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PATHOLOGY OF TESTES CELLS IN WHITE MICE AFTER IMPACT OF EPRINOMECTIN

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ABSTRACT

Avermectines are new class of antibiotics with acaricidal and anthelmintic activity. New medicine containing macrocyclic lactones is Eprimec. One milliliter of Eprimec contains 10mg of eprinomectin as active substance. The aim of our study was to determine the extent of karyopatic and cytotoxic effects of that preparation to cell division process in testes of white mice. The materials for the study were the non-linear white mice – males 18 - 22 g. Experimental group of mice received a dose of 200 micrograms of active compound per 1 kg of weight or 10 mg / ml of eprinomectin by single subcutaneous injection. The control group of animals remained intact. Each group consisted of 8 mice. The animals were slaughtered at 12 hours after drug administration. Testes of mice were isolated, smears – imprints were prepared that are fixed by May - Grunewald and stained with azure - eosin by Romanovsky. Glass slides with testes cells were viewed under a microscope and implemented to count the number of dividing cells, take into account the shape, size and color of the nuclei. Analysis of the frequency of occurrence of a karyopatic disorders and cytotoxic effects of cells were performed separately and lying sprawled cells with counting at least 1000 cells in each glass slide (Lazareva et al, 2016). Analyzing the data, we concluded that on the basis of eprinomectin formulations significantly reduce the activity of cell division, but it is toxic and cause serious karyopatic changes in the cells of the testes nonlinear white mice, indicating that the negative effects on the reproductive system of males.

Keywords: *antiparasitic medications, Eprinomectin, karyopatic effects.*

INTRODUCTION

Works of plenty of scientists evidence the kariopatic effect of chemical substances and antigens (Alov, 1972; Tolbert et al., 1992; Morgan, 2007; Lazareva et al., 2016). Among the latest antiparasitic drugs a special place has avermectins are new class of antibiotics possessing insecticidal, acaricidal and antihelminthic effect. This pharmacological group of substances widely used for the treatment and prevention of parasitic diseases of agricultural, small domestic and exotic animals. Ivomec and its copy – Baymak, Sevmek, Bisection, Pandeks, Ivermectin, Everton,

Novomec are most popular for veterinarians. The popularity of products containing macrocyclic lactones of the following generations increases, in particular drug Eprimec. Eprimec includes as an active ingredient eprinomectin – 10mg/ml, as an auxiliary substances dimethylacetamide – 50 mg/ml, benzyl alcohol 10 mg/ml and triglycerides up to 1 ml. In appearance the drug is transparent solution of yellowish color. The main target actions are eprinomectin glutamylcysteine chloride channels and receptors of gamma aminobutyric acid in nematodes, the larvae of botflies and ectoparasites. The magnitude of the change of chloride ions current through the membranes of nerve and muscle cells disrupts the conduction of impulses, resulting in paralysis and death of parasites. Eprimec is a low-hazardous substances (4 hazard class according to GOST 12.1.007-76), in recommended doses has no embryotoxic, teratogenic and sensitizing effect; toxic to fish and bees. Data on the effects on the reproductive system of males in literature is absent.

The aim of our this study was to determine the extent of karyopatic and cytotoxic effects of that preparation to cell division process in testes of white mice.

MATERIAL AND METHODS

Experiment was performed at the parasitology laboratory of Perm State Agricultural Academy in summer 2015.The materials for the study were the non-linear white mice – males 18 - 22 g. Experimental group of mice received a dose of 200 micrograms of active compound per 1 kg of weight or 10 mg / ml of eprinomectin by single subcutaneous injection. The control group of animals remained intact. Each group consisted of 8 mice. The animals were slaughtered at 12 hours after drug administration. Testes of mice were isolated, smears – imprints were prepared that are fixed by May Grunewald and stained with azure - eosin by Romanovsky. Glass slides with testes cells were viewed under a microscope and implemented to count the number of dividing cells, take into account the shape, size and color of the nuclei. Analysis of the frequency of occurrence of a karyopatic disorders and cytotoxic effects of cells were performed separately and lying sprawled cells with counting at least 1000 cells in each glass slide. To study the activity of cell division we used mitotic index (MI%) – the ratio of the number of dividing cells to the total number of cells that we see at the moment. Micronuclei were identified as rounded chromatin body with a smooth continuous edge, no larger than 1/3 of the nucleus, which was located separately from the main kernel, not refracted light, with the intensity of staining and pattern of chromatin as the basic core, and were in the same plane with the nucleus (Morgan, 2007). In addition, incorporate a dual-core cells, fragmentation and vacuolization core, early (premature) separation of chromatids in prophase, division of pathology associated with damage to the mitotic apparatus and a violation of cells division. For statistical data analysis we used the software package STATISTICA 6.

RESULTS AND DISCUSSION

Data analysis of the experiment is shown in the table.

Table. The comparison between the control group and the group of animals exposed to the drug Eprimecto cells of the testes nonlinear white mice

Parameter	Control group	Experimental group
PM/AT	11. 24±4. 75	5. 94±1. 29
MI (%)	27. 87±3. 87	21. 21±5. 30
Path(%)	1. 85 ±0. 28	12. 86±4. 49

PM/AT- prophase-metaphase/anaphase-telophase ratio
MI(%) – mitotic index, the ratio of the number of dividing cells to the total number of cells
Path (%) – pathology of meiosis (%) in number of devising cells

In the control group we detected high activity of cell's division MI (%), and prophases and metaphases none significantly prevailed to anaphases and telophases. Lagging of chromosomes and groups of chromosomes in metaphase leaded among meiosis pathology, however number of pathologies remained in the limits of the physiological norm (Morgan, 2007).

In the experimental group of animals activity of cell's division in testes after injection of Eprimec decreased slightly, but amount of pathologic meiosis increased in seven times. At the same time disturbed the balance of the ratio of prophases and metaphases to ana- and telophases. This fact indicate about the interlocking of normal division is still in its early stages and the simultaneous production of pathological cells (Tolbert et al, 1992).

In the comparison to control group in experiment these abnormalities of meiosis, as agglutination of chromosomes, formation of anaphase bridges, premature chromosomes in prophase and metaphase, lagging chromosomes and groups of chromosomes at all stages of division appeared (Il'inskih, 1984). Detected pathologies are connect with destruction of chromosomes and mitotic apparatus that disrupt meiotic cytokinesis and delays, and as a consequence is likely to lead to cell death (Motorna, 2001; Alov, 1972). In the experiment, we noted a significant number of cells with toxic vacuolization, and the appearance of cells with micronuclei.

CONCLUSION

Analyzing obtained data; we came to the conclusion that drugs based on eprinomectin slightly reduce the activity of cell division, but cause serious toxic and cariations caryations changes in the cells of the testes nonlinear white mice that speaks about the negative effects on the reproductive system of males. The using of drugs based on eprinomectin requires further study, as well as strict control of veterinary specialists.

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HABITAT TYPES OF EUROPEAN IMPORTANCE IN THE AREA OF WETLANDS GROMIZELJ (BOSNIA AND HERZEGOVINA)

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ABSTRACT

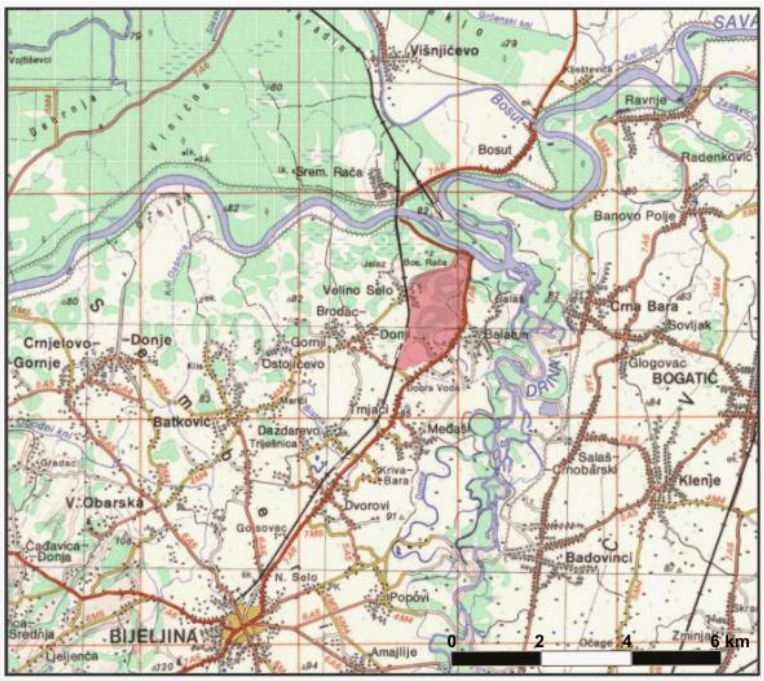
The paper presents the types of wetlands habitat Gromizelj which is of European importance. The review is made on the basis of studies of flora and vegetation and the Guide of the types of habitats according to the European Union (EU) Habitats Directive. Swamp Gromizelj is located in the northeast of Republic of Srpska (RS) and Bosnia and Herzegovina (BiH), in the municipality of Bijeljina. During the research the following habitats have been isolated: 3150 Natural eutrophic lakes with Magnopotamnion-or Hydrocharition-vegetation type, 3270 Muddy river banks with Chenopodionrubrip. p. and Bidention p. p. vegetation, 6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels, Reedbeds, tall sedges and vegetation of Phragmito-Magnocaricetea, 91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnionincanae, Salici-onalbae) and 91F0 Riparian mixed forests of *Quercus robur, Ulmus laevis* and *U. minor, Fraxinus excelsior* or *F. angusti folia,* along great rivers (*Ulmenion minoris*).

Key words: *habitat, wetlands, Gromizelj*.

INTRODUCTION

Connecting protected areas of RS and BiH in the European network of protected areas Natura 2000 is aimed to prevent the loss of biodiversity, preserve the habitat of endangered species and ensure their long term survival. For BiH, WWF MedPO started a project to support the implementation of the European ecological network Natura 2000 in 2007. Based on the literature data, the research of authors, supported by The EU, made the Habitat Types Guide of BiH according to the EU Habitats Directive.

Potentially protected area Gromizelj is located in the northeast of RS in the municipality of Bijeljina and GMT is between 44^{0}51'12, 5" and 44^{0}53'37" north latitude and 19^{0}18'04" and 19^{0}20'24" east longitude. Central coordinates of the point with coordinates are 44^{0}52'19" north latitude and 19^{0}19'15" east longitude. The surface of a natural resource is 831. 3 hectares. The map 1 shows the position of the natural resource.



Map 1. The situation of natural resource (geographical map 1: 200, 000)

The basic value of the natural resource "Gromizelj" is made of wetlands Gromizelj with Laketic sources and partially channelled stream Prugnjaca. Laketic source extends from southeast to northwest. According to average annual values issued, the length of the source ranges between 50 and 55 meters with a maximum width of 25 m. Laketic source islocated in the area of intensive agricultural production under significant anthropogenic pressure. The area is characterized by specific geological and hydrological phenomena and extraordinary biological diversity. This research was first recorded wetland grill (*Urticaki oviensis*) in BiH. *Urticaki oviensis*is on the Red List of Europe in the category of vulnerable species (VU).

MATERIALS AND METHODS

Floristic and vegetation research on areas are carried out from 2009 to 2011. Taking plant material and makingphytocoenological recordings were done at different habitats. Identification of species was based on floristic literature (Javorka and Csapody, 1979; Beck, 1903; Beck, 1927; Josifovic ed. 1970-1977; Domac, 1978; Sumatic et al., 1999). Phytocoenologically recordings were made by the method Braun-Blanquet (1965). During allocations European significant habitats in the wetlands Gromizelj used data research of flora and vegetation and Guide to the types of habitats according to the EU Habitats Directive (Milanovic et al., 2015).

RESULTS AND DISCUSSION

Based on explored flora, vegetation and habitats, according to the Review of the Habitats Directive of the EU, isolated habitats are shown in Table 1.

Table 1. Overview of habitat areas of swamp Gromizelj

Code	Name of habitats
3150	Natural eutrophic lakes with *Magnopotamnion*- or *Hydrocharition*-type vegetation
3270	Muddy river banks with *Chenopo dionrubri*p. p. and *Bidention* p. p. vegetation
6430	Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels
-	Reedbeds, tall sedges and vegetation of *Phragmito-Magnocaricetea*
91E0	Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnionincanae, Salicionalbae)
91F0	Riparian mixed forests of Quercusrobur, UlmuslaevisandU. minor, Fraxinus excelsior or F. angustifolia, along greatrivers (Ulmenionminoris)

3150 Natural eutrophic lakes with *Magnopotamnion*- or *Hydrocharition*-type vegetation

The habitat is characterized by significant biomass production, reduced mineralization and reduced amount of oxygen in the deeper layer of sludge. Natural well is located in the area of intensive agricultural production. In the water of lakes ammonia and nitrate nitrogen, are indicating that manufacturers perform intensive fertilizing arable land with NPK fertilizer. Saprobity index "S" was measured (Pant-Buck, 1955) which shows that the water source Laketic belong to class III and has a "moderate" ecological status. The concentration of dissolved oxygen in water ofLaketic source shows the requirements for class V indicating very poor status of water quality. The value of the saturation of water with oxygen is very bad. The water is rich in dissolved bases with a pH above 7 (Water Institute, doo Bijeljina, 2010). Habitat is populated with vegetation of submerged to class Potametea and order Potametalia which is articulated on alliance Magnopotamionauct (submerged plant communities that are not connected to the bottom); Nymphaeionalbae (community of plants with floating leaves) and Hydrocharition. From community of alliance Magnopotamionauct. there is *Ceratophylletumdemersi* belonging tosubmerged, aquatic vegetation not connected to the bottom and withoptimal development achieved during the summer. Species that participate in building of communities are: *Nuphar luteum, Myriophyllum spicatum, Hottonia-palustris*, *Potamogetonnatans* and *Lemna minor*.

Alliance *Nymphaeionalabae* includes community *Hottonietumpalustris* and *Myriophyllo-Numpharetum*. Association *Myriophyllo-Numpharetum* develops in the deepest zone of the source where the water is moderately enriched with organic and mineral substances. The main feature of the floristic community gives a dominant species *Nuphar luteum* and *Myriophyll umverticilatum*. There are also: *Potamo getonnatans*, *Myriophyllum spicatum*, *Certophyllumdemersum*.

Monodominant community *Hottonietumpalustris* occurs in Laketic source fragmentary. In the second half of the year, waters receded within forests of alder and ash, on the muddy and wet surfaces with a high level of ground water. After the withdrawal of water *Hottoniapalustris* takes emergent character growing together with species *Nuphar luteum*, *Calthapalustris* and *Siumlatifolium*.

Vegetation of hydrophytanot connected with bottom belongs to class *Lemnetea*, order *Lemnetalia*and alliance *Lemnionminoris*with community *Lemnetumminoris, Lemnetumtrisulcae* and *Hydrocharidietummorsus-ranae*. This type of vegetation rarelydevelops in the coastal zone of Laketic source, but it is more frequent in small depressions of swamps Gromizelj and in the part Prugnjace where water reserves in the first half of the year. At this habitat, intensive processes of decomposition of organic matter occur under the separation of methane, hydrogen sulfide, witha pH value above 7.

Community *Lemnetumminoris* belongsto submergedvegetation not connected to the bottom stands. It mainly develops sporadically along the coast of Laketic source in shallow irrigation canals and in parts Prugnjace. In that habitat, water is warm and moderately rich in organic matter and water flow is weak or absent. The water depth does not exceed 1 m, the bottom is muddy. Stands of the community are poor of species. In small depressions near Laketic source and the source of the coast stands of communities *Lemnetumtrisulcae* were recorded. There are species: *Lemna minor* and *Utricularia vulgaris*. Community of *Hydrocharidetummorsus-ranae* belongs to the type of floating not connected to the bottom aquatic vegetation observed in small depressions of Gromizelj near Laketic source, where the water is calm, standing, shallow, warm and eutrophic. Besides the dominant species *Hydrocharismorsus-healing*, there are also *Ceratophyllumdemersum*, *Lemna minor*, *Lemnatrisulca* and *Utricularia vulgaris*.

This type of habitat is the most representative in the upper part of the Pliva lakes, coastal zone of Spreca, at Bardaca, Svilaj, Vojskovi, Loncari, Sanicani, Prnjavor, but along the river Savait is largely absent (Redzic and Brudanovic, 2008-2009). This type of habitat is recorded in a number of permanent water surfaces ofBiH, ponds, canals and artificial lakes. (Milanovic et al., 2015).

3270 Muddy river banks with *Chenopodionrubri*p. p. and *Bidention p. p.* vegetation

Nitrophilic community of wetland Gromizelj belongs to alliance *Bidention tripartite* p. p.,order *Bidentetalia tripartite* and class Bidentetea tripartite. Itdevelopson wet and nitrated anthropogenic fluvisol and eugley. The soils have mainly base reactions. This type of vegetation is typical for autumn.

At the sites Sljunkara and Prugnjaca nitrophilous vegetation of muddy and wet habitats alliance *Bidentitiontripartitae* and communities *Polygonohydropiperis-Bidentetumtripartitae* and *Polygonetumlapatifoliae* were recorded. Short belt of flat and slightly inclined coast Šljunkara is covered with association *Polygonohydro-piperis-Bidentetumtripartitae.* This association is well developed in the Prugnjaca in the second half of the year, when water is withdrawn, which takes up significant area. In a community, the dominant species are *Polygonumhydropiper* and *Bidenstripartita.* There are also: *Menthaaquatica, Alismaplantago-aquatica, Veronica becabunga, Juncusatriculatus, Lycopuseuropaeus, Lythrumsalicaria, Lycopusexaltatus, Agrostis stolonifera, Salix alba, Eupatorium cannabinum*and others.

Association of *Polygonumlapathifolium* occurs more frequently in the study area by building monodominant stands that take up less surface area.

This vegetation is distributed in the flood zone of the Sava River on lands rich with nitrates. They are very developed along the river backwater. Its dominance increases going upstream towards Bosanska Raca (at the mouth of the Drina River). Nitrated stagnant water isin the wider area of Plivsko jezero, Modrac, Spreca, Gradiska, Sanicani, Prnjavor, Svilaj, Bosanski Samac, Odzak, Orasje, Ukrina, Zabar, Loncari, Raca (Redzic and Brudanovic, 2009).

6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels

The vegetation of tall herbs was developed in the hydrophile forests of alder, willow and poplar, and in the forests of oak and elm. Optimal location is on the wet nitrified soils and the openings ofhygrophile land. Depending on the degree of ecological conditions of humidity, shade and nitrified land vegetation of high green two alliance can be differentiated: *Senecionfluviatilis* and *Petasition officinalis.* Alliance *Seneciofluviatilis* includes typical Posavina hydrophilic-nitrophilic community. This vegetation, in ecological terms, continues to vegetation and alliance Magnocaricion and Phragmition. Some of the species that participate in the construction of high green vegetation are: *Angelica sylvesris, Barbarea vulgaris, Rorippaaustriaca, Althea officinalis, Potentillareptans, Menthalongifolia, Glycirhizaechinata* and other.

Vegetation of alliance*Petasition officinalis* is recorded in the vicinity of roads, dykes and settlements, in places rich in nitrates. It often occurs in degraded forests of alder, willow, ash and oak. As characteristic, the species of these habitats are: *Aegopodiumpodagraria, Eupatorium cannabinum, Parietaria officinalis, Galiumaparine, Artemisia vulgaris, Urticadioica, Sambucusebulus*. Those species are involved in building of communities: *Urtico-Aegopodietum, Urtico-Sambucetumebuli, Eupatorietumcannabini* and*Urtico-Parietarietum.* An important area of habitat isoccupied by invasive species *Polygonumcuspidatum*and *Echinocystislobata*.

Reedbeds, tall sedges and vegetation of Phragmito-Magnocaricetea

The belt of aquatic vegetation community builds on floodplains is dominated by emersal hydro-heliophytes of classPhragmitetea, order Phragmitetalia covered withalliance *Phragmition* and *Sparganio-Glycerion.* Alliance *Phragmition* belongs to the community *Scirpo-Phragmidium, Typhaetumangustifolia, Typhaetumlatifolia, Phragmitetumaustralis* and *Sparganietumerecti*. Community of reeds and sedgeshasan important role in preserving the overall plant and animal diversity of wetland habitats.

