the farm, inhabited by an owner, allowing friends to participate in activities on the farm. In 2007, McGehee, and Kim Jennings defined agroturism as rural household which jointly included working farms and commercial tourist component. Theoretical and practical understanding of agroturism is very different. According to the authors Vrsaljko, Viljac 2015, rural tourism is a primary branch, rural tourism secondary, and agroturism is tertiary branch of tourism in rural areas. The main difference between these terms is in the area of implementation.

Rural tourism facilities are located throughout the rural area, rural part in part of rural area, which belongs to the village, while agri-tourist are located on farms. On the basis of available sources of literature it can be concluded that terms and farm tourism agriturism often used in Western Europe, while the term agroturism is more used in Eastern Europe (Vrsaljko, Viljac 2015). Kušen, in his considerations, suggests that the term "rural tourism" can be replaced with two others "rural tourism" and "rustic tourism" or "tourism on family farms" (Brščić, Franić et al., 2010 according Kušen, 1995). Agro tourism implies services of supply, accommodation and meals, including a country atmosphere and the economic, cultural and other areas of activity (Grgić et al., 2011). Author (Clemens 2004) observes agroturism from the standpoint of possibility of additional earnings on farms, that have a relatively small arable land, high cost of labour and difficult conditions for agricultural production. Diversification of agricultural activities in the agroturism has an impact on increasing the income of farmers, but due to need of farmers to acquire new knowledge and skills regarding agroturism, it has affects on the identity of the farmers (Siti-Hajar et al., 2015). Although the rural development policy, pay more attention to agro tourism, this activity is still insufficiently valorised through tourism offer (Vogt, 2013). Linderhof and Reinhard (2012) researched both the impact of EU rural development policy on the development of agroturism and its influence on the area using econometric analysis. Regardless of the number and diversity of the definitions on agri-tourism, the essence is focused on rural (local) economy and activities in nature. Local population gains the greatest benefit in comparison to

the tourist agents and hotels. Rural tourism is such a form of tourism that includes the complexity of all activities and aspects of integrated tourism products (recreation in rural areas, enjoying in nature, cultural tourism, tourism on farms), (Jafari, 2000). In some countries, the term is understood differently in practice. In Finland, renting a small rural house to tourists also includes catering services. In Slovenia, the most important form of tourism is on agricultural households-farms. In the Netherlands is meant camping on family farms (Jelinčić, 2007). Common characteristics are key elements identifying the agroturisam are quiet environment, preserved nature, accommodation in traditional rural households and home-cooked food.

Rural areas, which is a basic resource for the development of rural tourism covers about 90% of the territory of Montenegro. According to the OECD methodology, Northern region encompasses 13 municipalities in its composition and it is predominantly rural (59.7% of population lives in rural areas), while the Coastal (41.7%) and Central (20.4%) belongs to the transitional one. Family farms play the most important role in rural areas. According to the last agricultural census of 2010, the total number of farms was 48 870, of which 48 824 are family farms, or 99%, while only 46 companies were engaged in agricultural activity (Census of Agriculture, 2010). Introducing tourism activities in rural areas provides opportunity to family farms to improve their economic efficiency. The paper will present the existing natural and socio-economic conditions for development of agroturism on family farms, with an emphasis on the northern region of Montenegro. It will also highlight the advantages and disadvantages, as well as limitations in the development of agrotourism.

MATERIAL AND METHOD

The paper analyzes the natural and socio-economic preconditions for the development of agroturism in Montenegro. The focus of research is in the Northern region of Montenegro, namely, family farms, which are recognized as potential for the development of agroturism. In order to assess the state of the natural and socio-economic preconditions, statistical data of the last Census of Agriculture (2010) year were used. As a complement to the statistical data surveys conducted on 60 subjects in the municipalities of Pljevlja, Bijelo Polje and Kolasin were used. Also, we used the domestic and foreign literature and online sources. The questionnaire was consisted of 35 questions and it was intended for agricultural farms. The data were analyzed and statistically analyzed by descriptive statistics and using SPSS program.