Community of *Scirpo-Phragmitetum* inhabits continuously or periodically flooded areas of Laketic source and Sljunkara. The basic characteristic of floristic community types comes from the following species: *Phragmitescommunis* and *Scripuslacustris*. The narrow coastal strip of Sljunkara is covered with species *Scirpuslacustris* which builds less facies. It was noted that the facies with *Scirpuslacustris* alternately change the community stands of *Typhaetumangustifoliae* and *Typhaetumlatifoliae*. Differences were observed in the floristic composition of communitiesbetweenLaketic source and Sljunkara. In the community stands *Scirpo-Phragmitetum*of Sljunkara, besides dominant species, there are also: *Lycopuseuropaeus, Menthaaquatica, Iris pseudacorus, Lythrumsalicaria, Polygonumlapathifolium, Bidenstripartita*. In the community of Laketic source, in a significant number, there are species: *Siumlatifolium*and*Sparganiumeraticum.*

Ass. *Phragmitetumaustralis* is present at the sites Prugnjaca, Sljunkara and Citluk where it occupies significant space, a slightly larger area under this community can be foundin Buline. The floristic composition is dominated by *Phragmitesaustralis,* which survives in conditions after the withdrawal of water where there is a high level of groundwater. Community of reeds is mainly developed in the forest belt of alder, willow, poplar and ash.

Hygrophilous community *Typhetumangustifoliae* is recorded at the site Sljunkara. It inhabits shallow water along the coastal part.

Ass. *Sparganio-Glycerietumfluitans* belongs to alliance *Sparganiumglycerion* and it is particularly developed in some parts of Prugnjaca. There is a wide ecological valence compared to hydric regime. Significant surface of Prugnjaca is covered with community *Sparganietumerecti* which joins withspecies *Urticakioviensis* and builds a specific community. It is necessary to monitor this "community" with the aim of describing and determining the appropriate status. For the territory of Bosnia and Herzegovina, *Urticakioviensis* was firstly recorded in Laketic source, on december 2008.

Alliance of *Salicioncinerea* includes a community *Salicetumcinerea*. It is presenton the coast of Laketić source with permanent flooding during the first half of the year. The community is dominated by wading willow bushes which reaches a height of 2-3 m, and there are species: *Telypterispalustris*, *Urticakioviensis*, *Carexremota, Sparganiumerectum, Menthaaquatica* and others. Particularly important species *Telypterispalustris, Urticakioviensis*are rare species for the area of BiH. The only, so far known, habitat of *Urticakioviensis*in Bosnia and Herzegovina is wetland of Gromizelj and Laketic source, as well asfor *Telypterispalustris* in RS.

The habitat of these species is not defined in "Interpretation manual of European Union habitats" but it is necessary to specify it as a literary data. Community of reeds and rushes are distributed along the flood areas of the Sava River and lower parts of its tributaries. Amongthem, the most important habitats arein the area of fishpond Sanicani, fishpond Bardaca, fishpond Prnjavor, hinterland of lake Modrac, the western part of the large Pliva lakes, backwaters zone in the area Odzak, Svilaj, Velikaand Mala Tisina, Loncara. In other places, this vegetation is sporadically developed in the form of small fragments (Redzic and Brudanovic, 2009).

91E0 Alluvial forests with Alnusglutinosaand Fraxinusexcelsior (Alno-Padion, Alnionincanae, Salicionalbae)

Hygrophilousforests with *Alnusglutinosa* (*Alnetumglutinosae*) sintaxonomic belong to the Central European alliance Alnionglutinosae, order Alnetaliaglutinosae and class Alneteaglutinosae. They arelocated in places where is a high level of ground water throughout the year, mainly on deep fluvisol soils, gleys and pseudogley. Surface water is retained until May when it withdraws. In the area of swamps Gromizelj this community occupies about 10 hectares of the study area. In the floor of the trees there is species withthe largest cover value *Alnusglutinosa*. Trees reach a height over 20 meters. From shrubs there are:*Cornussanquinea, Sambucusnigra*, *Ligustrum vulgare*, *Corylusavellana*, *Acer tataricum*, *Viburnum opulus*, *Euonymus europaeus*and other species. In the floor of the herbaceous plants there are: *Stachyspalustris*, *Carexelongata*, *Carexremota*, *Lysimachia vulgaris*, *Solanum dulcamara*, *Thelypterispalustris*. Forests of *Alnusglutinosa*have an important role in maintaining ecological balance, hydric regime, preservation of groundwater and the existing biodiversity. It should be noted that the communities in this area are well preserved, but there is always a danger of logging and the spread of invasive species *Echinocytislobata*, *Asclepiassiriaca* and *Amorphafruticosa*. The high importance of the forest ecosystem has resulted in putting habitat in priority in conservation (Annex 1 of the Habitat Directive). Community of *Alnusglutinosa,* alliance Alnionglutinosaeis widespread in Posavina, and in the coastal zone of large rivers, particularly in their lower courses (Una, Vrbas, Bosna, Drina). The forests of *Alnusglutinosa*, have been developed as azonal vegetation along other waterways in the continental part of BiH (Redzic and Brudanovic, 2009).

Community Frangulo-Alnetumglutinosae is on the smaller areas that have the mosaic distribution. Significant cover value in the floor of bushes has species *Frangulaalnus,* and there are other species such as: *Viburnum opulus*, *Rhamnuscathartica*, *Thelypterispalustris*, *Glechoma hederacea*, *Salix cinerea*. In the floor of the herbaceous plants there are species:*Solanum dulcamara*, *Symphytumtuberosum*, *Polygonumlapathyfolium*, *Stachyspalustris*, *Lythrumsalicaria*, *Iris pseudacorus*, *Siumlatifolium*and other.

A significant feature of alluvial forests vegetation of *Alnusglutinosa* and *Fraxinus excelsior* (*Alno-Fraxinetumangustifoliae*) has a high ecosystem importance which is resulting in separation of the community into the category of priority habitat conservation. In the trees floors, the dominant species is *Fraxinusangustifolia* and

there are also: *Populusnigra, Populusalba* and *Alnusglutinosa*, and on the higher ground there is *Quercusrobur*. In the floor of shrubs there are species: *Ulmuslaevis, Rhamnuscatartica, Frangulaalnus, Acer campestre, Salix fragilis*, and in the floor of herbaceous plants there are: *Leucojumaestivum, Ficariaverna, Crocus vernus, Arum maculatum, Lysimachianummularia, Menthaaquatica, Lycopuseuropaeus* and others. From community of alliance Salicionalbaet here are: *Salicetumalbae-fragilis, Populetumnigro-albae, Salici-Populetumnigrae, Salicetumalbae* and *Salicetumfragilis*. Community of *Salicetumalbae*is developed on the site Pristalovac and coasts of Prugnjaca. The dominant species in community is *Salix alba* and there are also species: *Salix fragilis, Populus alba, Populusnigra, Ulmuseffusus*, in shurbs there are: *Amorphafruticosa, Cornus mas, Crataegusmonogyna.* In the floor of herbaceus plants there are: *Menthaaquatica, Solanum dulcamara*and others.

Populusalba and *Populusnigra* build special community in smaller areas. Association *Populetumalbae* is recorded near Prugnjaca and wider area Pristalovca.

91FO Riparian mixed forests of Quercusrobur, UlmuslaevisandU. minor, Fraxinus excelsior or F. angustifolia, along greatrivers (Ulmenionminoris)

Alliance of *Alno-Quercionroboris* includes wet swamp lowland forest regions developed over periodically flooded fields or beyond, but a significant role in the maintenance of these forests has the presence of high levels of groundwater. Riparian forests of *Fraxinusangustifolia* are present on some dry habitats but with a high level of ground water. In riparian forests of *Fraxinusangustifolia* community *Leucojo-Fraxinetumangustifoliae* is developed which is registered on the site Ranisavljeva basca, on the surface of about 1 hectare in the depression which is strongly influenced by the flood and groundwater. In the floor of trees the dominated species is *Fraxinusangustifolia*, in the floor of herbaceus plants there are: *Leucojumaestivum, Galiumpalustre, Lycopuseuropaeus, Solanum dulcamara, Carexelongata*and others.

On the elevated grounds with short-term flooding or no flooding communities of *Quercusrobur* and *Genistaelata* (*Genistoelatae-Quercetumroboris*) were developped. Community belongs toalliance *Alno-Quercionroboris*, order *Alnetaliaglutinosae* and class *Alneteaglutinosae*. Due to excessive logging of *Quercusrobur* community has significantly changed the original appearance. Ground floor of community abounds with offspring of *Quercusrobur*. This community has a very important role in the preservation of biodiversity of Posavina landscape and deserves special priority in protection.

Mixed forests of *Fraxinusangustifolia* and *Alnusglutinosa* with *Quercus*occupy about 14 hectares of the study area. They occupy most hydrographic positions (beams) where flooding is short and affordable level of groundwater is located at a considerable depth. These forests are the first transition of hydrophilic alluvial vegetation according to climatogenic forests. Only *Quercusrobur* forests in the wide alluvial valleys belong to this type of habitat (Milanovic at el., 2015).

CONCLUSION

Connecting protected areas of RS and BiH in the European network of protected areas Natura 2000 aims to prevent the loss of biodiversity, preserve habitats of endangered species and ensure their long-term survival. For BiH, WWF MedPO started a project to support the implementation of the European ecological network Natura 2000in 2007.

The paper presents the habitat areas of Gromizelj which is of European importance. During the research and according to Guide to the types of habitats of BiH the following habitats were isolated: 3150 Natural eutrophic lakes with Magnopo-tamnionor Hydrocharition-type vegetation, 3270 Muddy river banks with Che-nopodionrubrip. p. and Bidention p. p. vegetation, 6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels, Reedbeds, tall sedges and vegetation of Phragmito-Magnocaricetea, 91E0 Alluvial forests with *Alnusglutinosa*and *Fraxinusexcelsior* (Alno-Padion, Alnionincanae, Salicionalbae) and 91F0 Riparian mixed forests of *Quercusrobur, Ulmuslaevis*and *U. minor, Fraxinus excelsior* or *F. angustifolia*, along large rivers (*Ulmenionminoris*).

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SOCIAL NETWORK SITES UTILIZED IN AGRICULTURAL EXTENSION SERVICES IN KINGDOM OF SAUDI ARABIA

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ABSTRACT

The purposes of this research are to: i) Measure the degree of utilization of Social Networks (SNS) (Facebook, Twitter, Instgram, Google Plus, LinkedIn, Flicker, Tumblr, Hi5, Whatsapp, Snapchat, and Youtube) among agricultural extension personnel in Kingdom of Saudi Arabia (KSA), ii) Identify the role of SNSs in provision of extension services from the viewpoint of respondents, and iii) Recommend interventions needed to enhance the utilization of SNSs in provision of extension services. An online survey with 55 agricultural extension personnel represent 22% of the total number of extension personnel in KSA (250 personnel) was conducted in April-May 2016, frequencies and percentages were used for data presentation. Sample was young (82% are less than 45 years old) and rather highly educated (75% were have university degree or postgraduate studies). Results show that the majority of respondents (60%) have a medium level of SNS utilization, while the remaining 40% were divided equally between low and high levels of utility. More than two-fifths (46%) declared that they highly prefer using SNS in contacting farmers, and the same percentage indicated that SNS is highly facilitate their work circumstances. Most of respondents (85%), stated that SNSs have positive impact in facilitation of extension personnel communication capacities, most of them (96%) indicated the high and medium positive impact of SNSs in the extension employee's acquiring knowledge. Also, most of respondents (91%) stated that SNSs were helped them in achieving their work tasks with medium and highly base. Study recommends and concludes some interventions for better SNS-based extension services in KSA.

Keywords: *social media, online survey, agricultural extension, KSA.*

INTRODUCTION

Agricultural extension services in Kingdom of Saudi Arabia (KSA) are provided to growers through thirteen general agricultural administrations, twelve agricultural directorates, and 107 agricultural branches all over the kingdom (AlSaghan, 2011).

Agricultural extension describes the services that provide rural people with the access to knowledge and information they need to increase their productivity and improve their quality of life, it includes, but is not limited to, the transfer of knowledge generated by agricultural research using communication channels (NRI, 2014). Social network sites (SNSs) or social media have become essential necessities of present day life; they provide knowledge, communications, and a means for sharing (Al-Daraiseh *et. al*., 2014; Diab & Abdel-Rahman, 2016).

By 2015, the internet users in the world reached about 3. 4 billion users (46. 4% of the total world population), Asia gained about 1. 6 billion of them (40. 2% of Asian population). The KSA have 18. 3 million internet users represent 65. 9% of the total number of nation's population (Internet World Stats, 2015). Since their appearance, Social Network Sites (SNSs) have attracted millions of users, many of whom have integrated these sites into their everyday practice. SNSs are computer-mediated instruments that allow individuals or organizations to create, share, or exchange information, career interests, ideas, and pictures/videos in virtual communities and networks (Boyd, and Ellison, 2008; Petersen & Johnston, 2015).

The first Arab social media report indicated that the SNSs impacted on Arab society as well as business and economy, these impactsinclude: connecting people together and shortening distances between them, instant platform to get news and information, aid in learning new things, offers entertainment features, allows for "cheap" means of communication, offers opportunities for job hunting andcareer growth, enables faster business growth and expansion, direct revenue generation through social media, improving corporate image, marketing and advertisement platform, talent hunt, and encourage consumer-centric and transparent approaches(TNS, 2015; AlShahry, 2012).

The e-government program indicated that Facebook, Twitter, and Youtube are most popular platforms in governmental institutionsin KSA (Saudi Arabia e-government, 2015). Saudi Arabia had 2. 4 million active Twitter users in 2014, making it the fastest growing Twitter nation in the world (BTI, 2016). Facebook's mobile users have increased more than 150%, Saudi's LinkedIn users have grown 30% since January 2012 (The social clinic, 2013). Dubai School of Government (2012) and Shoaib & Shoaib (2016) stated that Saudi Arabia is among the top Arab countries according social media users in terms of penetration and numbers. There are 90 million video views in Saudi per day (the highest number of YouTube views in the world per Internet user). Saudi Arabia leads the region with the most playbacks followed by Egypt, Morocco and UAE. YouTube uploads jumped 200% and views increased 260% in 2011 versus 2010.

Relying on the previous quick discussion, it's obvious that determining NSNs utilized by agricultural extension personnel in order to enhance agricultural extension personnel'sbehaviour relevant to modern communication methods. From this starting point, the purposes of this research are to: i) Measure the degree of utilization of Social Networks Sites among agricultural extension personnel in KSA, ii) Identify the role of SNSs in provision of extension services from the

viewpoint of respondents, and iii) Recommend interventions needed to enhance the utilization of SNSs in provision of extension services.

MATERIALS AND METHODS

The current study focuses in eleven social network sites in KSA, namely: 1) Facebook allows users to create their profiles, add other users as friends, exchange messages, post status updates and photos, share videos, use various apps, and users may join common-interest user groups, 2) Twitter enables users to send and read short 140- character messages called "tweets", 3) Instagram is an online mobile photo-sharing video- sharing, and social networking service, 4) Flickr help people make their photos available and organizing photos and video. 5) Hi5 had many features, such as friend networks, photo sharing, user groups, status updates, and gaming and entertainment, 6) Tumblr allows users to post multimedia and other content to a short-form blog.,7) WhatsApp allows users send text messages, documents, images, video or audio messages to other users, 8) Snapchat used for creating multimedia messages called "snaps"; snaps can consist of a photo or a short video, and can be edited to include filters and effects, text captions, and drawings, 9) You tube allows users to upload, view, rate, share, and comment on videos, 10) Google Plus: is an interest-based social network, and 11) Linkedin is a business-oriented social networking services, mainly used for professionalnetworking.

An online survey with 55 agricultural extension personnel represent 22% of the total number of extension personnel in KSA (250 personnel) was conducted in April-May 2016. The survey was designed for achieving the study objectives. The survey included sets of questions to measure the study variables, as follows: 1) Age: measured by respondents' years of old, 2) Education: measured by respondents' official education degree, 3) Extension work experience: measured by number of years spent by respondents from starting current carrier till now, 4) Job Class: respondents were asked to indicate their job class in the organizational hierarchy, 5) level of SNSs' utility: respondents were asked to state whoever they use or not the eleven social network sites; scores were assigned to responses as yes = 2, no = 1. Accordingly responses combined to each respondent, the theoretical range reached 11 to 22scores. Then responses were classified to three levels of SNSs utilization as follows: low utilization (11 – 14 scores), medium utilization (15 – 18 scores), and high utilization (19 – 22 scores). 6) Respondents preferences of SNSs to communicate with farmers, SNSs helping in acquiring knowledge, and role of SNSs in achieving work tasks: respondent were asked to indicate their opinions. Responses ranged from agreement to disagreement as follows: high = 3, medium = 2, low = 1, no = 0. Therefore, total number ranged from the minimum of 0 to the maximum of 3. Finally, frequencies and percentages were used for data presentation.

RESULTS AND DISCUSSION

Figures in table 1 represent the main characteristics of the respondents. Among the main findings is that majority (81%) are less than 45 years old, three- quarters (75%) have a university degree or post graduate degree. These results indicate that agricultural extension system in the KSA is still youthful and highly educated- this result is agreed with the official Saudi's demography data- that may help in fostering adoption and diffusion of modern technology (i. e. social media) within the organization. With regard to work experience, data show that majority of respondents (60%) have more than five years of work experience. The majority (85%) are located in a middle position of job class hierarchy (6^{th} - 9^{th} classes). This implies that extension employees are highly expertise and have the willingness to job promotion.

Table 1. Distribution of respondents according to their characteristics

Variable	**Freq.**	**%**	**No.**	**Variable**	**Freq.**	**%**
Age				Work experience		
≤ 24years old	1	1. 82		< 5 years	22	40. 00
25 - 35	6	10. 91		5 -	18	32. 73
35 - 45	38	69. 09		10 -	7	12. 73
45 – 55	10	18. 18		15 -	7	12. 73
Job class				20 and more	1	1. 82
≤5^{th} class	3	5. 5		Education		
6^{th} class	7	12. 7		≤secondary	3	5. 45
7^{th}class	9	16. 4		Agricultural Diploma	11	20. 00
8^{th}class	13	23. 6		College	33	60. 00
9^{th}class	18	32. 7		Post graduate degree	8	14. 55
10^{th} class	4	7. 3				
11^{th} class	1	1. 8				

**Source: Study's survey*

Utilization of Social Networks Sites

Figures in table 2 show scores of respondents' utilization level of social network sites. The majority of respondents (60%) have a moderate level of utilization compared with 20% of them have high and low scores for each. This finding implies that the utilization of SNSs has anacceptable potentiality with extension employees, also the category of high level could be increased if the study were concentrated in limited number of sites.

Table 2. Distribution of respondents' level of utilization of social network sites

Range		Mean	S. D.	High (19 – 22 scores)		Moderate (15 – 18 scores)		Low (11 – 14 scores)	
Min.	Max.			Freq.	%	Freq.	%	Freq.	%
13	21	16. 44	2. 39	11	20	33	60	11	20

**Source: Study's survey*

Results in table 3 show that WhatsApp, Twitter, and Facebook are the most popular sites, on the other hand, Flicker, Tumblr, and Hi5 are less preferred sites. Most of respondents (86%) are subscribed inWhatsApp, almost 55% (or 6 out of 11) of users stated that WhatsApp is the most preferred site for them; this indicated that WhatsAppis now the leading social media platforms in Saudi Arabia.

In respect of Twitter, findings in table 3 show that majority of respondents (69%) are Twitter users. Near to fifth (16%)of SNSs users indicated Twitter as the almost preferred site for them. With respect to Facebook, findings in table 3 show that half of respondents (50%) are subscribers in such platform, of each 7 subscribers there is 1 stated that Facebook is the most preferred site for him. The progress of WhatsApp, Twitter, and Facebook are agreed with the previous studies related to the Saudi's social media penetration and numbers, and may attributed to ease of use and mobile-based of such platforms.

Regarding the less preferred network sites, finding in table 3 revealed that majority of respondents (72. 7%, 70. 8%, and 78. 2%) were none users of Flicker, Tumblr, and Hi5 respectively. These sites also received the low preference behavior of respondents, since none of them stated any of these platforms as top preferred SNS.