For displaying the data, the statistical tables and graphs were used. Available data were analyzed by methods of description and comparison. The work is held according to the SWOT analysis in order to demonstrate the advantages and disadvantages better, as well as threats and constraints for the development of agroturism in Montenegro. It was used and the method of research desk "desk research".

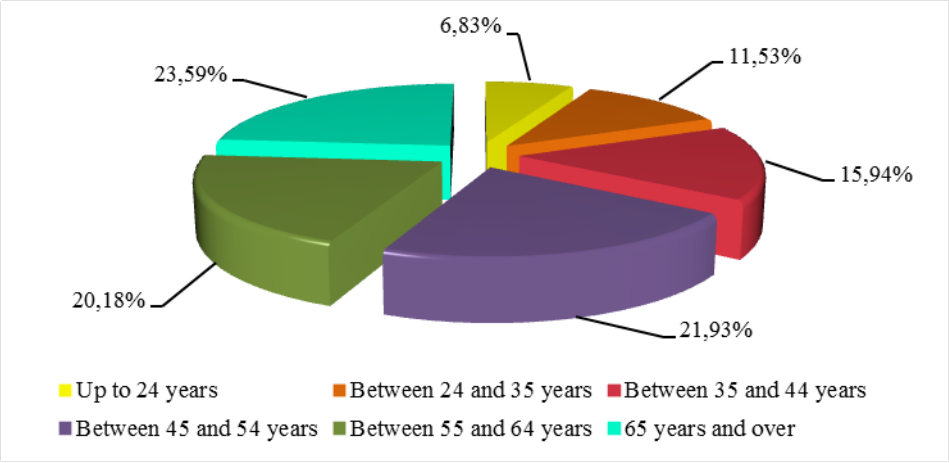
The paper aims to draw attention to the fact that agroturism is important for overcoming regional disparities, as well as to increase the income of the farms and the increasing employment of women.

RESULTS AND DISCUSSION

Last sixty years have witnessed major changes in the way of life in rural areas of Montenegro. Such changes resulted in an uneven development of the regions. According to the Regional Development Strategy of Montenegro, three regions are classified: Northern, Central and Southern region. Depopulation of the Northern region is greatly expressed, as well as the high concentration of population and economy in the central and southern region. Rural area of Montenegro remains discharged, while on the other side - in Europe rural areas are becoming increasingly important alternative of living and working in relation to cities. Tourists are constantly looking for new and different forms of tourism. Agrotourism covers individual regions and it is becoming a regional macroeconomic challenge. It is not only economic but also sociological, geo-strategic and political category (Pejanović 2008). Agrotourism gains its importance primarily due to the needs of the modern consumer to stay in the rural environment and live in a harmony with nature. Author (Pejanović 2008) points out that the experience of rural and regional development in the EU shows that the development of agrotourism enables: stabilization of the regional development, resource utilization, preservation of tradition, optimization of the rural and urban regions and raising the competitiveness of certain regions as tourist destinations. Through the indirect economic impacts on agriculture indirectly, tourism stimulates development of other activities. Economic effects of rural areas can be numerous: development of abandoned rural communities through the process of valorisation of rural houses, abandoned farms, through return of young people to the countryside, revival of traditional crafts, etc.

Average farm in Montenegro has 4.6 ha of agriculturally used land (Statistical Office of Montenegro - MONSTAT 2011). Taking into account the size of households, development of agrotourism provides an opportunity for diversification of their activities and achieving favourable economic results. It could be a significant supplementary activity on the farm, which ensures better usage of production capacity and labour of household members. The main characteristic of family farms is high proportion of older working-age population on the farm and a small number of young people. Aged households cannot be a basis for the development of tourism in the country. According to (Franjić and Grgić, 2002), the owners of farms who would like to operate in field of agroturism should assess whether their household is suitable for providing tourism services and evaluate properties of their household members, age and willingness of the family to kindly treat the guests. Development of agrotourism is an opportunity to stop emigration of young people from rural areas and it generally affects on decrease in migration flows. On family farms in Montenegro 6717 persons were under 24 years old, which makes 6.83% of the total workforce

(Figure 1). On family farms 23198 working-age population is aged 65 and over, which makes 23.58% of the total workforce.