Table 3. Distribution of respondents' ranking of social network sitesutilization

Response / SNSs		Use	Rank of preference											Not use	Score
			1	2	3	4	5	6	7	8	9	10	11		
Whats App	%	85. 5	54.6	10.9	7. 3	9. 1	1. 8	0.0	0.0	0.0	1.8	0.0	0.0	14.6	10.1
	Freq	47	30	6	4	5	1	0	0	0	1	0	0	8	
Twitter	%	69. 1	16.4	32.7	18.2	0. 0	1. 8	0.0	0.0	0.0	0.0	0.0	0.0	30.9	9. 9
	Freq	38	9	18	10	0	1	0	0	0	0	0	0	17	
Facebook	%	49.1	11.6	9. 1	14.6	5. 5	3. 6	0.0	1.8	0.0	0.0	0.0	0.0	50.9	9. 4
	Freq	27	8	5	8	3	2	0	1	0	0	0	0	28	
Google Plus	%	43. 6	1. 8	10.9	9. 1	14.6	0. 0	5.5	1.8	0.0	0.0	0.0	0.0	56.4	8. 5
	Freq	24	1	6	5	8	0	3	1	0	0	0	0	31	
Instagram	%	61. 8	7. 3	7. 3	16.4	10.9	9. 1	7.3	1.8	1.8	0.0	0.0	0.0	38.2	8. 3
	Freq	34	4	4	9	6	5	4	1	1	0	0	0	21	
Youtube	%	78. 2	1. 8	16.4	16.4	16.4	7. 3	5.5	7.3	1.8	1.8	3.6	0.0	21.8	7. 7
	Freq	43	1	9	9	9	4	3	4	1	1	2	0	12	
Linked In	%	29. 1	1. 8	3. 6	1. 8	3. 6	12.7	0.0	1.8	1.8	1.8	0.0	0.0	70.9	7. 3
	Freq	16	1	2	1	2	7	0	1	1	1	0	0	39	
Snapchat	%	49. 1	1. 8	3. 6	3. 6	14.6	9. 1	5.5	1.8	0.0	5.5	3.6	0.0	20.9	6. 8
	Freq	27	1	2	2	8	5	3	1	0	3	2	0	28	
Flicker	%	27. 3	0. 0	5. 5	0. 0	3. 6	3. 6	9.1	0.0	3.6	1.8	0.0	0.0	72.7	6. 7
	Freq	15	0	3	0	2	2	5	0	2	1	0	0	40	
Tumblr	%	29. 1	0. 0	0. 0	3. 6	0. 0	5. 5	3.6	9.1	1.8	3.6	1.8	0.0	70.9	5. 5
	Freq	16	0	0	2	0	3	2	5	1	2	1	0	39	
Hi5	%	21.8	0. 0	0. 0	1. 8	1. 8	0. 0	5.5	1.8	9.1	0.0	1.8	0.0	78.2	5. 2
	Freq	12	0	0	1	1	0	3	1	5	0	1	0	43	

**Source: Study's survey*

Social media and Agricultural extension

Respondents were asked to indicate the degree they prefer to use SNSs in communication with farmers, most of respondents (85. 45%) stated that theyhighly and medium prefer social mediain communication with target groups (table 4). Due to the distance between farms, foreign farm workers, the administrative response-bilities of extension personnel, and the large amount of area to be served by each personnel, extension personnel prefer social media platforms to communicate with farmers and farm owners regardless time, distance, effort or cost. Respondents also were asked to indicate how SNSs helped them in acquiring knowledge and information, results in table 4show that most of respondents (96. 37%) indicated thatSNSs have helped them in acquiring knowledge and information. The social network sites play a vital role in exchange knowledge and information between individuals and organizations. With regard to the role of social media in achieving work tasks, findings in table 4 revealed that most of respondents (90. 9%) stated

that SNSs were helped them in achieving their work tasks with medium and highly effect. Because social media are wonderful windows of communication, creating strong friendships, discover the local leaders and retrieve conversations between farmers and extension personnel.

Table 4. Role of social network sitesin provision of extension services

Categories / Item	Mean	S. D.	Highly		Medium		Low		No	
			No	%	No	%	No	%	No	%
I prefer it to contacting with farmers	2. 31	0. 72	25	45. 45	22	40	8	14. 55	0	0. 00
It helped in acquiring knowledge	2. 47	0. 63	29	52. 73	24	43. 64	1	1. 82	1	1. 82
It helped in achieving work tasks	2. 33	0. 75	25	45. 45	25	45. 45	3	5. 45	2	3. 64

**Source: Study's survey*

Enhancing the utilization of SNSsin provision of extension services

Findings in table 5 indicated eight recommendations for better SNS-based extension services in KSA. The most frequent recommendation were: Providing theextension personnel with smart devices and web accessibility (55%), this implies that extension personnel use their own smart devices and personal internet access to communicate with farmers through personal social media profiles, they recommend that official internet access and smart devices should be offered form them from the department of extension. They also stated the lack of trainings concerning social media, the emergence communication channels in advisory services, so the continuance internal and external trainings for staff relevant to electronic extension have the second needed intervention stated by near half (44%) of respondents). However, establishing linkages among various stakeholders in agricultural sector became in the third position and indicated by more than one-fifth (22%) of respondents.

Table 5. Interventions needed to utilize SNSs in provision of agricultural extension services

No.	Interventions	No.	%
1.	Providing the extension personnel with smart devices and web accessibility	30	54. 55
2.	Continuance internal and external trainings for staff relevant to electronic extension	24	43. 64
3.	Establishing linkages among various stakeholders in agricultural sector	12	21. 82
4.	Competitive incentives for personnel	8	14. 55
5.	Convenient transportation means for extension personnel	8	14. 55
6.	Establishing database for farmers, activities, and extension service	7	12. 73
7.	Building multimedia (audio/video) agricultural recommendations ready for share	7	12. 73
8.	Publishing pages, groups, accounts, and channels for agricultural extension services	6	10. 91

**Source: Study's survey*

CONCLUSION

This study highlighted the significance of social network sites for agricultural extension and advisory services, since the social network sites has changed the way we think, talk, watch TV, listen to music, search potential employer and employee and sometimes start a revolution. The social media in KSA is perceived as having numerous positive aspects that enhance the provision of agricultural extension, business profitability and exchange knowledge and information. Some of needed interventions could be summarized as follows:

1. Many efforts are required(from training department of the ministry) to increase the awareness and skillsof farmers, researchers, and extension workers regarding the potential role of social media in provision of extension and advisory servicesthrough training programs.
2. Since, young people and teenagers (futuristic farmers, extension workers, researcher, and investors) are the most subscribersof social media, extension services could influences on their agricultural socialization.
3. In order to enhance agricultural extension personnel's behaviour relevant to modern communication methods, efforts should be directed towards providing them with tools relevant to deal with electronic extension such as smart devices and internet access.
4. A series of reform interventions are necessary to integration of digital tools (i. e. smart devices, mobile applications, and social media) into agricultural extension communication channels.
5. One of reform alternatives could be based on social networking services, by creating official pages, groups, or accounts in Whatsapp, Twitter and Facebook to facilitate recommendation transfer and needs identification, and

A web-based forum for agricultural extension and advisory services could be established to bring all stakeholders together (research and development, extension providers, agricultural industry, decision makers, farmers, investors, and traders etc.), this forum could start virtually (on-line) as a pre-step to bring it into reality.

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COMPARATIVE CHARACTERISTICS OF PHOTOSYNTHETIC ACTIVITY OF NECTARINE CULTIVARS AND FORMS WITH DIFFERENT COLORED LEAF PLATE

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ABSTRACT

Genotypes of nectarine with red leaves were created in Nikita botanical gardens. They have commercially valuable properties and biological characteristics (resistance to powdery mildew, large-fruited, the ability of transmitting the trait of red leaves with constant result to progeny seed, etc.). The aim of our researches was to study the main indicators of photoactivity of leaf apparatus of nectarine with green and anthocyanin leaves coloration. Investigations have been carried out in 2010-2015 on intact leaf plates for three cultivars and forms of nectarine with green leaves – Rubinoviy-8 (control), Chemus, Krymtsuht 53-85 and 2 forms with anthocyanin coloration of leaf plate (NektadianaKrasnolistnaya 996-88, Krasnola 495-86). Photosynthetic activity was characterized by chlorophyll fluorescence parameters (Kautsky effect). The content of chlorophyll *a* and *b* were determined by spectrophotometry. The cultivar Rubinoviy-8 and the form - Krasnola 495-86 were selected according to intensity indicators of the primary reactions of photosynthesis. Anthocyanin forms concede cultivar Rubinoviy-8 in efficiency of the primary reactions of photosynthesis an average of 48-50%; but they exceed nectarines Chemus and Krymtsuht 53-85 with green color of leaves of 23-25%. The efficiency of energy supply in the "dark" reactions of photosynthesis in all varieties and forms of nectarine maintained within 41-46%. But the further ability to utilize received energy is most efficiently implemented at the nectarine cultivar Rubinoviy-8 and form Krasnola 495-86. The forms of red-leaved nectarine in comparison with traditional cultivars are distinguished by a great potential for retaining the stability and productivity of the functioning of the photosynthetic apparatus.

Keywords: *nectarine, leaves, photoactivity, fluorimetric indicator, chlorophyll.*

INTRODUCTION

The Nikita Botanical Gardens collected a large collectionof nectarine of cultivars and forms. It contains more than 156 genotypes. The collection comprises cultivars and formsfrom own breeding well as the introduced samples obtained from various

regions of the world. Genotypes with red coloration of leaves account for 15%of total number.

An important input to the development of nectarine provided Shoferistov E. P.,doctor of biological sciences and leading researcher. Under his leadership there were elaborated basic principles of reference of this culture, introduced cultivars from natural regions of the Europe, China and America, alongside with greenleaved plants were created nectarine genotypes with red colored leaf. Our team bred a range of promising cultivars with excellent combinations of economically valuable traits for using in the Crimean farm-garden industry. In 2015, we received the plant patents for 3 cultivars: (Rubinoviy 8, Nikitskiy 85, Krymchanin), setting out their growing in Russian nurceries (Shoferistov, 1999; Smykov, 2015).

It should be noted that the department of horticultural crops of Nikita botanical garden newly produced hybrids of red leaf peach with nectarine (P. *vulgaris* Mill. subsp. *Atropurpurea*(Schneid.) which were characterized by intense anthocyanin coloration of fruit pulp and leaves.

As a result of long term research there are allocated: 1) red colored leaves nectarines called Krasnola 179-81, Krasnola 436-85, № 703-89 and others capable to transmit constantly the trait of leaf red coloring to seed progeny; 2) red colored leaves nectarines that are resistant to powdery mildew (*Sphaerothecapannosa* Lev. var. *persica*Woronich) – 485-86, 495-86, 500-86, 501-86, NectadianaKrasnolistnaja 996-88 (Figure 1.)and others; 3) red colored leaves nectarines with large size fruit (110-130 g) – 487-89 (Figure 2.), 703-89, 485-86 and others (Shoferistov, 1995;Shoferistov E. and Ovchinnikova Iu.,2006).

a) b)

Figure 1. Red leaves (a) and flowers (b) of nectarine Nektadiana Krasnolistnaja 996-88.

Figure 2. Fruit of nectarine 487-86

In world practice plants with anthocyanin coloration of leaves are widely used in ornamental gardening and small-stature clonal rootstocks are widely used (Usova, 1997; Romanov and Usova, 2007). Red leaf color is caused by anthocyans which alongside with chlorophyll and carotenoids are the main plant colorants of flavonoid group (Harborne, 1967; Harborne, 1976;Kulikov and Ivanova, 1976;Brouillard, 1993). The feature of red leaf color hasn't been studied sufficiently but at the same time it could be regarded as a universal method to increase agricultural plant productivity due to anthocyanins that take part in photosynthetic process of vegetal pigment. The interest in such non-traditional forms in horticulture arises in connection with the peculiarities of their functioning in the conditions of high temperature, moisture deficit and overall stability relating to productivity. Anthocyanins protect plants from ultraviolet radiation, increase frost resistance, rooting ability, asphyxiation and diseases and pests resistance (Romanov and Usova, 2007; Trutneva et al., 2012).

Current photosynthetic activity researches are commonly performed on greenleaved plants. In the main such researches were conducted on peach, nectarine, apricot, apple and others fruit species. These characteristics weren't studiedon nectarines withred leaves. Present research article represent the results of experiments conducted on green and red colored nectarineleaves of using fluorimetric indicators, indirectly characterizing the photosynthetic activity of pigmented structures.

The fluorescence and its kinetic components are often used for analysis of efficiency of photosynthetic processes in the leaves of higher plants (Edwards and Uoker, 1986). The most interesting is the technique based on photoinduction of chlorophyll fluorescence (Kautsky effect). Its essence lies in the fact that with the reducing of fluorescence intensity grows the intensity of photosynthesis, the higher the level and the more the duration of fluorescence, the less effective functions the photosynthetic apparatus of leaf. The indexes of intensity of photo-induced chlorophyll fluorescence (F) were evaluated on the basis of following principles, which, at the root, do not go against modern ideas about photoactive properties of leaf apparatus:

- the indexes F_m, F_0, F_v/F_0 – characterized the efficiency of primary of photo-synthetic reactions in the leaves, related to functioning of light-harvesting chlorophyll-protein complex. The higher index value, the larger the potential of relative cultivar or form as to exercising of photosynthesis and photoactivity;
- the index $(F_m-F_{st})/F_m$ – served to display the degree of exercising secondary or "dark" photosynthetic reactions: the higher index, the more efficient function photosynthetic reactions related to productivity;
- indexes F_{st}, $T_{0,\ 5}$ – are used for indirect characterizing the efficience of secondary photosynthetic reactions, their value is in inverse proportion to photoactivity of this stage of photosynthesis

The aim of our study was to examine the main indicators of photoactivity of varieties and leaf apparatus of nectarines with green and red leaves coloring.

MATERIALS AND METHODS

The studies were conducted in laboratory conditions on intact leaf plates at 3 greenleaved nectarine cultivars and forms - Rubinovyi-8 (control), Chemus, Krimzucht 53-85 and 2 forms of nectarine with red colored lamina (Nektadiana Krasnolistnaja 996-88, Krasnola495-86). Changes in fluorescence intensity were carried out on a portable fluorometer "Floratest". Leaves were taken in triplicate of each cultivars and before measuring fluorescence parameters they were adapted to the dark during 8 minutes. In the spectral range of operation of photosynthetically active forms of chlorophyll (690 nm) multi photoinduction fluorescence curves were recorded (Kautsky effect) (Buschmann, 1986; Romanov et al., 2007; Stirbet, 2011). Following parameterswere recordedduring the experiment: F_0 -background or zero level of fluorescence; F_m - maximum fluorescence level, coincides the start of productive photosynthetic processes, CO_2 fixation and activation of enzymes of the Calvin cycle; F_{st} - fixed level of fluorescence, indicating the establishment of a stable and most intense level of photosynthesis; F_v - variable fluorescence, which indicates the difference of the maximum and fixed levels (F_m-F_0), specifying the ability of the chlorophyll-bearing apparatus to provide photosynthesis; $(F_m- F_{st})/F_m$ - ratio of fluorescence induction; F_m/F_{st} - fluorescence decay rate (Brionet al.,2000; Korneyev.,2002). The content of chlorophyll a and b was determined by spectrophotometry(Gavrilenko et al.,1975).

RESULTS AND DISCUSSION

Using the indexes of efficiency of primary photosynthetic reactions, we could select nectarine cultivars Chemus, Rubinovyi-8 and the form Krasnola 495-86 (Table 1.). They differed from the other cultivars and forms by an increased pool of light-harvesting complex (Fm), as well as by more significant content of photo-synthetically active chlorophyll forms (Fv/F_0). Its value was high at the green-leaf nectarine as well as at red-leaf form Krasnola 495-86. The average value of F_v/F_0 at these cultivars is higher by 32 % than at nectarine Krimzucht 53-85 and the red-leaf form Nectadiana 996-88. As we can see, among studied nectarine cultivars and forms there are plants, contrasting at photoactivity.

Table 1. Indexes of photosynthetic activity of nectarine cultivars and forms.

Cultivar/form	Photoactivity indexes, rel. un.						
	F_0	F_m	$\frac{F_v}{F_0}$	$\frac{F_m F_t}{F_m}$	F_v	F_t	$T_{0,5}$
Rubinoviy-8 (c)	43. 7±3. 2	75. 2±5. 4	0. 9±0. 2	39. 8±3. 8	38. 3±1. 2	48. 3±1. 2	28±2. 0
Chemus	17. 2±1. 3	50. 0±4. 1	1. 9±0. 4	46. 3±2. 5	32. 3±2. 6	26. 9±2. 1	19±1. 5
Krimzucht 53-85	17. 1±2. 5	35. 8±2. 8	1. 1±0. 3	42. 2±2. 3	18. 7±3. 2	20. 7±3. 0	24±3. 0
Nectadiana Krasnolistnaja 996-88	18. 6±1. 2	40. 6±4. 0	1. 2±0. 3	43. 0±1. 8	22. 2±1. 7	23. 1±4. 1	38±2. 0
Krasnola 495-86	25. 2±2. 3	62. 8±4. 7	1. 5±0. 2	42. 0±2. 6	37. 8±2. 2	36. 4±2. 6	53±1. 0

The high photoactivity level, peculiar to selected green-leaf nectarines Rubinoviy 8 and Chemus, is caused by an increased pool of light-harvesting complex (F_m). Moreover, the Rubinoviy 8 showed the highest value of F_0, averagely 2. 5 times more than other nectarine cultivars and forms. The light-harvesting complex volume (F_m) and the content of photosynthetically inactive chlorophyll forms, shown by the red-leaf nectarine Krasnola 495-86, is 1. 5 times higher than that of all others, exclusive Rubinoviy 8. It is to be noted that a higher light-harvesting complex volume does not affect the quantum photosynthesis efficiency (maximum photochemical quantum yield photosynthesis, Fv/ F_0). If anything, the light quantums utilization efficiency, displayed by Rubinoviy 8, is much weaker. Approximately, 1. 5-2. 0 times lower than that of the green- / red -leaf cultivars Chemus / Krasnola 495-86. As seen from the table 1, there appears to be no clear difference between the green- and red-leaf nectarine groups after the indicator Fv/ F_0. The efficience of energy transfer to secondary or "dark" photosynthetic reactions (index $(F_m-F_{st})/F_m$) remains in the range between 41 to 46 %. But the capacity for productive using of energy coming from the light-absorbing complex, is being realized in a most effective way by genotypes of nectarines Chemus, Krimzucht 53-85 and NectadianaKrasnolistnaja 996-88. On average, they use the energy arrived from primary photosynthetic reactions1. 5 to 2 times more effective for productive processes. It is being reached by means of the faster ($T_{0,5}$) and lower fall of intensity of the photo-induced fluorescence (F_{st}). At red-leaf forms NectadianaKrasnolistnaja 996-88 and Krasnola 495-86 was noted a less speedy fall ($T_{0,5}$) of the intensity of fluorescence. It is 1. 5 times slower than at control cultivar Rubinoviy and almost 2 times higher than at green-leaf cultivars Chemus and Krimzucht 53-85. Most probably this is caused by blocking energy transfer from light-harvesting complexes and by energy redirection towards the unproductive photosynthetic processes. A difference between green- and red-leaf nectarines in the light-harvesting complex volume, the quantum photosynthesis efficiency and the following "dark" photosynthesis processes suggest an effect of the red-leaf-

pigments on the leaf apparatus photoactivity. The advantage (their increased concentration) in leaves, probably, affects the stability level of the chlorophyll-protein complexes in photosynthetic systems, which are exposed to a significant deactivation at high illuminance levels. That may cause an increased volume of light-harvesting complexes and a large number of photosynthetically inactive chlorophyll forms at red-leaf nectarines. We could detect that the red-leaf nectarine forms have a reduced content of chlorophylles*a* and*b*, their quantitative relationship were 1. 2 times lower than at green-leaf genotypes (Figure 3).

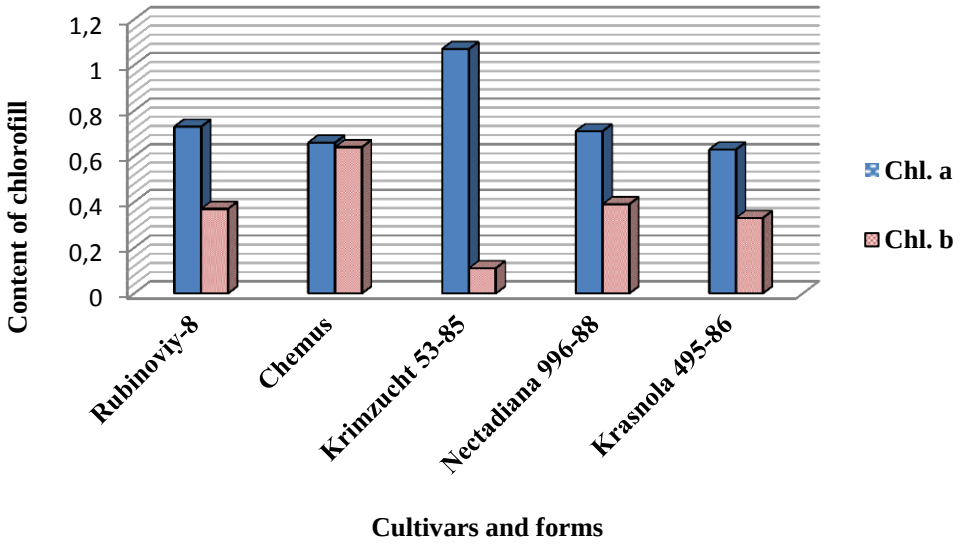

Figure 3. Content of chlorofill forms *a* and *b* in the leaves of nectarine cultivars and forms.

CONCLUSION

All together, both nectarine forms, with the red and with green leaves, are notable for sustainable parameters of photosynthesis. The red-leaf nectarine forms, in comparison with traditional cultivars, show reduced chlorophyll content but they have a great potential for maintaining sustainability and productivity of the function photosynthetic apparatus. The future research could clarify the role of red-leaf forms in productivity forming and remove defects in reducing photoactivity at the stage of secondary photosynthetic reactions.

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SEED GERMINATION RATES OF DIFFERENT COOL SEASON LEGUMES

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ABSTRACT

Temperature is the main factor affecting plant growth and development. Seed germination and seedling establishment are the most critical stages in the life cycle of plants For the purposes of the study, the germination temperatures of six cool-season legumes (*Vicia faba, Lens esculentus, Vicia sativa, Lupinus albus, Pisum sativum, and Lathyrus sativus*) important cultivars were determined. Seed germination rate was measured at different temperatures in the range 4-10 °C in a growth chamber with constant parameters (light, water) at three-day intervals for a period of three weeks. Each treatment was repeated four times for each temperature value, where 100 seeds were placed in five different separate glass made containers. The measurements were taken at three-day intervals. A seed was deemed to have germinated when at least 1 mm of radicle was visible. It was found that pea germinated faster than the rest cultivars under low temperatures, whereas *Vicia sativa* and *Lathyrus sativus* showed the slowest germination rates. The descending order of germination rate *Pisum sativum> Vicia faba > Lens esculentus> Lathyrus sativus >Vicia sativa>Lupinus albus*. Germination rate increased with increasing temperature for all cultivars. *Lupinus albus* was the legume seed with the less germination percentage. Therefore, temperature is the most limited factor on germination process and the increase of 4 degrees is enough to give better germination results. As a general conclusion, *Pisum sativum* and *Vicia faba* may satisfactorily germinate in rather cool micro-environments, and their sowing in the fall may be postponed for some weeks without substantial germination risk comparing to the rest legume cultivars.

Keywords: *germination rate, cool season legumes, temperature*.

INTRODUCTION

There are many factors affecting plant growth and most of them are important, but there is a primary one affecting the rate of plant growth and development and this is the temperature. There is a range of temperatures that differ among crop species throughout their life cycle and are primarily the phenological stages. Seed germination and seedling establishment are the most critical stages in the life cycle

of plants (Walck et al., 2011; Baskin and Baskin, 2014). For each species, a defined range of maximum and minimum temperatures form the boundaries of observable growth. There is an index of cardinal temperature values for selected annual crops which are given fromHatfield et al. (2008, 2011, 2015) for different species. Legumes are crops that develop in cropping systems with relatively low inputs and are suitable to a more sustainable agriculture. Successful crop establishment, which is crucial for reliable plant production, depends on seed quality, environmental factors and genotypes. A review of the literature on legumes confirmed that the differences in the responses of the legume seeds and seedlings to different temperatures were associated with their geographic origin. The need for more sustainable cropping systems has attracted interest in growing such crops over larger areas in Europe. Seedling establishment is a crucial stage in crop production which influences variations in yield. A major change in the past 20 years in legume production has been to adapt earlier sowing dates to shift successful crop establisment (Vocanson and Jeuffroy, 2008). A consequence of earlier sowing is the exposure of seeds and seedlings to stressful cold conditions. A trend in the crop improvement is to breed seed legumes for frost tolerance during winter to enable even earlier sowing (Bourion et al., 2003; Vocanson and Jeuffroy, 2008).

Besides ecological factors, some seed characters are effective in legume crops growing in order to obtain a desirable yield and quality.

Since germination speed is very important for about earliness in the plant growing it can differ according to species, soil structures, sowing methods and especially temperature and soil moisture ratios. Knowledge of seed germination response to environmental factors is required not only for understanding and predicting the ecological adaptation of the species but also for formulating effective strategies for restoration.

The effect of temperature can be modeled by thermal time and predict seed germination progress well but also to provide “a measure of physiological time” and yield coefficients (Bradford, 2002).

The purpose of this study was to quantify the germination temperatures of six cool season legumes (*Vicia faba, Vicia sativa, Lens esculentus, Lupinus albus, Pisum sativum and Lathyrus sativus*) in a range of temperatures.

MATERIALS AND METHODS

This research was carried out in the laboratory of Agronomy and Applied Crop physiology in the University of Thessaly, Greece. The study was performed in a growth chamber adjusted to 4 different temperatures (4, 6, 8, 10°C) for six cool season legumes. Seeds were exposed daily to 12 hours of light with a mean photon flux density of 60 μmol m^{-} s^{-} (400-700nm). Each treatment was repeated four times for each temperature value. For each temperature treatment, 100 seeds were placed in five different separate glass made containers -11cm diameter- lined with 2 sheets of Watman No. 1 filter paper and closed with drying paper. The filter paper was moistened with a solution of 2 mmol/L $CaCl_2$ to facilitate imbibition. The trial was

commenced as soon as the seed was exposed to the moist filter paper. The measurements were taken at three-day intervals. A seed was deemed to have germinated when at least 1 mm of radicle was visible. The number of germinants was measured at frequent and regular intervals by the rate of germination. The maximum germination percentage for each temperature treatment was calculated as the average of the four replicates. The rate of germination was taken as the reciprocal of the time at which 50% of this maximum germinant number was reached.

RESULTS AND DISCUSSION

As shown in Figure 1, the germination of six cool season legume seeds (*Vicia faba, Vicia sativa, Lens esculentus, Lupinus albus, Pisum sativum and Lathyrus sativus*) at 4 ^{o}C varied at different levels. The pea presented top germination as from the 3rd day showed rapid germination of seeds (about 23%), and on day 15 reached the final percentage. All the other legume seeds hierarchical followed: *Vicia faba > Vicia sativa > Lathyrus sativus >Lens esculentus >Lupinus albus*. Their germination percentage was about 98, 92, 65, 62, 58 and 50% respectively in the testing period of 21 days.

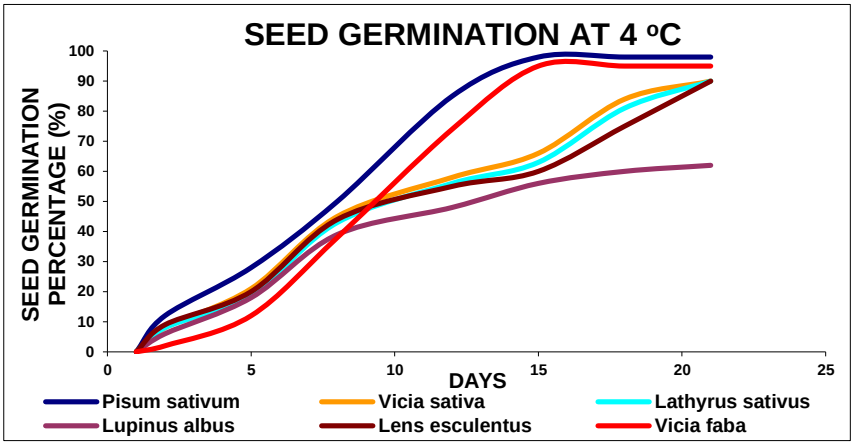

Figure 1. Seed germination percentage of six cool season legumes (*Vicia faba, Vicia sativa, Lens esculentus, Lupinus albus, Pisum sativum* and *Lathyrus sativus*) at 4°C.

Increasing the temperature by 2 degrees of Celcium (up to 6°C), the germination of the above six legume seeds (Figure 1) varied at different levels. Pea presented top germination as from the 3rd day increased the germination percentage up to 30%, while on the 15th day 15 reached the final percentage (98%). During the period of the first week (seven days) the germination of the seeds followed the above scheme: *Pisum sativum >Lens esculentus > Lathyrus sativus > Vicia sativa > Vicia faba >Lupinus albus*. The above scheme changed at the final percentage to: *Pisum sativum > Vicia faba >Lens esculentus > Lathyrus sativus > Vicia sativa >Lupinus albus, and their* germination percentage was about 98, 95, 95, 84, 74 and 65% respectively in the testing period of 21 days.

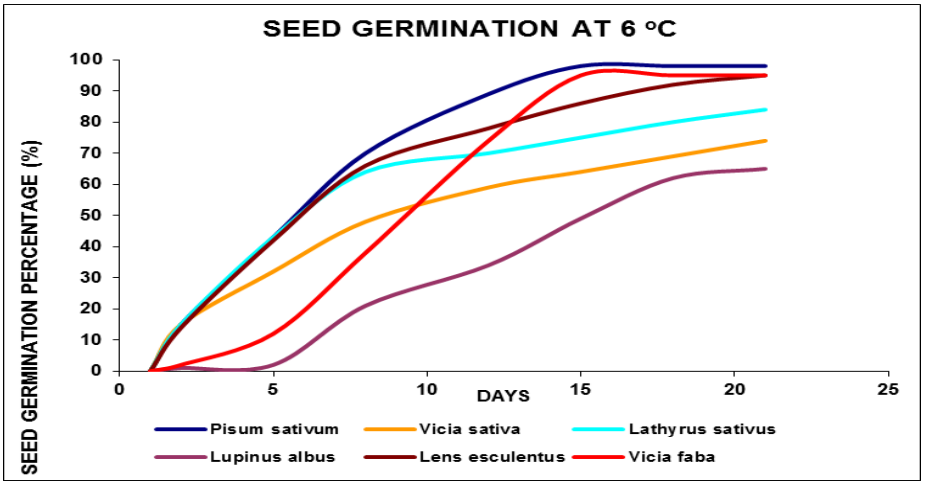

Figure 2. Seed germination percentage of six cool season legumes (*Vicia faba, Vicia sativa, Lens esculentus, Lupinus albus, Pisum sativum* and *Lathyrus sativus*) at 6°C.

As shown in Figure 3, the placement of seeds at a temperature of 8 °C showed similar results for the pea where at the 9th day germinated 83 % of seeds while in case of vetch only the 50% of the seeds were germinated and in case of *Lupinus* the germinated percentage was even lower (25%). The hierarchy followed *Pisum sativum* at day 21 was: *Lens esculentus* > *Lathyrus sativus* > *Vicia faba* > *Vicia sativa* >*Lupinus albus,* while the germination percentages were 98, 91, 90, 84 and 81 %.

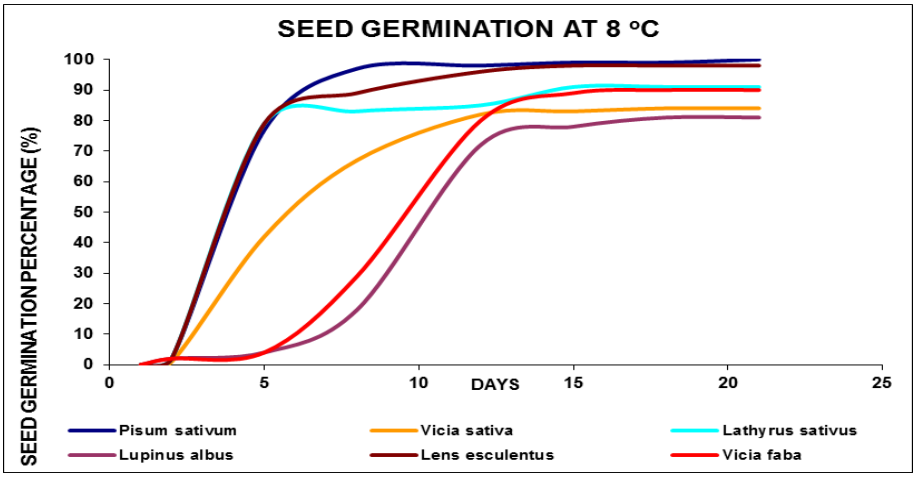

Figure 3. Seed germination percentage of six cool season legumes (*Vicia faba, Vicia sativa, Lens esculentus, Lupinus albus, Pisum sativum* and *Lathyrus sativus*) at 8°C.

Finally, increasing the temperature at 10°C as shown in Figure 4 accelerate the germination rate for all species. Specifically, all legume seeds reached the 50% of

germination on the 4th day. In this case, the hierarchy that was noticed is: *Pisum sativum* >*Vicia faba* > *Lens esculentus* > *Lathyrus sativus* > *Vicia sativa* >*Lupinus albus.* In each temperature was noticed that pea reached the maximum germination while *Lupinus* the minimum. Moreover, only in the case of 10°C*Lupinus* was able to reach the final germination percentage of 82%.

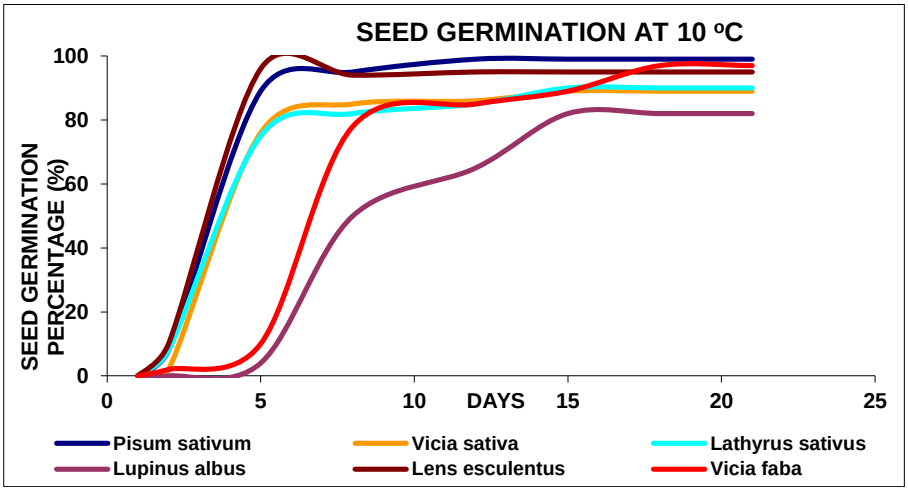

Figure 4. Seed germination percentage of six cool season legumes *(Vicia faba, Vicia sativa, Lens esculentus, Lupinus albus, Pisum sativum* and *Lathyrus sativus*) at 10°C.

CONCLUSIONS

Pisum sativum is the most vigorous seed at tested temperatures (4, 6 8, 10°C) of cool season legumes. On the other hand, *Lupinus albus* was found to have less seed germination percentage, and only at temperatures of8 and 10°C reached 80%.

In case of *Vicia faba* an important postpone appeared (a period of week to reach 50%) in germination. According to the above results, it is concluded that the temperature of 6°C represents the limit for the 50% of seed germination in a period of 15 days for *Vicia sativa, Lens esculentus, and Lathyrus sativus.*

The descending order of germination rate was *Pisum sativum*>*Vicia faba*>*Lens esculentus*>*Lathyrus sativus*>*Vicia sativa*>*Lupinus albus*.

As a general conclusion, *Pisum sativum* and *Vicia faba* may satisfactorily germinate in rather cool micro-environments, and their sowing in the fall may be postponed for some weeks without substantial germination risk comparing to the rest legume cultivars

Finaly, mostly *Pisum sativum* could be proposed as cultivation in Greece, especially in areas with altitude above 400m where the average temperature during the seedling period is about 5 to 6°C.

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PRE-SOWING SEED INOCULATION IN THE BIRDSFOOT TREFOIL SEED PRODUCTION

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ABSTRACT

A prerequisite for the improvement of birdsfoot trefoilproduction is the production of sufficient quantities of good quality seeds. The aim of the study was to analyze the impact of the seed inoculation with mesorhizobial bacteria on yield and yield components of birdsfoot trefoil seed (*Lotus corniculatus* L.). In experiment birdsfoot trefoil cultivars Zora, K-37 and Rocco, and two strains of *Mesorhizobium loti* were used. A trial was carried out in spring 2012 on a private estate in the village of Ivanovci near Ljig (Serbia) and were set up on brown forest soil type, using a randomized block design with three replications and plot size of 5 m (5x1m). Seed inoculation was performed immediately before sowing. Sowing was carried out at a distance of 20 cm row spacing with the amount of seed of 10 kg ha^{-}. Irrespective of inoculation, cultivars significantly differed in terms of the number of stems m^{-}. Two-factorial experiment showed significant influence of *M. loti* strains as well as interaction between the strains and the cultivars on seed yield and yield components investigated (with exemption on seed number per pod). Generally, both strains of *M. loti* have had a positive impact on yield components and seed yield of the birdsfoot trefoil cultivars. Inoculation of seeds has significantly influenced the increase ofthousand seeds weight in the cultivar Rocco, number of stems m^{-} and seed yield of cultivars Zora and Rocco.

Key words: *birdsfoot trefoil, inoculation, seed yield, Mesorhizobium loti.*

INTRODUCTION

In the area of Southeast Europe in less favourable growing conditions, especially in terms of climate and soil, to produce sufficient quantities of forage, a special attention is given to the cultivation of birdsfoot trefoil (*Lotus corniculatus* L.). In The Republic of Serbia there are no reliable statistics on yields and areas where the trefoil is grown, although it is believed that among the perennial legumes by abundance it takes the third place, after alfalfa and red clover (Đukić et al., 2007). It is particularly important species in the hilly and mountainous regions of Serbia (Petrović et al., 2011). A precondition for the improvement of production of

birdsfoot trefoil is the pro-duction of sufficient quantities of quality seeds. According to Gullien (2007) the average yields of birdsfoot trefoil at a global level are below 200 kg ha^{-}. According to Miladinović (1967) with full agricultural technology seed yields of birdsfoot trefoil in our conditions can reach more than 350 kg ha^{-}. Analyzing the seed yield of birdsfoot trefoil in three locations McGrew et al. (1986b) found that there are significant interactions genotype x environment, so as to test the potential for seed yield should be carried out in an environment where the seed is commercialized. According to Steiner et al. (1995) the seed yield of perennial legumes is mainly determined by the genetic base of the cultivar, environmental conditions of the area, time of cutting the first cut, the presence of insect pollinators, as well as the interaction of genotype/environment. In providing legumes with nitrogen, the symbiotic nitrogen fixation plays an important role, which can be realized in symbiosis of legumes with the soil bacteria from the family *Rhizobiaceae* (Marinković et al., 2010; Delić et al., 2013). On the activity of these bacteria affects the physical and chemical properties of soils particularly soil pH, climatic factors, seasons, agricultural technology, especially fertilization as well as biotic factors (host plant, plant cover, plant deseases, insects, viruses, nematodes) (Bašić, 2014; Delić, 2014). Application of inoculation of nodule bacteria *Mesorhizobium loti* before sowing stimulates root nodulation in legumes and affects better supply of plants with nitrogen (Jarvis et al., 1982; 1997). The aim of the study was to analyze the impact of sowing seed inoculation with bacteria *M. loti* on the yield components and seed yield of birdsfoot trefoil.

MATERIALS AND METHODS

The experiment was set up in the village of Ivanovci near municipality of Ljig (Serbia) 2012 on the cambisol soil type (according to the WRB classification), with the medium amount of nutrients. The main tillage was carried out to a depth of 30 cm. Together with the main tillage, the soil was entered with 300 kg ha^{-} $N_{15}P_{15}K_{15}$. Two factorial experiment was set up as a completely randomized block design with three replications and plot size of 5m (5x1m). Three cultivars of birdsfoot trefoil were used: K-37 (The Institute for Forage Crops Kruševac), Zora (Institute for Agricultural and Technological Research, Zaječar) and Rocco (Italian cultivar). In addition, two *M. loti* strains (608 and Z) used as plant inoculants. *M. loti* strain 608 isolated from the root nodules of the plant *Lotus corniculatus* from the pasture on the mountain Ozren, a collection of strains of the Institute of Soil Science, Belgrade while *M. loti* strain Z isolated from the soil at the site in Eastern Serbia, a collection of strains of the Institute of Soil Science, Belgrade. The cultivars were inoculated with single mesorhizobium strains resulting in inoculated treatmants, R_1 (strain 608) and R_2 (strain Z) which were compared with controled treatmants (uninoculated cultivars). Seed inoculation was performed immediately before sowing. Sowing was done on row spacing of 20 cm, with a quantity of seeds 10 kg ha^{-}. Weed control was done mechanically on two occasions. The crop was grown without irrigation.

Yield and seed yield components were determined from the second growth in the second year of cultivation when the seed production is usually performed. The

following yield components were determined: number of stems m^{-} (counting the area of 0. 2 m per elementary plot), number of pods per stem (counting on ten randomly selected stems from the elementary plot). In laboratory were determined: the number of seeds per pod (a sample of ten randomly selected inflorescences per elementary plot) and thousand seed weight (based on the weight of 5x100 of the seed). The potential seed yield is determined on the basis of yield components (number of stems m^{-}, number of pods per stem, number of seeds per pod, thousand grain weight), and recalculated on the seed yield in kg ha^{-}. The results were analyzed by the analysis of variance of the two-factorial trial using SPSS software (1995). The significance of differences in mean values was tested by LSD test.