Source: Agricultural Census 2010, Structure of agricultural holdings by municipalities

Graph. 1. Labour force family agriculture holdings

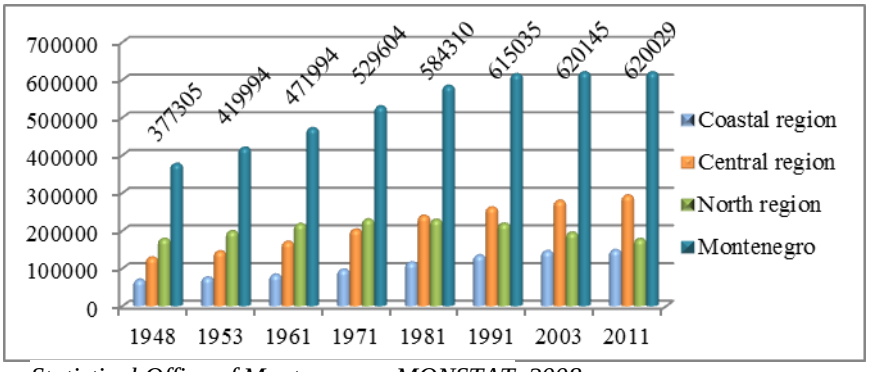
Employability of women in agriculture accounts for only 4% of total employment. The main reasons for lack of interest of women to remain in rural communities are the following: women in rural areas rarely own property, are rarely found in the position of holders of holdings and rarely have the role of decision-making on agricultural production and the role of responsibility for the economic risks of operations on the farm. According to the Census of Agriculture, women comprise only 12.87% of holders of family farms. Approximately 33.24% of holders of holdings are aged over 65 or more and they are mostly men. Unfavourable educational structure creates a low potential for successful economic participation of women and significantly reduces their employability. In fact, development of agroturism can positively affect not only the development of women entrepreneurship, but also entire agroturism offer. Rural tourism in Greece is used as important means of social promotion of female population (Grgić et al. 2015).

The best example of the use of agroturism as "tools" in the development of rural areas represents Austria. In fact, Austria is in the area of the Alps and it began with the revitalization and development of non-agricultural activities in 50s of the 20th century. Today Austria is the leading European country in agrotourism with a long tradition and great experience, especially in maintaining farms in mountain regions. Italy is the only country in the European Union which has a special law on agrotourism, while the rest of the countries of this form of tourism include in the general provisions. In neighbouring Croatia, the first registered farms have emerged in 1998. In the period 2000-2005, the number of

registered farms has doubled. In 2013, there were 447 farms, which are engaged in agrotourism, but they are very unevenly distributed in the counties. The biggest advancements are made in the field of agrotourism in Istria (Grgić et al. 2015).

From the standpoint of education of the labour force on family farms in Montenegro, and according to available data, the highest share belongs to people with 4 years of high school (33.74%), of which 66.88% are men and 33.22% are women. The share of people with secondary or higher agricultural education is at the level of 1.47% of the total workforce of family farms. Men with secondary or higher agricultural education accounted for about 72.89% and 25.63% women. Human resources play an important role in the development of agrotourism. All those who work on farms and who are involved in agrotourism, apart from knowledge needed to carry out agricultural activities, must possess a certain entrepreneurial and communication skills, and also culture of communicating with people (Grgić et al. 2015). If we look at the number of members on the farm, most of those farms have 1 to 2 members, and they accounted to 76.8%. Analysis of the situation on family farms indicates that apart natural conditions, human resources are of a great importance for the development of agroturism. A special advantage of agrotourism, in relation to other forms of tourism is its perennial character, or functionality of 365 days (Pejanović 2008).