RESULTS AND DISCUSSION

Perennial forage legumes as nitrogen fixing plants are minimaly fertilized with nitrogen fertilizers, whose remains easily leach from the soil, causing contamination of groundwater and local water course (Janzen and McGinn, 1991). The pursuit of environmental protection, given the current climate change, indicates on the necessity of using measures such as pre-sowing inoculation of the birdsfoot trefoil seed.

Table 1. The effect of seed inoculation (Ø - control, R_1– *Mesorhizobium loti* (strain 608) *i* R_2– *Mesorhizobium loti* (strain Z)) on the seed yield and yield components of birdsfoot trefoil cultivars

	Treatmants	Stem number per m^{-}	Pod number per infleorence	Seed number per pod	Thousand seed weight(g)	Seed yield(kg ha^{-})
Cultivars	Zora	353. 3 b	13. 02	24. 03	1. 25	1364
	K-37	399. 6 a	11. 89	23. 18	1. 16	1266
	Rocco	373. 7 ab	11. 84	22. 71	1. 22	1236
Mesorhizobium	Ø	346. 2 b	12. 61	23. 62	1. 16 b	1186 b
	R_1	370. 9 b	12. 91	23. 54	1. 27 a	1422 a
	R_2	409. 4 a	11. 23	22. 76	1. 20 ab	1257 ab
Zora	Ø	300 c	12. 87	24. 5	1. 29 a	1221 bc
	R_1	335 bc	13. 9	25. 1	1. 33 a	1561 a
	R_2	425 a	12. 3	22. 5	1. 12 bc	1311 ab
K-37	Ø	427. 7 a	12. 2	24. 9	1. 11 bc	1418 ab
	R_1	362. 7 abc	12. 07	23. 3	1. 12 bc	1136 bc
	R_2	408. 3 ab	11. 4	21. 27	1. 25 ab	1243 bc
Rocco	Ø	311 c	12. 77	21. 5	1. 06 c	919 c
	R_1	415 a	12. 77	22. 2	1. 34 a	1571 a
	R_2	395 ab	10. 0	24. 5	1. 24 ab	1218 bc
ANOVA	Cultivars	*	ns	ns	ns	ns
0, 05	Mesorhizobium	*	ns	ns	*	*
	Cultivars x Mesorhizobium	*	ns	ns	**	**
CV (%)		11, 5	9, 7	9, 4	6, 9	14

The values denoted with different small letters within columns are significantly different at (P<0. 05) in accordance with the LSD test; *- F test significant at p<0. 05; **- F test significant at p<0. 01; ns - F test non-significant; CV - coefficient of variation.

In our experiment, a significant positive impact of pre-sowing seed inoculation on the number of stems m^{-} compared to the control treatment was recorded in treatment R_2 with cultivar Zora and in treatments R_1 and R_2 with cultivar Rocco (Table 1). The cultivar K-37 in control treatment had a significantly higher number of stems m^{-} as compared to the other cultivars of birdsfoot trefoil.

Irrespective of the pre-sowing seed inoculation, the cultivars did not significantly differ between themselves in terms of the number of pods per stem and the number of seeds per pod. The cultivars in average had 12. 2 pods per stem and 23. 3 seeds per pod. McGraw et al. (1986a) showed largely influence of number of inflorescences per stem as a component of yield on seed yield of birdsfoot trefoil. The pre-sowing seed inoculation of birdsfoot trefoil with bacteria *M. loti*, had no significant impact on the number of pods per stem and the number of seeds per pod regardless of the cultivars.

In our experiment the thousand seed weight ranged in average from 1. 16 in cultivar K-37 to 1. 25 in cultivar Zora. Grant (1967) states that in one gram is about 750 to 800 seeds of birdsfoot trefoil, or the mass of thousand seeds is about 1. 3 g. Pre-sowing seed treatment with bacteria *M. loti* positively influenced the thousand seeds weight only in the cultivar Rocco.

According to Turkington and Franco (1980) and Gullien (2007), the potential seed yield of birdsfoot trefoil is estimated at over 1200 kg ha^{-}. In our research in the second growth in the second year of cultivation, the average potential seed yield of birdsfoot trefoil of 1289 kg ha^{-} was achieved. According to McGraw et al. (1986a), the average harvest seed yield of birdsfoot trefoil varies from 50-175 kg ha^{-}, or about 100 kg ha^{-} of seed. Seaney and Henson (1970) point out that seed yields of birdsfoot trefoil vary from 50-560 kg ha^{-}. Seed yields of birdsfoot trefoil in USA range between 50 and 170 kg ha^{-} (Fairey and Smith, 1999), in Uruguay between 120 and 150 kg ha^{-} (Garcia et al., 1991; Artola, 2004) and Argentina between 25 and 150 kg ha^{-} (Mazzanti et al., 1988). According to Vojin et al. (2001), in agroecological conditions of the Republic of Srpska, in the area of Banja Luka, the seed yield of birdsfoot trefoil of 272 kg ha^{-} was achieved. In the Republic of Serbia the seed yield of birdsfoot trefoil varies from 100-280 kg ha^{-} (Vučković et al., 1997). At the full maturity trefoil pods easily burst and spill the seeds. The problem of bursting pods significantly limits the successful production of seeds (Fairey, 1994). According to Winch et al. (1985) losses at the seed harvest of birdsfoot trefoil are large, ranging up to 85% of potential yield. In our paper, shown the seed yield of birdsfoot trefoil is recalculated on the basis of the yield components, without taking into the account losses due to uneven ripening and bursting of pods. Uninoculated cultivar K-37 like the control treatment had a significantly higher yield than uninoculated cultivar Rocco, while there were no significant differences between other uninoculated cultivars. In general, seed inoculation with bacteria *M. loti*, had a positive effect on seed yield of birdsfoot trefoil. However, a significant positive effect of the pre-sowing seed inoculation on the yield was recorded only in the treatment R_1 of the cultivars Zora and Rocco. This is mainly due to the positive impact of inoculation on the seed yield components, primarily on the number of

stems m^{-} and thousand grain weight, thanks to the better repletion of the plants with nitrogen.

CONCLUSION

Inoculation of seed birdsfoot trefoil strains of *M. loti* significantly affected the seed yield components and seed yield. The both strains of bacteria of *Mesorhizobium loti* have made a positive impact on the aforementioned seed yield components of the birdsfoot trefoil cultivars. Inoculation of seeds had a significant effect on the increase of number of stems m^{-} in the cultivars Zora and Rocco and the thousand seed weight in the cultivar Rocco.

In terms of seed yield, the cultivars differed significantly among themselves in the control treatment. Cultivars Zora and Rocco had a higher seed yield only in the treatment of R_1 as compared to the control variant. The results indicate that the cultivar Rocco more positive reacted to the seeds inoculation with the bacteria *Mesorhizobium loti* as compared to the other cultivars and that thelowest worst reaction had the cultivar K-37. This further indicates that between the cultivars of birdsfoot trefoil there were differences in preferences for symbiotic relationships with different strains of bacteria. This tendency is conditioned by the degree of compatibility between the different cultivars and strains of rizobial bacteria. The results of ANOVA showed that the mutual interaction cultivar x strain, significantly affected the number of stems per m^{-} and the number of pods, and a very significant on the thousand seed weight and potential seed yield.

Therefore, further work should be focuced on finding the most appropriate combination of cultivars, species and strains of bacteria, in order to improve the production to the maximum in the given circumstances.

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THE CONTENT OF ESSENTIAL ELEMENTS IN THE FLOWERS AND FRUITS OF CHAENOMELES (Chaenomeles Lindl.)

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ABSTRACT

Chaenomeles sp. (*C. cathayensis, C. japonica, C. spesiosa* and *C. x superba*) are characterized by rich chemical composition of all plant parts and the wide variability of the accumulation of separate components in different species and cultivars. For the expansion of ideas about the chaenomeles content nutritional value of 7 essential macro- and microelements in the flowers and fruit of 8 cultivars from the Nikita Botanical Gardens collection have been studied. Analysis of plant samples was carried out by dry ashing with subsequent determination of mostelements on the atomic absorption spectrophotometer. Ca and Mg were determined by complex metric method. It was revealed that essential elements were accumulated in the flowers and fruits in different quantities. The maximum amount of K, Fe, Mg, Zn, Cu and Mn is contained in the flowers. The largest amount of Ca was detected in fruits, whereas Zn, Mn and Cu were most presented in seeds. The studied cultivars differ significantly in accumulation of essential elements. According to the studied complex components, the accession P-8-3 was allocated. Flowers were characterized by the highest content of Ca, Zn, Mn, Cu and high content – of K; fruits were rich in K, Ca, Zn, Mn and Cu. In the jam from the chaenomeles fruit, the high content of K (2087 mg 100 g^{-}, i. e. more than 2%) was revealed. It is seven fold higher than daily rate for human. Thus, chaenomeles flowers, fruits and seeds are a valuable raw material, enriched with vital macro- and microelements.

Key words: *nutritional value, breeding, macro and microelements.*

INTRODUCTION

The chaenomeles (Chaenomeles Lindl.,fam. Rosaceae Juss.,subfam. Amygdaloideae Arn.,syn. Maloideae C. Weber) is a popular ornamental, but relatively rare in Europe fruit culture. The interest of researchers to it as a medicinal and food plant increases in the last decades more and more not only in the countries of South-East Asia where it originate, but also in other regions of cultivation. Thanks to the rich chemical composition, high content of ascorbic acid, phenolic compounds, pectin, fiber and other substances the chaenomeles fruits are a valuable raw material for a different of processed foods rich in biologically active substances: juice, puree,

aroma extracts, syrups, liqueurs, carbonated soft drinks, jams, candies, pectin, dietary fiber blends (Lesinska and Kraus, 1996; Rumpunen, 2002; Tarko *et al.*, 2014). Fruits, flowers and leaves of chaenomeles are also of great interest as a pharmaceutical raw material and widely used in traditional Chinese medicine. Many research works devoted to the study of medicinal properties of various parts of chaenomeles, experimental drugs which have anti-inflammatory, hepatoprotective, antibacterial and other health effects are obtained and tested (Komar-Tyomnaya and Tarachtiev, 1999; Lim, 2012; Dzhan *et al*., 2010b).

Minerals are important for human life support as proteins, fats, carbohydrates vitamins and other biologically active substances. They participate in the most important metabolic processes in the human body, are the building blocks of cells. Therefore, study of the composition of essential macro- and micronutrients of food and medicinal plants is actual. The former conducted study of the chaenomeles elemental composition revealed a significant content of potassium, iron, calcium and other essential and conditionally essential elements in the flowers, leaves and fruits (Komar-Tyomnaya *et al*., 2000; Dzhan *et al*., 2010a; Lesinska and Kraus, 1996). Chaenomeles is characterized by the wide variability of the accumulation of separate components in different taxa and different plant parts (Komar-Tyomnaya, Paliy, 2015). Therefore, a number of studies are carried out on specific varietal or breeding material distributed in research area. The aim of this study is reconnaissance analysis of the contents of 7 essential macro- and microelements in the flowers and fruits of the 8 chaenomeles genotypes from the Nikita Botanical Gardens collection for extendingideas about food and biological value of this crop.

MATERIALS AND METHODS

Plant material. The material for the study were the flowers and fruits of 8 chaenomeles selected forms of Nikita Botanical Gardens collection, originating from species *C. cathayensis* (XK-2-1), *C. japonica* (PX-3-10, PX-4-4, PX-5-15), *C. spesiosa* (P-1-2-1) and *C. x superba* (P-8-3, P-5-11, P-5-9). The analyzed sample of seeds was collected from several genotypes. All the plantsareseedlingsfromopen pollinationof the best selected formsand cultivars, the seeds of whichwere obtainedfrom differentbotanical gardens andpomologicalinstitutions.

Analysis of the plant materials. Analysis of plant samples was carried out by dry ashing (Grishina and Samoilova, 1971) with subsequent determination of most elements on the atomic absorption spectrophotometer S-115 PKS in the absorption mode (Fe, Mn, Cu and Zn) or in the emission mode (K). Ca and Mg were determined by complex metric method (Yagodin, ed., 1987). In the analysis absolutely dry plant material in an amount of 100 g was placed in a porcelain dish, slowly warmed in a muffle furnace to 500°C and was ashed for 4-6 hours until complete disappearance of residues carbon in the ash. After cooling in a desiccator, cup with ash was weighed and then the ash was moistened with several drops of distilled water. 10% hydrochloric acid solution was poured to moistened ash by drops until the termination of the boiling reaction. After that, the ash solution was

transferred from the cup through a filter in 100 ml flask. Residue on the filter was washed with distilled water several times, and the volume of solution in the flask was brought to the 100 ml. The obtained ash solution was usedfor the analysis of macro- and microelements in the plant material.

RESULTS AND DISCUSSION

The study revealed that the quantity of essential elements in different parts of plants, and in chaenomeles accessions differs significantly. In the chaenomeles flowers higher content of the vast majority of essential macro- and microelements was observed than in fruits and seeds. Their total amount is 2730. 5 mg 100 g^{-}of dry matter, which are 2. 5 times larger than in the fruits and 1. 5 times larger than in the seeds (Table 1). The maximum amount of K, Fe, Mg, Zn, Mn and Cu was found in the flowers (Fig. 1). The average amount of K in the flowers was 2180. 7 mg 100 g^{-}and exceeds its content in the fruits for 1. 6 times. The Mg content was 376. 8 mg 100 g^{-}, which is 4. 1 times higher than in the fruits. Zn and Fe had the greatest superiority in amount among microelements in the flowers. Their amounts were 3. 77 mg 100 g^{-} and 6. 05 mg 100 g^{-}, respectively, which are 4. 5 times larger in the fruits (Fig. 2). The average Cu content in the flowers was 1. 0 mg 100 g^{-}, which exceed the amount in the fruits for 4. 2 times. The amount of Mn was 0. 3 mg 100 g^{-} which on average was 2. 3 times more than in fruits.

Table 1. The average content of essential elements in different parts of *Chaenomeles* plants, mg 100 g^{-} (on dry weight basis).

Plant material	K	Ca	Mg	Fe	Zn	Cu	Mn	Σ*
Flowers	2180. 7	161. 8	376. 8	6. 05	3. 77	1. 01	0. 30	2730. 5
Fruits	1378. 6	289. 7	91. 8	1. 38	0. 83	0. 24	0. 13	1761. 3
Seeds	558. 8	268. 2	234. 9	2. 50	3. 69	1. 62	0. 46	1070. 2

* – The total content of macro- and microelements.

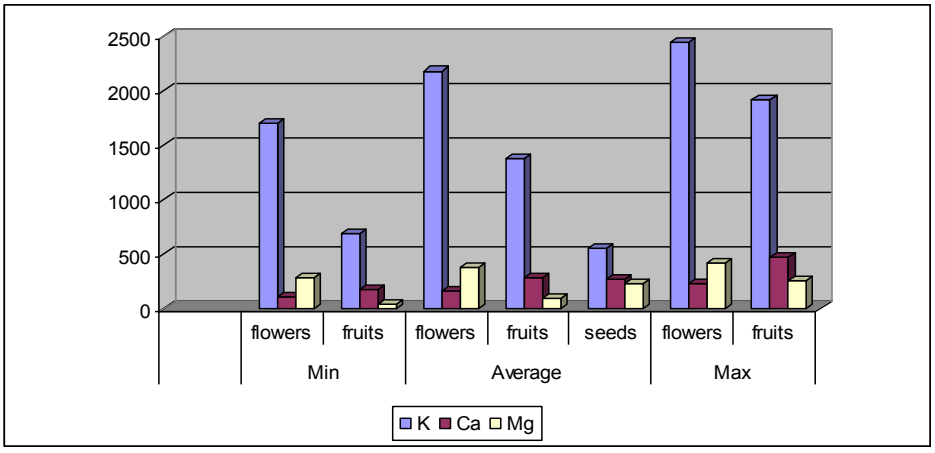

Figure 1. Content of macroelements in the flowers and fruits of Chaenomeles, mg 100 g^{-}.

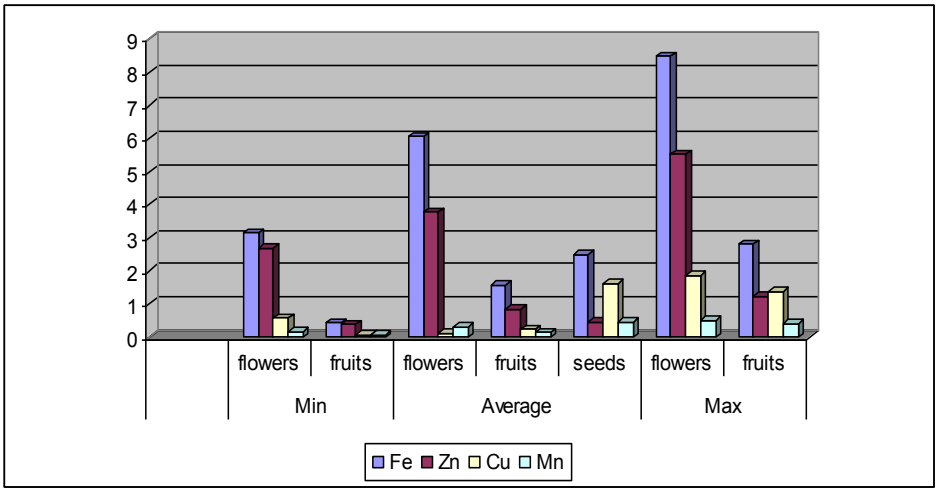

Figure 2. The content of microelements in the flowers and fruits of Chaenomeles, mg 100 g^{-}.

Table 2. Content of essential elements in the flowers and fruits of *Chaenomeles*, mg 100 g^{-} (on dry weight basis).

Breeding forms	Plant material	K	Ca	Mg	Fe	Zn	Cu	Mn	Σ*
XK-2-1	flowers	2433. 9	136. 1	393. 4	8. 48	2. 78	0. 57	0. 36	2975. 6
	fruits	1173. 6	179. 6	40. 8	0. 42	0. 72	0. 28	0. 09	1395. 5
P-8-3	flowers	2386. 1	186. 0	413. 4	7. 31	5. 51	1. 87	0. 49	3000. 7
	fruits	1450. 0	479. 1	63. 2	1. 61	1. 23	0. 37	0. 19	1995. 7
P-5-11	flowers	2279. 8	221. 0	334. 6	3. 14	3. 83	0. 92	0. 37	2843. 7
	fruits	1175. 7	344. 5	128. 4	2. 09	0. 87	0. 33	0. 25	1652. 1
P-5-9	flowers	2443. 4	113. 6	412. 9	3. 74	5. 26	0. 67	0. 32	2979. 9
	fruits	1205. 4	275. 3	163. 2	1. 18	0. 84	0. 37	0. 11	1646. 4
PX-4-4	flowers	2030. 9	155. 0	375. 5	3. 41	2. 69	0. 77	0. 15	2568. 4
	fruits	1601. 2	228. 9	72. 3	1. 45	0. 67	0. 07	0. 07	1904. 7
PX-5-15	flowers	1699. 8	139. 4	422. 3	7. 54	3. 11	0. 83	0. 26	2273. 2
	fruits	1432. 8	251. 3	108. 9	0. 98	0. 77	0. 11	0. 17	1795. 0
PX-3-10	flowers	1750. 0	226. 2	291. 1	8. 67	3. 14	0. 79	0. 17	2280. 1
	fruits	1915. 5	266. 1	116. 1	2. 1	0. 76	0. 28	0. 10	2300. 9
P-1-2-1	flowers	2421. 8	116. 8	371. 3	6. 15	3. 81	1. 71	0. 31	2915. 8
	fruits	1074. 4	292. 6	41. 1	1. 32	0. 81	0. 14	0. 09	1410. 5

*The total content of macro- and microelements.

Table 3. Contents of essential elements in jams, mg 100 g^{-}

Product	K	Ca	Mg	Fe	Zn	Cu	Mn
Apricot jam*	152	12	9	1	**	**	**
Apple jam*	129	14	7	13	**	**	**
Chaenomeles jam	2087. 2	39. 8	9. 7	0. 8	0. 08	0. 04	0. 02
The daily human need in mg ***	300 – 3000	800 – 1600	500 – 750	10–20	12–20	1, 0 – 2, 0	2, 0 – 5, 0

* –data on the requirements of SOSD.

** –data of SOSD are not provided.

*** – daily consumption rate depends on the age, sex, state of health and physical activity of the person (Scalny, 2003).

The total amount of essential elements in the fruit is 35% less than in the flowers. In addition, it decreases during the ripening, although for the separate elements it is not impossible to say definitely. The content of K, Ca and Zn is reduced, and the Fe and Cu is particularly increased (Dzhan *et al*.,2010a). The largest amount of Ca was detected in chaenomeles fruits among the studied plant parts. It reaches an average 289. 7 mg 100 g^{-}. By the amount of Ca and Fe chaenomeles fruits exceed the apples, pears, cherries, apricots, strawberries inseveral times, and can serve as a source of these elements (Komar-Tyomnaya *et al.*,2000, Iliashenko, 2012). It is thought thatthechaenomeles fruits are far superior to the apples on the content of K, Ca, Mg, and a less superior on the amount of Fe (Skalny, 2003).

The average total content of essential elements in the seeds decreases even more. It is 60% less than in flowers, and 39% less than in the fruit. However, seeds remain an important source of microelements. The highest amount of Mn (0. 46 mg 100 g^{-}) and Cu (1. 62 mg 100 g^{-}) has been found in seeds. They are approaching to the fruits on the Ca content and to the flowers on the content of Zn. As well as flowers, they are characterized by relatively high accumulation of Mg in contrast to the fruit.

The studied genotypes differ significantly in accumulation of essential elements. Such featureallows to conduct the breeding for these characteristics. Among the chaenomeles accessionsthe highest content of K in the flowers was observed in P-1-2-1, XK -2-1 and P-5-9, Ca – in the P-5-11 and PX-3-10, Mg – in the XK-2-1, Fe – in the XK-2-1 and PX-3-10, Zn – in the P-8-3 and P-5-9, Mn – in the P 8. 3, Cu – in the P-8-3 and P-1-2-1. According to the maximum content of these elements in the fruits the selection forms were distributed as follows: K – PX-3-10, Ca – P-8-3, Mg – P-5-9, Fe – PX-3-10, Zn – P-8-3, Cu – P-5-9, Mn – P-5-1. According to the studied complex components the P-8-3 is allocated. Its flowers are characterized by

the highest content of Ca, Zn, Mn, Cu and high – K, fruits are rich in K, Ca, Zn, Mn and Cu.

Considering the difference in the accumulation of micro- and macroelements in the flowers and fruits of some genotypes, we can assume the prospectivity of chaenomeles breeding on the maximum content of separate elements or complex elements.

In the jam from the chaenomeles fruit, high content of K (2087 mg 100 g^{-}, i. e. more than 2%) was revealed, which represents almost 7 maximum rate of daily human needs (Table 3). This is significantly higher than industrial products: 13. 7 times higher than in the apricot jam, and 16. 2 times higherthan in the apple jam. That is, 143. 7 g of jam from the chaenomeles fruit made in Nikita Botanical Gardens laboratory contains the maximum rate, and 14. 4 g – the minimum rate of daily human needs in this important element. Concerning the content of Ca, the chaenomeles jam had 3. 3 times higher calcium content than the industrial standard of apricot jam and 1. 4 times higher than apple jam. The amount of Mg in the chaenomeles jam is 1. 4 times higher than in apple jam and a little higher than in the jam from apricots. In regard of Fe, the chaenomeles jam is slightly inferior to industrial products. This element is contained 1. 2 times less than in the apricot jam and 1. 6 times less than in apple jam. Besides these elements, the chaenomeles jam contains microelements zinc, manganese, belonging to the essential group.

CONCLUSION

It was revealed that essential elements accumulated in the flowers and fruits in different quantities. The maximum amount of K, Fe, Zn, Mn and Cu is contained in the flowers. The largest amount of Ca was detected in chaenomeles fruits and Zn, Mn and Cu – in seeds. The studied genotypes differ significantly in accumulation of essential elements. According to the studied complex components, P-8-3 was allocated. Its flowers are characterized by the highest content of Ca, Zn, Mn, Cu and high content – of K; fruit are rich in K, Ca, Zn, Mn and Cu. In the jam from the chaenomeles fruit, high content of K was revealed, which represents almost 7 fold maximum rate of daily human needs. Thus, chaenomeles flowers, fruits and seeds are a valuable raw material, enriched with vital macro- and microelements.

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EXPLORING HOUSEHOLD FOOD WASTE ISSUE IN ALGERIA

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ABSTRACT

Food losses and waste (FLW) is a phenomenon that has been underestimated, little studied and poorly documented in the Mediterranean countries. Actually, no policies, laws, strategies and action plans have been implemented to reduce FLW in Algeria. This exploratory study aims to evaluate household FW in Algeria. An online survey with 323 Algerian households was conducted in February-April 2015 to assess the knowledge and relative importance of FW; attitudes towards FW; impacts of behaviors regarding food and food management; FW quantity and value; as well as barriers and willingness to behavioral change. Sample is gender-balanced (54% female and 46% male) and rather young (93% are less than 44 years old) while most of respondents have high education level. Results show that household planning and shopping activities are significant forecasters of FW. Attitudes may change according to periods especially in Ramadan (88% of respondents declare that FW increase during this month) and to the category of food (most wasted product groups are fruits and vegetables, cereals and bakery products). Most of the respondents have a good understanding of "use by" food date label while they still confuse the meaning of "best before". It seems that FW is widespread in Algeria as only 1% of respondents declare that they do not waste any food. About 15% declare that their households throw away at least 250 g of still consumable food each week. Even though Algeria is considered as a developing country, an important part of food wastage occurs at consumer level as in high and middle income countries. In order to reduce food waste, efforts should be directed towards providing consumers with skills and tools to deal with their food-related activities and to better consider the impact of food waste on the environment and economy.

Keywords: *food waste, online survey, household behavior, Algeria.*

INTRODUCTION

Food losses and waste (FLW) issue has long been underestimated, little studied and poorly documented. Nevertheless, its reduction is nowadays considered as a key factor to improve the sustainability of the Mediterranean food system and achieve food and nutrition security in the region (UNEP/MAP, 2015). In the Mediterranean area, food systems are confronted to major sustainability challenges related to food consumption and production patterns (Lacirignola *et al*., 2014; Capone *et al*., 2014; CIHEAM and FAO, 2015). In fact, food insecurity and malnutrition are still present in some countries of the area. Population is steadily and rapidly increasing in the South as well as food demand all over the region. At the same time, agricultural production in the area has to deal with limited natural resources, principally in the South. Moreover, agriculture is the main water user in a region where water scarcity is the most critical development problem and a main factor limiting agricultural growth (CIHEAM, 2008).

Food losses and wastage occur in each step of the food chain; harvesting, transport, storage, packaging, processing, wholesale and retail trade, and where food is consumed (Institution of Mechanical Engineers-UK, 2013; Gustavsson *et al.*, 2011). FLW occur between the moment when a product is ready to be harvested or harvested and the moment when it is consumed or removed from the food supply chain (Parfitt *et al*., 2010). Losses in the first part of the food chain, which are due to poor harvesting, transport, storage, are more important in developing and low income countries (Venkat, 2011; Lundqvist *et al*., 2008), while in industrialized, high and middle income countries most food losses occur at the retail and consumer level (Gustavsson *et al*., 2011). Food waste can be even classified as either avoidable or unavoidable. Avoidable food waste consists of products that could have been eaten, such as leftovers, food left to go bad and food that past its sell-by date. Unavoidable food waste consists of non-edible waste such as peels, bones, shells and coffee grounds (Schott *et al*., 2013).

The report of the High Level Panel of Experts on Food Security and Nutrition (HLPE, 2014) has underlined that FLW are very dependent on food systems. Understanding differences among food systems can help identify critical areas as well as means for improvement. It stresses the need to improve knowledge on FLW for understanding their causes within the diversity of countries and food systems. That means that solutions have to be adapted to local situations. However, basic information is lacking on the types and quantities of food lost and/or wasted. Available data are scarce and fragmented.

The Economist Intelligence Unit (2015) released a report ranking Algeria 68th of 109 countries in terms of food security. Algeria is one of the biggest world wheat consumers while its national production covers only 25% of its needs. The same applies to the milk powder; 60% of milk needs are covered by imports. The import bill of food has quadrupled since 2000; it went from 2. 6 billion US$ in 2003 to more than 10 billion US$ in 2013 (Si Youcef, 2013).

Unfortunately, the main institutions (ministries, agencies) justify this situation by causes related to low food production and lack of effective strategy to meet

people's food needs; no analysis has been developed regarding FLW by the Algerians and their questionable food habits.

To deal with food insecurity problem, the state implemented new policies; Renewal of Agricultural and Rural Economy and new legislation as the Agriculture Act Law No. 08-16 of August 3, 2008 (MADR, 2008), ignoring an obvious problem, discussed informally by all spheres of Algerian society, including the press and intellectuals, which is food waste in Algerian homes.

The Ministry of Commerce addresses this problem only at Ramadan eve, through a formal speech broadcast by the media to the public, but no campaigns or actions were launched to fight against waste nor during Ramadan neither during the rest of the year.

The actions of The National Union of Algerian Traders and Artisans (UNPC), the national consumer protection association, its local representations, ministries of agriculture, trade, health and all government and nongovernmental organizations related to the topic of food waste are limited to raising the awareness of consumers, producers and traders on the quality of food products on the market (e. g. storage, expiration date, packaging). Even these campaigns are launched at the beginning of each summer, because of the heat that characterizes the country during this season and which causes the loss of big quantities of food due to inappropriate cold chain management.

The government represented by the Ministry of Agriculture and the Ministry of Trade, focus on food quality and availability, dealing with FLW problem as a secondary issue. Actually, no policies, laws, strategies or action plans have been implemented since the independence of the country in 1962. The only actions are done by NGO's and even the latter are very limited.

During the last week of Ramadan (Friday July 10th, 2015), the National Consumer Protection Association (UNPC), in collaboration with many local associations and with financial support by a food company (CEVITAL) and the city of Algiers, organized for the first time a dinner called "*Chaabi-ftour*", which means "*popular fast breaking meal*", prepared only with foods (dishes, fruit, vegetables. . .) that were not consumed by people. Leftovers were collected from households of Algiers. The action has as objective, food waste reduction during the holy month, but, unfortunately, such actions are not repeated out of Ramadan.

In Algeria the main foodstuffs such as cereals, milk, cooking oil, sugar, etc. – which are subsidized by the state - are available on the market at low prices. These prices accessible by almost all of the Algerians make waste of bread, milk, as well as all products prepared with these subsidized foods a marginal issue.

The present work aims to analyse household food waste in Algeria focusing in particular on: knowledge of and perceived relative importance of food waste; attitudes towards the environment, waste and food waste; impacts of behavior regarding food and food management on food wastage; quantity and value of food wasted; and barriers and willingness to behavioural change.

MATERIALS AND METHODS

During the last years the Department of Sustainable Agriculture, Food and Rural Development of the International Centre for Advanced Mediterranean Agronomic Studies in Bari (CIHEAM-Bari) has undertaken different activities on the sustainability of the Mediterranean food system. In the framework of these activities, a particular attention was devoted to the issue of food waste in the Mediterranean region. In fact, precise and accurate data regarding FLW should be enhanced. In the final declaration of the 10th meeting of the CIHEAM member states' agriculture ministers - held in Algiers in February 2014 - the relevance of food waste issue in the Mediterranean countries was strongly stressed (CIHEAM, 2014).

The present paper was based on the results of an exploratory survey performed in Algeria using a questionnaire that was adapted to the Algerian context from previous questionnaires and studies on food waste carried out in Australia (OEH, 2011) and Italy (Last Minute Market, 2014).

The tool used to conduct the food waste survey is a self-administered questionnaire. It was designed and developed in English, French and Arabic languages in December 2014. The survey was performed from February 17th to April 4th, 2015, through the *Survio* website. About1529 persons visited the survey site. Participation was entirely on a voluntary basis and responses were analysed only in aggregate.

The questionnaire consisted of 26 questions. It included a combination of one option and multiple-choice questions. It was developed into six sections:

- Food purchase behaviour and household food expenditure estimation: questions related to this section aimed to check behaviour and attitude of Algerians towards shopping.
- Knowledge of food labelling information: this section informed about different interpretations of food date labels by Algerian consumers.
- Attitudes towards food waste: the questions of this part highlighted different points of view regarding food waste at households.
- Extent of household food waste: this section interrogated participants about their awareness of food wastage.
- Economic value of household food waste: this part of the questionnaire evaluated economic value of food wastage
- Willingness and information needs to reduce food waste: the last section gave recommendations of how one can reduce food waste in Algerian households.

In the introductory part of the questionnaire, the concept of FLW was introduced to inform the respondents.

Various institutional communication channels were used to disseminate the questionnaire such as institutional websites (e. g. www. uhbc. dz), social media (e. g. Facebook, Twitter) and personal e-mails.

Data were analysed using descriptive statistics (*e. g.* means, max, min), in order to get a general picture of frequencies of variables, using Microsoft Excel.

Out of 339 questionnaires received, 16 were not considered because there were missing data. Therefore, the total number of the sample is 323 adult Algerians. Respondents were from 43 different cities covering the whole country. Fifty-four percent of respondents were females. As for age group, participants in the survey who are aged from 25 to 34 years accounted for 54. 5% of all interviewed individuals. About two thirds of respondents (67. 5%) have a high education level (masters, doctorates). Among all interviewed persons 46. 7% were employed full time/part time. The majority of respondents (65. 9%) live with their parents (Table 1).

Table 1. Profile of Respondents (n= 323).

Items		Percentage (%)
Gender	Male	45. 8
	Female	54. 2
Age	18-24	24. 4
	25-34	54. 5
	35-44	13. 9
	45-54	4. 3
	55 and over	2. 8
Family status	Single person household	4. 3
	Living with parents	65. 9
	Partnered	5. 6
	Married with children	20. 4
	Shared household, non-related	2. 2
	Other	1. 5
Level of education	Primary school	1. 2
	Secondary school	1. 5
	Technical qualification	2. 2
	University degree	25. 7
	Higher degree (MSc, PhD)	67. 5
	No formal schooling	1. 8
Household composition (number of members)	1 to 3	16. 7
	4 to 6	53. 6
	7 to 10	26. 3
	> 10	3. 4
Occupation	In paid work (fulltime or part-time)	46. 7
	Student	36. 5
	Unemployed and looking for work	13. 3
	Home duties	2. 2
	Retired/Age pensioner	1. 2

**Source: Authors' elaboration based on survey results*.

RESULTS AND DISCUSSION

According to answers of respondents, only 12. 5% buy their foods from hyper/super markets. However, the majority (56%) buy food from mini-markets, and shops (bakers, butchers, grocers...), while those who buy weekly or daily from markets represent 30%, the direct purchase from farms is rare. The low rate of people purchasing their foods from hyper/super markets is due to the low number of this kind of markets in Algeria, except in big cities. Small shops are still dominant in rural areas and small villages.

According to the survey, 39% of respondents do their food shopping every day, 15% every two days, while those who buy their food once or twice per week represent 19% each, the surveyed persons who buy their food two times per month or monthly represent only 3 and 4%, respectively.

The survey revealed that 30% of participants spend between 15, 000 and 20, 000 Algerian dinar (1 AD = 0. 0092 Euro), while the rate of those whose food costs between 10, 000 and 15, 000 AD as well as between 5, 000 and 10, 000 AD per month represents 27%. Thirty percent of respondents revealed that they spend more than 20, 000 AD monthly. This means that many Algerians spend all or half of their payroll in food because SNMG (average national guaranteed wage) in Algeria is 18, 000 AD, whereas our neighbours (Moroccan) spend about the half (900 MAD) (1 MAD = 0. 1 Euro) and Egyptians respondents spend about 37% of their income on food (Abouabdillah *et al*., 2015; Elmenofi *et al*., 2015).

The rate of respondents who use a list when shopping and people who do not use list is the same (30%), while 40% sometimes use a shopping list. The use of the shopping list is especially frequent in case of monthly shopping.

About two fifths of respondents (37%) said that the deals (*e. g.* buy one get one free, etc.) do not attract them and only 24% are interested in special offers, while 38% said that the offers are sometimes attractive. Actually, these kinds of offers are common in the hyper/super markets; however, their number in Algeria is still limited.

Consumers' poor understanding of "use by" and "best before" date labels may encourage food waste at home. This behaviour is made largely by the confusing system that some countries adopted for the expiration date of the products. Many studies have called the attention to inconsistencies that exist in the labelling of products, which cause many products to be discarded for this reason. In the United States (USA), federal law requires the manufacturers of processed products to use three different dates on the packaging: "sell-by" or "best if used by" and "use-by" being the first the deadline for the product to remain on the supermarket shelf, the second the best date for consumption and the third the deadline from the point of view of food safety. In Europe there are two types of legally required date marks (Directive 2000/13/EC) addressed to consumers: "best-before", which relates to food quality and indicates the "*date until which the foodstuff retains its specific properties when properly stored*" and "use-by" which relates to food safety, for "*foodstuffs which, from the microbiological point of view, are highly perishable and are therefore likely after a short period to constitute an immediate danger to*

human health". The European Commission wants to help consumers reducing food waste by making "best before" and "use by" dates clearer on the packaging (HLPE, 2014).

Various studies in the USA (NRDC, 2013), Europe (Bio Intelligence Service, 2010), the United Kingdom (WRAP, 2011) and Spain (HISPACOOP, 2012) have underlined that food date labelling, and confusion about it, are a major indirect cause of FLW at retail and consumer levels, as consumers tend to assume that dates are linked to food safety when they are in reality more often grounded on food quality. Date labelling is also a major cause of FLW and of economic loss at retail level as retailers often anticipate dates to preserve their good image (MAGRAMA, 2013; NRDC, 2013).

The survey participants were asked about their knowledge regarding the date label "use by". Eighty percent think that this statement indicates to consume or throw food before this date, while 15% understand it as food products are still fit for consumption after that date, if they are not damaged, spoiled or deteriorated, and only 4% believe that food should be sold at a discount after that date.

Regarding the "best before" date label, 70% of the respondents still have confusion as they think it means that food must be consumed before or discarded after that date. About 26% said that this label means that food is still suitable for consumption after the date if it is not spoiled or damaged, and only 4% see that food must be sold at a discount after that date. Abdouabdillah *et al.* (2015) found that 65% of Moroccan respondents confuse between the two labels.

About the question of the awareness of food waste problem, 86% of interviewed persons admit they worry and try to avoid when they can. Only 5% answered that they are aware of the problems associated with food waste, but they do not think that their behaviour will change in the near future, and 5% do not consider food waste as a crucial problem. Just 3% confirms that they had an interest in the issue of food waste in the past, but now they do not care about it. Most of people interviewed showed a high level of awareness about the issue, which is an important condition for a future behaviour change.

The survey provided some interesting information about the amounts of uneaten food wasted. Interestingly, 47% of respondents declare that they are wasting a very small amount of food, and 29% say they waste a reasonable amount, but 4% admit they are throwing away much more than they should, while 6% argue that this issue has a greater importance than it should. Unfortunately, only 1% of respondents said that they waste no food in their homes. Even if food waste is obvious for all survey's participants, a wide percentage said that they throw away a very small or a reasonable amount of food; on the contrary, just few of the interviewed persons confirm wasting big quantities.

The participants were asked about the final use of uneaten food; 46% confirmed that it ends up in the trash, while 47% said that the remaining food is given to animals. About 13% donate it to the needy people and 6% transform it into compost (Fig. 1). The final use of uneaten food is similar in Egypt and Morocco;

uneaten food that ends up in the garbage is 34. 8% in Egypt (Elmenofi *et al.*,2015) and, unfortunately, 69% in Morocco (Abouabdillah *et al.*,2015).

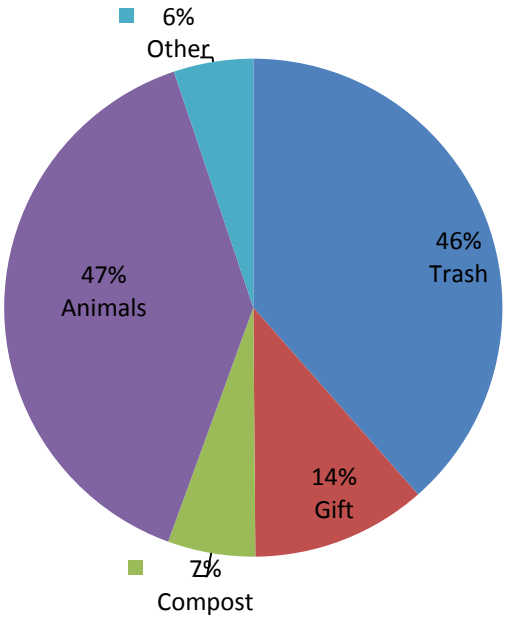

Figure 1. Final use of wasted food.