The development of agrotourism can significantly influence and lead to a reduction in regional differences in Montenegro that are highly expressed. The graph 2 shows the movement of the population by region in the period 1948-2011.



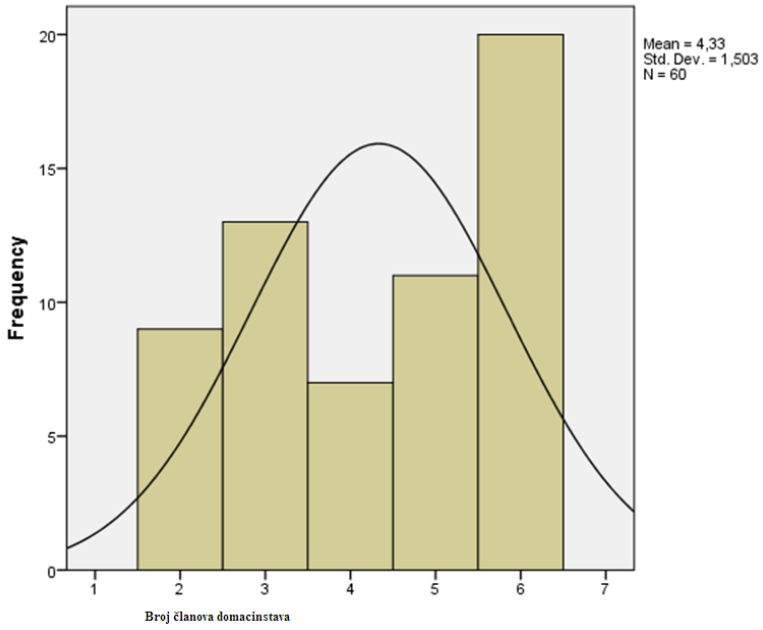
Source: Statistical Office of Montenegro - MONSTAT, 2008

Graph 2. Movements in the total population of Montenegro by regions, 1948-2011

Since the Census of 1981, share of the population of the Southern and Central regions of the total population of Montenegro has been increasing, while share of population of the Northern region is declining. Population of the Coastal region has increased by 29.73%, the Central by 38.30%, while the North was reduced by 39.92% (Despotović, et al., 2015). This trend has resulted in the weaker pace of development of agriculture. Northern region (mainly rural)

represents more than 50% of the country, but is inhibited by less than a third of total population. On the other hand, nearly one-quarter of the population in Montenegro inhabit slightly over 10% of the territory of Montenegro. EU experiences indicate that the development of agrotourism ensures stabilization of the regional development and increase of the competitiveness of the region as a tourist destination.

In Montenegro, the agrotourism is still seeking its place in regional development and development in general. The essence of offer in rural areas is a stay in the renovated village houses with the possibility of preparing food, familiarizing with traditions and revival of many traditional activities. Tourism would encourage the development of other activities in rural areas, which would be directly or indirectly involved in providing services to tourists, such as: transport, trade, production of food, drinks and other activities. However, there are little concrete activities that were directed towards the development of agrotourism. The reasons should be sought in the fact the potential for developing this type of tourism has not still been recognized, but also in a lack of knowledge of those subjects that should work on the promotion of agrotourism. Therefore, we can conclude that the agrotourism in Montenegro is still in its infancy and it is necessary to be worked on its affirmation. In order to learn what farmers think and know about agrotourism, a survey on 60 family households in the Northern area of Montenegro, or more precisely in Kolašin, Pljevlja and Bijelo Polje was conducted. The survey implied 35 questions, which included socio-economic characteristics of households and their production status.