**Source: Authors' elaboration based on survey results*.

The survey revealed that 10% of participants throw leftovers more than twice a week, about 46% do it less than once per week, and 21% discards leftovers once to two times per week, while 23% said that they never throw leftovers. Almost a quarter (24. 5%) of the interviewed people prepare their main meal with fresh raw materials, while those consuming the remaining meals of the previous day less than 3 to 6 times a week represent 30% and about a half (52%) of respondents consume leftovers less than twice a week.

The survey showed that Algerians prefer to eat at home as only 15% of respondents eat outside 7 to 10 times a week, while 35% never eat out-of-home. For prepared or frozen meals, 80% say that they have never bought them; only 12% consume them twice a week. The survey shows that Algerians prefer to have lunches and dinners at home. Prepared dishes and frozen foods are not widely eaten.

Regarding the month of Ramadan, people were asked about the relative extent of food wastage noticed during the holy month for Muslims with respect to other months; 88% admit that food waste significantly increases and reaches its peak, even though many weeks before the holy month the government and NGO's launch several campaigns to fight against this negative phenomenon. There are some similarities in this regard also with Morocco and Egypt. In fact, 87% of Moroccan and 75. 7% of Egyptian respondents confirm this negative phenomenon (Abouabdillah *et al.*, 2015; Elmenofi *et al.*, 2015).

According to participants' opinions, the main reason that contributes to the wastage of food at homes is that usually foods are left in the fridge for a long time (58%), while 47% say that leftovers are thrown. Moreover, expiration date is another reason listed by 35% of respondents. Surprisingly, 39% of respondents believe that organoleptic and food quality are the causes, because some foods do not have a good smell or taste.

A half of the interviewed persons confirmed that they never throw away still consumable food, however, 21% of surveyed people say they throw less than 250 g per week, while 13% throws between 250 g and 500 g. Those that throw away larger quantities are a minority; only 2% of respondents wastes more than 2kg of food per week.

As expected, cereals and bakery products are the most wasted products; according to the survey 22. 9% of respondents declared that they waste more than 20% of purchased cereals and bakery products. Also wastage of vegetables and dairy products is significant in Algerian households (Fig. 2).

Table 2. Percentage of food groups wasted (n= 323).

Items	Less than 2%	3 to 5%	6 to 10%	11 to 20%	Over 20%	Total (%)
Cereals and Bakery products	38. 8	14. 2	13. 3	10. 8	22. 9	100
Roots and tubers	60	20. 8	8. 3	4. 6	6. 3	100
Pulses and oilseeds	68. 3	15	7. 9	5. 4	3. 3	100
Fruits	77. 1	8. 8	6. 3	5. 4	2. 5	100
Vegetables	59. 2	19. 6	11. 7	4. 6	5	100
Meat and meat products	83. 3	6. 7	5. 8	2. 5	1. 7	100
Fish and seafood	85. 4	8. 8	3. 8	1. 3	0. 8	100
Milk and dairy products	63. 8	17. 5	9. 2	4. 6	5	100

**Source: Authors' elaboration based on survey results*.

The survey showed that only 52% of respondents waste the equivalent of 400 AD (about 5 US$) per month in terms of food, while 40% wastes between 500 and 1500 AD (6 to 20 US$) monthly. Those whose economic value of household food

wasted exceeds 4000 AD (51 US $) are only 5% (Fig. 2). According to the survey done in Egypt, 78. 5% of Egyptian respondents admit that the economic value of the monthly wasted food is less than 35 Egyptian pounds - EGP (5US$) and 14. 9% of respondent revealed that the economic value of wasted food is between 42 and 140 EGP (6 to 20US$) (Elmenofi *et al*., 2015).

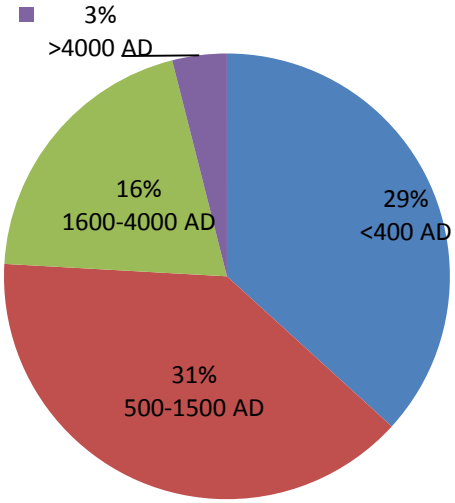

Figure 2. Economic value of food wasted by Algerian households.
**Source: Authors' elaboration based on survey results*.

Respondents were asked about what they wished it exist to avoid waste. About 45% say they would throw less food if they were better informed about the negative impacts of food waste on the economy; while 44% thought that they are misinformed about the negative impacts of food waste on the environment; and 35% believe that additional taxes against the wasters could reduce food waste.
Finally, respondents were asked about information they need to reduce food waste. More than the half say they need tips on how to store food properly, while 45% need recipes prepared with leftovers, 39% need to be better informed about organizations and initiatives that address the prevention and/or reduction of food waste (e. g. food banks), and 33% require more information about the freshness of food.

CONCLUSIONS

The survey shows that household food waste in Algeria is a real concern considering way of food shopping (daily), the big part of salaries dedicated to food. In fact, it seems that there is still some confusion regarding date labels, which increases the amount of FW. This result represents a big problem as the sample has good education. Bread and cereals products are the most wasted food category by Algerian households followed by vegetables and dairy products. Actually, the Algerian cuisine is mainly based on bakery products (couscous, *chakhchoukha*,

etc.) and bread. Nevertheless, the survey revealed also some positive aspects such as the high level of awareness among Algerian households about food waste negative economic and environmental impacts at short and long time as well as readiness of more than 90% of Algerians to change their behaviour to reduce food wastage. Current support policies of food price had a very negative effect on consumption behaviour among Algerians. However, fall in oil prices and the declared austerity policy in the public budget are likely to change bad habits in terms of food consumption and consequently also wastage. That being said, awareness raising campaigns about the economic and environmental consequences of food waste are key to improve food consumption behaviour among Algerian people. Public, religious and academic institutions can play a key role in raising awareness about the far-reaching implications of food wastage on the present and the future of the country.

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SANITARY CONDITION *OF PICEA ABIES* (L.) KARST. YOUNG FOREST STANDS DEPENDING ON THE SPATIAL SPECIFICITY

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ABSTRACT

As a result of nature resources intensive use, most of ecosystems have been converted. Anthropogenic impact includes changes of forest stands structure and their spatial specificity in the forest area. Accordingly the sanitary state of Norway spruce young forest stands can be affected by different risk impact factors of management. The aim of the research was to analyze the spruce *Picea abies* (L.) Karst. young forest stands sanitary condition depending on forest plots spatial specificity and location in the forest areas. The data were collected in 4 regions of Latvia in spruce young forest stands (1 - 40 years old). The research was conducted in young natural and artificial stands (pure – 44, mixed – 42). In total 502 sample plots with a total area of 28250 mwere installed. The particular plot size (25, 50, 100 and 200 m) were selected depending on the stand average tree height, while their number depended on the forest stand area. A total area of investigated forest stands were 127. 5 hectares. Results showed that the expression of spatial specifics depended on risk factors and their intensity, as well as the environmental characteristics. Damages caused by abiotic risk factors at different forest stands were not the same regarding intensity, nature and volume, but more or less closely were related to all site conditions. Spatial specificity of forest stands area (regular and irregular), as well as their location in the forest massif significantly affects the spruce young forests sanitary status (respectively p=0. 027 and p=0. 002). Different risk factors damage to forests, bordering with spruce or pine young growths, cutovers and various types of infrastructure, were identified as much more important.

Keywords: *forest stand, risk factor, sanitary condition.*

INTRODUCTION

Young forest stands of Norway spruce (*Picea abies* L. Karst.) fromone to forty years take a relatively large area in Latvia - 5751. 1 hectares (State Forest Service, 2016). It is easy to regenerate onmedium fertile and fertile soil (Ošlejs, 2005). However, in recent years, forest owners have difficulties to grow high-quality young stands because of different risk factors of management which worsening sanitary state (Laiviņš, 2005). Different risk factors will always affect stands more or less and they cannot avoid (Ruba *et al.,* 2013). For example, Slovakia stands are

most affected by the biotic factors, such as beetle (mainly, *Ips typographus*) and pathogenic fungi (mainly, *Armillaria*), who causes the most damage, so their activity, population dynamics and relations are widely studied.

The climatic conditions are regarded as one of the abiotic factorsthat mainly influence tree growth. Tree response to weather conditions may change depending on the species, origin, age, competition and location forest tract (Boddy and Jones, 2008). Forest owners managing spruce young stands are faced not only with the biotic and abiotic, but also with anthropogenic risk factors. The most of young forest stands of spruce in Europe have been modified by human activities and as result of it has changed the original structure of forest stands, in Central Europe decrease of spruce stands stabilityalso was detected (Lamedica *et al.,* 2011). To minimize the impact of human activities and maintain the naturally developed stands in many places it is prohibited or limited an economic activity and the stand have turned into nature reserves or natural parks (Motta *et al.,* 2010). However, there are other opinions - that natural forest cultivation without human intervention is not acceptable (Zālītis, 2006). As is often the case that incorrect forest management has changed stands spatial structure, as the result increase the number of damage in young forest stands, worsen the health status of trees and the natural ecosystem transformation of unnatural. Therefore, changes in the spatial structure of the forest, to a greater extent, may affect the formation of the crown, tree growth and competition in the stand (Moeur, 1993). Changes in the forest structure are closely related to the different infrastructure objects creation in the forest (forest roads, drainage systems). Forest road network is one ecosystem, which is managed by a human, and it can positively or adversely affect the stands next to the existing plants and trees(Demir, 2007), therefore is important to assess the magnitude of the anthropogenic impact on this ecosystem(Gadow *et al.,* 2013). Irregular, naturally created shaped areas transformation into a regular and incorrectly performed thinning can lead to a deterioration of the health status in the young stands, causing a variety of diseases and insect invasion(Ruba *et al.*, 2014).

The aim of the research was to analyze impact of forest plots spatial structure on management of Norway spruce young forest stands). Tasks to achieve goal: to analyze impact of forest stands area spatial structure on sanitary condition of Norway spruce young stands (forms – regular, square, trapezium and irregular; to analyze young forest stands sanitary condition depending on the forest stands locationin the forest tract; to determine impact significance on sanitary condition in young forest stands depending on form and location in the forest tract. Hypothesis:the form of forest area and location in the forest tract significantly impact the sanitary condition ofNorway spruce young forest stands.

MATERIALS AND METHODS

The main indicator for choosing the number of sample plots was area of forest compartment. The average tree height (H) of the stand was the main indicator for choosing the type of sample plots. In the stand with$H \leq 12.0$ m - 50 m sample plots were created with a circle radius of 3. 99 m, while with $H \geq 12.0$ m - sample

plots 200 m with a circle radius of 7. 98 m. All trees in each of the temporary sample plots were counted, and each of them diameter at breast height (DBH) was measured. For DBH measuring electric calliper or simple calliper were used, tree heights for 20 – 30 trees were measured using VERTEX measuring instrument. In young forest stands was detected the following kinds of damage: biotic (insects, browsing, diseases), abiotic (frost, summer droughts)and anthropogenic (thinning), which were summed up and calculated the average parameters of tree damage occurrence (P) and intensity (R). The damage caused by various risk factors was divided into six damage degrees (Table 1).

Table 1. Evaluation of damage caused by biotic, abiotic and anthropogenic factors

Damage evaluation	Damage degree
Trees without indications of weakening or growth disturbances	0
Economically insignificant damage or faults	1
Economically significant damage	2
Highly damaged	3
Trees died in the current year	4
Dead trees	5

*According to Miezite et al., 2013; Ruba et al., 2013.

The following formulawasused to determine the number of trees per hectare (1):

$$N = \frac{N_p \cdot 10000}{L}, \quad (1)$$

where N - number of trees per hectare according to the measured sample plot data (ha^{-}), Np - number of trees on the sample plot and L– area of the sample plot (m). Damageoccurrence proportion was calculated using formula (2):

$$P = \frac{n \cdot 100}{N}, \quad (2)$$

where P–damage occurrenceproportion (%), n–the numberof damaged trees (ha^{-}) and$N$–total number of trees (ha^{-}). The following formulawasused tocalculate the intensity of tree damage (3):

$$R = \frac{\sum n_i b_i \cdot 100}{N \cdot k}, \quad (3)$$

where R–damage intensity proportion (%), n_i–the numberof damaged trees (ha^{-}), b_i–damage degree, N - total number of trees (ha^{-})and$k$- higher degree of damage (Mieziteet al. 2013; Rubaet al. 2013).

Correlation between forms of forest areas and tree damage occurrence and intensity was determinated using analysis of Anova with replication. Young forest stands of Norway spruce were selected in the age groups (1-10; 11-20; 21-30; 31-40).

Location in the forest massif was fixed to find out impact of the adjacent stands on sanitary condition of Norway spruce young forest stands. For research population 12 saplings objects were selected and analyzed: 6 artificially created (3 with square and 3 with trapezoidal shape) and 6 naturally developed (3 with irregular and 3 with square shape). All these forms were determinated using forest digital map of Latvia State Forest Service (State Forest Service, 2016), while increases of each young forest stand were calculated by the following formula (4):

$$Z_{Hvid} = \frac{H_{vid}}{a}, (4)$$

where Z_{Hvid} –mean increment of forest stand height, H_{vid} – average height of the stand (m); a - age (Liepa, 1996).

RESULTS AND DISCUSSION

Working on this study, authors had encountered for such hardships: there are many difficulties to find similar studies in literature, still lack of comprehensive knowledge aboutchanges in spatial structure andinformation about current theme is not always publicly available. Other authors also had confronted the same problems (Griffiths *et al.*, 2014).

It's not enough to explore only the forest area, it is necessary to understand all processes taking place in the forest. One of the most important activities is to find out the impact of different disturbance regimes on ecosystem (Griffiths *et al.*, 2014).

In this study authors tried to find risk factors that most affect young forest stands of Norway spruce. One of the major risk factor is anthropogenic, because the following human activities like: naturally form of forest stands areas turning into a regular, various infrastructures objects creation in the forest massif increases the risk of biotic and abiotic damage and affects the sanitary condition in the stands.

Debeljak *et al.* (2014) have made statements that the initial state of forest stands and applied forest management could cause changes in the forest structure and composition in a short period of time (a few decades).

Previousand this researchhave shown that that forest areas, bordering with spruce or pine young growths, cutovers and various types of infrastructure (forest roads, block rides, amelioration system ditches) were identified as much more important (Ruba *et al.*, 2013). It was found that in regular square/artificially and square/naturally created areas of forest stands was recognized different types of damage: insects, diseases and browsing. Such damage also was detected in trapezoidal/artificially and undetermined/naturally created forest areas, only with a lower intensity of tree damage (Figure 1).

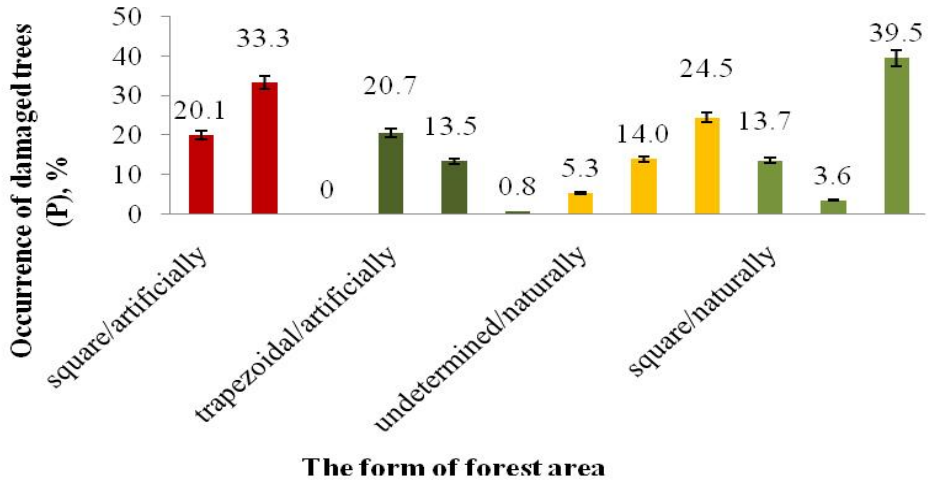

Figure 1. The relationship between the damage occurrence and forms of forest areas

Assessing tree damage and the stand height increases, it was concluded that there is no significant difference between the occurrence of damaged trees in pure and mixed stands($p = 0.375 > \alpha = 0.05$), while it is significant in different age groups: in mixed ($p = 0.003 < \alpha = 0.05$) and pure stands ($p = 0.017 < \alpha = 0.05$). There is no significant difference between the damaged tree intensity and height increases in pure stands ($p = 0,388 > \alpha = 0.05$), and between age groups ($p = 0.127 > \alpha = 0.05$), as well as in mixed stands between the damaged tree intensity and height increases ($p = 0.343 > \alpha = 0.05$), and age groups ($p = 0.094 > \alpha = 0.05$). The occurrence of damaged trees varies greatly between age groups (18. 1 - 119. 7%) in pure stands, while in the mixed stands (4. 0 - 59. 3%) (Table 2).

Table 2. Variation coefficients of tree damage occurrence and damage intensity in pure and mixed young stands according to age groups

Age groups	The occurrence of tree damage		The intensity of tree damage	
	In pure stand	In mixed stand	In pure stand	In mixed stand
1-10	119. 7	50. 1	10. 7	4. 6
11-20	18. 1	59. 3	2. 5	3. 0
21-30	110. 0	4. 0	19. 4	1. 2
31-40	111. 8	15. 2	18. 6	3. 0

* S

The sanitary condition is worse in the pure stands which height increases reaches 0. 2 - 0. 3 in the age group of 1-10 (average occurrence - 8. 6%, intensity - 4. 0%), in the same age group (average occurrence - 21. 3%, intensity - 5. 1%) height increases 0. 4 - 0. 5, but nevertheless in the mixed stands sanitary condition is better because of low damage (highest damage occurrence - 12. 6%, intensity – 2. 7%), in the age group of 21-30 and height increases 0. 2 - 0. 3 (Table 3).

Table 3. Values of tree damage occurrence and damage intensity in pure and mixed young stands according to age groups

The occurrence of tree damage					The intensity of tree damage				
Z_{Hvid}	1-10	11-20	21-30	31-40	Z_{Hvid}	1-10	11-20	21-30	31-40
0. 2-0. 3	8. 6	0. 7	0	6. 5	0. 2-0. 3	4. 0	0. 3	0	2. 6
0. 4-0. 5	21. 3	3. 6	0. 5	10. 3	0. 4-0. 5	5. 1	1. 5	0. 3	2. 6
0. 6-0. 7	1. 9	2. 2	6. 9	0	0. 6-0. 7	0. 6	0. 7	2. 9	0

**Source: Authors' elaboration*

This once again proves that better to create mixed stands, because these are more resistant to windthrows (Lüpke and Spellmann, 1997), less suffer from various diseases (root rot) (Piri et al.,1990) and have better nutritional content than pure stands (Sverdrup and Stjernquist, 2002).

CONCLUSION

As much more important risk factors damaging forests, bordering with spruce or pine young growths, cutovers and various types of infrastructure. Spatial specificity of forest stands area (regular and irregular), as well as their location in the forest massif (α=0. 05 > p=0. 002) significantly affects the spruce young forests sanitary status (p=0. 027 and p=0. 002). Damages caused by abiotic risk factors at different forest stands were not the same regarding intensity, nature and volume, but more or less closely were related to all site conditions, which either promotes or limits for certain damage risk factor in the likelihood and magnitude.

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GENETIC VARIABILITY OF THE CEREAL (POACEAE) GERMPLASM COLLECTION MONITORED BY PROTEIN AND MOLECULAR MARKERS

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ABSTRACT

All the new challenges that food production has been experienced, requires adequate response not only in wide agricultural practice, but also in modern breeding programs. Broadening genetic variability is indispensable to meeting the border set by climatic changes, land erosion, human population growth, and sustainable agriculture. Gathering genetic variability, forming and examining genetic collection are integral part of the task. A germplasm collection of 220 entries, consisting of cereal (*Poaceae*) genotypes has been formed. Genetic variability of wheat (*Triticum* sp.), barley (*Hordeum* sp.), and goat grass (*Aegilops* sp.), samples from the germplasm collection was analysed using gliadin blocks as protein markers, as well as, Random Amplified Polymorphic DNA (RAPD) markers. Gliadin allelic variation was notable within *Triticum* sp. samples, revealing not only genetic divergence, but also the origin and the structure of populations. Genotype variation and structure of populations of *Hordeum* sp. was followed by hordein allelic variation. Sampled population expressed heterogeneity from two to five genotypes per population sample. Landraces, old and modern varieties were separated in collection using hordein allelic variation, as well. A small, pilot, investigation was conducted on *Aegilops* sp. polymorphism using RAPD primers. Number and percentage of polymorphic loci, effective number of alleles, expected heterozygosity and Shannon's information index were used to estimate genetic variation.