Source: Survey, 2016

Graph 3 Number of household members

The survey covers respondents aged over 18. The sample included randomly selected households, which are located in areas suitable for the development of agrotourism. Agricultural producers accounted for 62.3% of respondents, while the rest were pensioners, housewives and workers. Regarding the gender structure of the survey - 90% of men and 10% women were included. The paper will present the results of a number of key issues important for the development of agrotourism. More than 50% of respondents are personal owner of the farm, i.e. of the available land, while statistically significant number of respondents stated that about 34% of land in the ownership possession belongs to the parents of the respondents. Over 30% of respondents said that they live in a community with more than 5 household members (Graph 3), which is significantly higher than the average in Montenegro.

Table 1. Comparison of strengths, weaknesses, opportunities and constraints in the development of agroturism in Montenegro

Advantages (Strengths)	**Disadvantages (Weaknesses)**
Preserved natural heritage Favourable geographical position Favourable natural conditions Diversity of agricultural production Hospitality of local population Traditional and authentic products Rich gastronomic offer Rich historical and cultural heritage Conditions for organic production	Depopulation, or migration of young people to the cities Mostly elderly population in rural areas Low quality of life in rural areas Underdeveloped infrastructure Lack of heritage Low level of knowledge in the field of agrotourism Lack of funding Unclear legislation Inadequate incentives for development
Possibilities (Opportunities)	**Limitations (Threats)**
Increase in demand for rural households Increased demand for organic products New tourism trends Raising awareness of local people about the importance of agrotourism Preservation of the environment Stimulation for entrepreneurship Valorisation of cultural heritage Placement of own agricultural products Incentives by the EU	Migration of youth and women Aging population Low interest in the development of agrotourism Low incentive for balanced regional development Lack of cooperation between tourist agencies and agricultural producers Increased pollution Lack of cooperation between local authorities and manufacturers Insufficiently defined strategy of development of agrotourism. Unstable tourism market

Source: Synthesis of the authors based on several sources (Grgić,2015, Vrsaljko et al.,2015)

However, the average number of household members was 4 members, while the most frequent number of repetitions went to the side of those with more than 5 members.

That the concept of working in field of agro industry is a relatively new phenomenon in Montenegro shows the fact that 85% of surveyed households are not familiar with the regulations to be observed when initiating/running agrotourism. Only 15% of households are familiar with the regulations. Although knowledge of the regulations is not a strong side of households, yet 76.7% of them showed willingness to be participating in agrotourism in the next five years. The motive for agrotourism activities in the majority of interviewee is easier placement or sale of their own products (39.3%), as well as increasing of income, reducing the risk of agricultural production, employment of family members 26%. Other interviewees were not precise in their responses. About 50% of households stated that funds are major constraint in dealing with agrotourism, but also they see demanding administrative procedures as an obstacle. 88.10% of them considered that the development of agrotourism can significantly boost socio-economic development of rural areas. The results obtained in the survey correspond to the literary sources, indicating the importance of agrotourism in terms of socio-economic development of certain regions. In order to promote its development, it is necessary to educate producers and simplify administrative procedures. Also, SWOT analysis indicates advantages, disadvantages, opportunities and constraints in the development of agrotourism in Montenegro.

CONCLUSION

Rural tourism in Montenegro is underdeveloped, and it is the result of years of neglect of rural areas. Family farms on the one hand are the backbone of the development of agroturism, and on the other hand represent a limiting factor. The structure of the land, precisely, fragmentation of holdings and insufficient number of trained persons on the farm, unfavourable age structure of the staff are just some of the factors that influence the development of agritourism. All of these limiting factors should be viewed realistically when designing agroturism offer. Results of surveys suggest that manufacturers are willing to work in agroturism if financing and permanent education is secured.

All of the above should be observed realistically keeping in mind that it is necessary to take into account the guidelines of sustainable development, supported by performance of special forms of tourism.

With the development of agrotourism, agricultural production is not only food production but it receives multifunctional role of agriculture. Eco-social model of agricultural development can create a platform for the development of policies that will improve agriculture and support the development of agroturism as an additional activity.

Experiences from other countries such as Austria, Italy, Greece, Croatia and other countries can help Montenegro when designing further development of agritourism.

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Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

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The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

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- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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