Key words: *biodiversity, wheat, barley, Aegilops* sp., *genetic marker.*

INTRODUCTION

Genetic resources, in the part of wild relatives, have always been important for broadening genetic variability in contemporary breeding programs, as well as, for food production for special requrements. Green revolution, the industrialization of primary food production, would not be possible without gene introduction from a

wide range of local populations, wild relatives and other plant genetic resources that changed the phenotype of cultivars dramatically (Hedden, 2003). Finally, intergenus hybridization that spontaneously occurred in nature has led to cultivated plants we are using in food production of today. Bread wheat (*Triticum aestivum ssp. vulgare* L.) has, for example, only one third of its genome originated from direct ancestors of *Triticum* genus (Arrigo *et al*., 2011; Matsuoka, 2011). Two third of wheat genome came from wild relatives belonging to *Agilops* genus that are absolutely non-edible. The "secret" of gene recombination of genotypes qualitative and quantitative non-suitable as food that gave the revolutionary outcome in hexaploid wheat genome is still the subject of investigations (Gogniashvili, 2016). However, a task that modern age and the future set up to agriculture requires new "green revolution". In order to take a food production to higher level a new plant ideotype is required (Petrović et al.,2008). Hence, new genetic variability requires new gene recombination and new sources of genetic variability (Dimitrijević and Petrović, 2015). That is the reason genetic resources have come in focus in the last decades (Monneveux *et al*., 2000; Colmer *et al*., 2006; Dimitrijević *et al.*, 2011). An *ex situ* conservation gene bank has been established consisting of about 200 entries of landraces and wild relatives in cereals, collected at SW Balkans during the first decade of XXI Century (Petrović et al., 2006).

The aim of the article is to present results of genetic variability study using protein and molecular marker analysis.

MATERIALS AND METHODS

The germplasm collection has been gathered for several years in Southern Adriatic, consiting of cereal landraces and wild relatives Aegilops sp. (goat grass). Samples of Triticum sp.,and Hordeum sp. collection has been examined for genetic variation using gliadin blocks. The screening and the results analysis was done at the Vavilov Institute of General Genetics, Russian Academy of Science, Moscow. Gliadin seed storage proteins were extracted from single seed wheat meal by 70% ethanol for 30min at 400oC. Gliadin separation was conducted using 8. 33% polyacrylamide gel electrophoresis (12. 5g acrilamid, 0. 62g N, N'-methylenebisacrylamide, 0. 15g ascorbin acid, 200µl 10% ferosulfate heptahydrate, diluted in 150 ml Al-lactate buffer pH=3. 1) after Novoselskaya et al. (1983), and Metakovsky and Novoselskaya (1991). Genetic variation in barley samples was followed by electrophoretic spectra of storage protein grains - hordein (HRD), using Pomortsev and Lyalina (2003) method. A discontinuous genetic variation of Aegilops sp.,was examined using 6 RAPD primers. DNA extraction has been done using the method of Somma (2004). In order to test amplification profiles for polymorphism, readability and reproducibility, six decamer (10 nucleotides length) primers from ROTH®GmbH kits were tested. PCR was carried out in a 25-µL reaction volume containing 2. 5 µL buffer; 0. 2 mM of each dNTP; 0. 5 µM of primer; 2 units of Taq polymerase (Fermentas) and 30 ng of DNA. Reactions were performed in Tpersonal PCR (Biometra) and Mastercycler ep gradient S (Eppendorf) thermocyclers with amplification profile: denaturation at 94°C for 4

min, followed by 40 cycles with 94°C for 2 min, 36°C for 1 min and 72°C for 2 min, with final elongation on 72°C for 10 min. PCR products were separated on 1. 2% or 1. 7% agarose gels containing 0. 005% ethidium bromide and visualized under UV light. Each fragment amplified using RAPD primers was treated as binary unit character and scored "0" for absence and "1" for presence. Estimation of genetic variation was carried out by using the POPGENE software package version 1. 32 for calculation of the following parameters: number of polymorphic loci and their percentage, effective number of alleles per loci, expected heterozygosity based on allelic frequencies and Shannon's index of phenotypic diversity based on marker frequencies. Calculations of all parameters were done separately for each species, and overall for all samples.

RESULTS AND DISCUSSION

Genetic variation within samples of *Triticum sp*. Several samples chosen from germplasm collection have been screened for genetic variation using gliadin loci. The sample arbitrary labeled as No. 1, represents the spring wheat (*T. aestivum*) landrace collected in Western Montenegro, the area of Piva River, municipality of Plužine, in the village of Zabrđe(43, 13°N+18, 76°E) at an altitude of 1381m. That wheat has been traditionally sown for decades using seed inherited from the ancestors. Harvest is in the middle of August. The host claimed that the yield was low, but the quality of flour and bread is quite good. According to gliadin allelic formula, this landrace appeared to be one of the oldest and unique genotype. The allelic variation of special interest comprises two alleles, *Gli-B1s*, and *Gli-A2z*, that were not originated anywhere in Europe and Caucasus, but had been found in the Mid-Asia and the Palestine. Another sample (No. 2) was the landrace "Grbljanka", collected under the very top of the Rumija Mountain (SE-Montenegro) nested the small settlement Lunje (42, 04°N+19, 18°E, alt. 718m) consisting of three houses of Lunjic family. That remote and hard accessible place grew the last remnants of landrace Grbljanka (*T. turgidum*) that had been grown all along the Montenegrin coast, from Ulcinj to Lastva Grbaljska, decades ago (Petrović, andDimitrijević, 2012). Gliadin allelic composition reveals that the population that was gathered consisted of 70% *T. turgidum* (4x), and 30% of *T. aestivum* (6x). Within *T. turgidum*, four biotypes were detected. A hexaploid component of the mixture showed alleles - *Gli-B1k*, *Gli-A2new2*, *Gli-B2t* that occur in landraces, old varieties and are rarely found in modern varieties. It is believed that such a mixture of tetraploid and hexaploid wheat sown by the ancient farmers. The wheat sample No. 3, represents the wheat population from the small settlement of about 30 inhabitants, named Ujniče (43. 09°N+19, 70°E, alt. 1157m) near the town of Bijelo Polje at the Northern Montenegro. The wheat (*T. aestivum*) sample, exhibited about 60% of the allele *Gli-B1g* within total allelic variation of this locus. The *Gli-B1g* is typical for *T. macha*, Caucasus endemic, and could be often found in *T. spelta*, but very rarely in *T. aestivum*. The allele *Gli-D1b* (80% of allelic variation on this locus) appears, as a rule, in landraces, as well as, very old varieties (tab. 1).

Table 1. Identified gliadin allelic variation of the samples from cereal germplasm collection

Sample No.	Genotype	Alleles of gliadin coding loci and their frequencies [%]					
		Gli-A1	***Gli-B1***	***Gli-D1***	***Gli-A2***	***Gli-B2***	***Gli-D2***
1	***T. aestivum***	q-50 i-36 m-14	s-48 b-42 f-5 e-5	a-100	z-45 t-32 k-13 c-5 f-5	?	a-85q-10 w-5
General formula		**q+i+m**	**s+b+f+e**	**a**	**z+t+k+c+f**	**?**	**a+q+w**
2	***T. turgidum*** (70%) 4x	4 biotypes					
	T. aestivum (30%) 6x	f-100	k-100	a-90f-10	f-90 new2-10	t-100	f-100
General formula		**f**	**k**	**a+f**	**f+new**	**t**	**f**
3	***T. aestivum***	f-75a-25	g-60f-15b-15 e-10	b-80 k-15 d-5	g-100	b-60 ?-40	b-50a-30 new1-20
General formula		**f+a**	**g+f+b+e**	**b+k+d**	**g**	**b+?**	**b+a+new1**

Genetic variation within samples of *Hordeum sp*. A seed sample of spring barley that goes under the local name "Bushket", was obtained in a small household in a village of Palež (43, 18^{o}N and 19, 14^{o}E) at 1431m of altitude, in Montenegro. That particular barley has been grown on that household for 70 years, according to the host. The sampled population consists of three genotypes where genotypes 1 and 2 are old varieties, and the genotype 3 is a modern barley variety (fig. 2A). A barley sample collected at the site of the village Kovčica (43, 09^{o}N and 19, 12^{o}E), at an altitude of 1437m, in Montenegrin mountain Durmitor area, appeared to be a mixed population of local cultivars (1 and 3), and HRD allelic composition (2) that could be found in modern varieties (fig. 2B). A barley sample obtained from Šćepan polje, Montenegro, located at 43. 35^{o}N + 18. 85^{o}E (alt. 764m), exhibited the highest genetic variability consisting of 5 genotypes with different hordein loci allelic variation where genotypes marked as 2 and 3 refer to modern varieties, while the rest (1, 4, and 5) originated from old local populations (fig. 2C). In the village of Pitomine, Montenegro, at the position (43, 09^{o}N and 19, 06^{o}E, alt. 1536m), a population of spring two-rowed barley was found. The host claimed that the seed had been inherited from his father. However, according to hordein allelic variation results, the sample belongs to modern barley variety (fig. 2D).

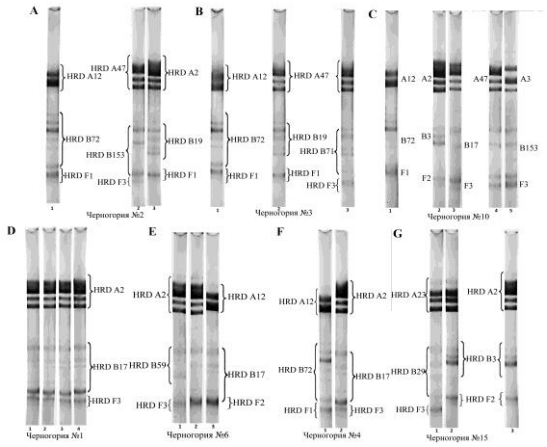


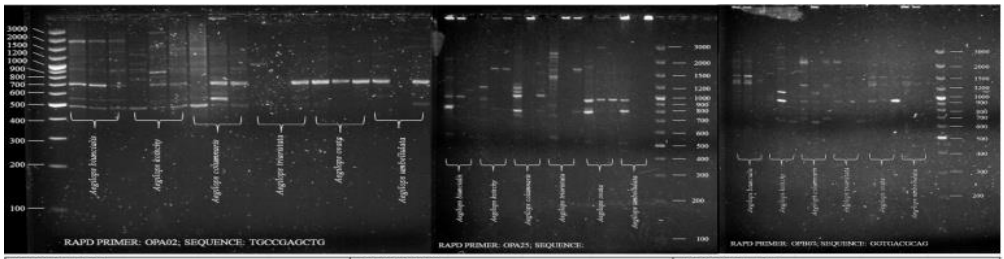
Figure 1. Seven samples of barley out of germplasm collection were analyzed using hordein allelic variation

In the village of Gradina (43, 10°N + 19, 29°E, alt. 1232m), the slopes of Kričак Mountain, Montenegro, samp-les of barley, beside rye and buckwheat, were collected. The sampled population appe-ared to be a mixture of modern varieties (genotypes 2 and 3), and genotype labeled as 1, showing the pattern that corresponds to an old barley population (fig. 2E). The sample of two-rowed barley collected in Montenegro at the site of Mala Crna Gora (43, 12°N i 19, 00°E), at an altitude of 1932m - the population consists of two genotypes; the first one appeared to be old local variety (1), the second (2) refers to modern barley variety, (fig. 2F). A seed sample of barley obtained on the small farm in scattered Montenegrin village Podgora (43, 07°N + 18, 18°E, alt. 1456m), that the farmer claimed to be an old inherited seed, appeared to be a mixture of three varieties of modern barley according to hordein allelic variation (fig. 2G).

Table 2. RAPD primers used for screening goat grass genotypes

Primers	**Nucleotide sequence**	**Usability**
OPA-02	5'-TGCCGAGCTG-3'	+
OPA-05	5'-AGGGGTCTTG-3'	-
OPA-08	5'-GTGACGTAGG-3'	-
OPA-25	5'-GACAGACAGA-3'	+
OPB-06	5'-TGCTCTGCCC-3'	-
OPB-07	5'-GGTGACGCAG-3'	+

Genetic variation within samples of *Aegilops sp*. Three RAPD primers, out of six tested, have been selected (OPA02, OPA25 i OPB07) to determine the polymorphism of different species of *Aegilops* genus (tab. 2). A total of 25 polymorphic bands were generated, ranging from 500 to 3000 bp. The highest number of polymorphic bands (10 bands)was achieved with primer OPA02. The result is in accordance to previously reported resultswhere RAPD primer OPA-02 was detected as U-genome specific marker (Cenkci et al., 2008). All the tested samples of *Aegilops* sp.,have a common U genome - *Aegilops biuncialis* (UM), *Aegilops kotschy* (SU), *Aegilops columnaris* (UM), *Aegilops triaristata/Ae. Neglecta* (UM), A*egilops ovata/Ae. geniculate* (MU), *Aegilops umbellulata* (U), fig. 2.



Aegilops biuncialis			
Primers / Molecular size (bp)	OPA02	OPA25	OPB07
500			
600			
700			
800			
900			
1000			
1200			
1500			
2000			
3000			

Aegilops kotschy			
Primers / Molecular size (bp)	OPA02	OPA25	OPB07
500			
600			
700			
800			
900			
1000			
1200			
1500			
2000			
3000			

Aegilops columnaris			
Primers / Molecular size (bp)	OPA02	OPA25	OPB07
500			
600			
700			
800			
900			
1000			
1200			
1500			
2000			
3000			

Aegilops triaristata			
Primers / Molecular size (bp)	OPA02	OPA25	OPB07
500			
600			
700			
800			
900			
1000			
1200			
1500			
2000			
3000			

Aegilops ovata			
Primers / Molecular size (bp)	OPA02	OPA25	OPB07
500			
600			
700			
800			
900			
1000			
1200			
1500			
2000			
3000			

Aegilops umbellulata			
Primers / Molecular size (bp)	OPA02	OPA25	OPB07
500			
600			
700			
800			
900			
1000			
1200			
1500			
2000			
3000			

Legend: Unique band; Shared by two; Shared by three; Shared by four; Shared by five; Shared by six

Figure 2. Amplification patterns with the selected RAPD primers (upper photographs) and DNA fingerprints of *Aegilopssp.* genotypes.

Number and percentage of polymorphic loci, effective number of alleles, expected heterozygosity and Shannon's information index were used to estimate genetic variation. *Aegilops kotschy* had the highest values for all tested parameters, exhibiting the highest level of variation, whereas variety *Aegilops biuncialis*exhibited the lowest (tab. 3).

Table 3. Estimates of genetic variation in *Aegilops* sp. using RAPD markers

Species	P (No.)	P (%)	Ne±SD	He±SD	I±SD
Aegilops biuncialis	2	7. 69	1. 044±0. 159	0. 028±0. 098	0. 042±0. 148
Aegilops kotschy	15	57. 69	1. 308±0. 314	0. 195±0. 180	0. 299±0. 268
Aegilops columnaris	9	43. 62	1. 249±0. 383	0. 140±0. 204	0. 204±0. 293
Aegilops triaristata	7	26. 92	1. 180±0. 306	0. 107±0. 181	0. 158±0. 266
Aegilops ovata	3	11. 54	1. 070±0. 214	0. 042±0. 122	0. 063±0. 181
Aegilops umbellulata	4	15. 38	1. 132±0. 270	0. 082±0. 157	0. 124±0. 235

P (No.): number of polymorphic loci; P (%): the percentage of polymorphic loci; Ne: effective number of alleles; He: expected heterozygosity; I: Shannon's information index; SD:standard deviation

CONCLUSION

A small number of samples from germplasm collection were tested using gliadin and hordein loci variation, as well as RAPD primers. Samples of wheat landraces tested using *Gli* loci variation shoved some unique alleles commonly presented in genotypes of ancient landraces. The sample of particular interest is spring wheat collected in Zabrdje (sample 1), having allelic variation *Gli-B1s*, and *Gli-A2z* that is very rare and cannot be seen in Europe and Caucasus, even, but in Central Asia. Barley samples, screened by HRD loci variation, appeared to be mixtures of landraces and modern barley varieties. *Aegilops* sp.,showed distinct patterns tested by RAPD primers. The results confirmed that RAPD markers, could be of value in germplasm collections management for identification and measurement of variation.

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INFLUENCE OF AGROCHEMICAL REHABILITATION ON THE HEAVY METAL MIGRATION TO THE WATER

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ABSTRACT

Soil plays the main role in the sustaining life of Earth ecosystems –it is the fundamental foundation of agriculture resources, food security, economy and environmental quality. The heavy metal pollution has been increasing in agricultural soils worldwide. For example, Cu is widely used as a pesticide against fungal and bacterial diseases in crops or as a contaminant in organic amendments, or for irrigation as pig manure or sewage sludge. Soil and water pollution have the great impact on food safety and to human health: polluted soils have direct health risks, and secondary risk is connected to contamination of water supplies. The articlepresents the lysimetric experiment with the chemical composition results. This exploratory study aims to evaluate the influence of agrochemical rehabilitation on the heavy metal migration to the water. The chemical composition of intra soil water has shown that contaminated black soil has a high absorption capacity of heavy metals. The bulk of heavy metal brought about in a form of water-soluble salts wasabsorbedand converted by soil colloids of podzolized chernozeminto relatively stable compositions. Results of the analytical research showed that organic and organic-mineral systems, where phosphates were used in the average volume of 60 kg of P_2O_5per hectare a year, reduced intake of cadmium in the subsurface water. Mineral systems also impeded migration of zinc and copper to the ground water. On the contrary, high doses of superphosphate in the fertilizer system increased the leaching of Cd, Pb and Cu to the infiltration waters.

Keywords: *heavy metals, pollution, water, soil, detoxification methods.*

INTRODUCTION

Environmental pollution and food safety are two of the most important issues of our time. Soil and water pollution, in particular, have historically impacted on food safety which represents an important threat to human health (Lu *et al*., 2015). It has been widely accepted that soil plays a key role in sustaining life of Earth

ecosystems with the huge abundance of soil microbiota(Young and Crawford, 2004). Soils polluted with heavy metals have become common across the globe due to increase in geologic and anthropogenic activities (Chibuike and Obiora, 2014). Soils may become contaminated by the accumulation of heavy metals and metalloids through pesticides, wastewater irrigation, spillage of petrochemicals, leaded gasoline and paints, animal manures, sewage sludge, emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, land application of fertilizers, coal combustion residues, and atmospheric deposition (Khan et al., 2008; Zhang et al., 2010).

Heavy metal pollution is increasingly threatening to crop production in agricultural soils (Weber *et al*., 2001). In the environment, Cu is widely used as a pesticide against fungal and bacterial diseases in crops or as a contaminant in organic amendments such as pig manure or sewage sludge (Christie and Beattie, 1989). Microorganisms are generally described as more sensitive to Cu and other heavy metal stresses than other organisms in soil biocenosis (Giller *et al*.,1998). While crop production in agricultural lands has been increasingly threatened by heavy metal contamination (Weber *et al*., 2001), long term impacts of heavy metal pollution on soil microorganisms have been increasingly concerned. Metals can exist in various chemical forms (or species). These forms often exist in a complex equilibrium governed by many soil factors and properties. For any given heavy metal, only a fraction is bioavailable and thus potentially it is only this fraction that can be taken up by the plants. More of the metal could be converted to the bioavailable fraction as it is gradually removed by the plant but the extent to which this happens and the kinetics of such processes are not known and would invariably be soil specific (Khan *et al*., 2000). Microbial biomass has been shown sensitive to increased heavy metal concentrations in soils(Giller *et al*., 1998). Heavy metal Cu and Cr pollutants can be attributed to various anthropogenic activities related to the industry; nevertheless, natural weathering process may also play a role as a source of these heavy metals (Ismail et al., 2016). The concern over soil contamination stems primarily from health risks, both of direct contact and from secondary contamination of water supplies (Soil Contamination, 2008). Migration of contaminants into non-contaminated sites as dust or leachate through the soil, and the spreading of sewage sludge are examples of events that contribute towards contamination of our ecosystems (Khan et al., 2000).

Contaminated soil can be remediated by chemical, physical or biological techniques (McEldowney et al., 1993). The rehabilitation technologies of agricultural land polluted with organic substances, are based on biodegradation of pollutants "in situ" and the cultivating of tolerant plants, parallel to implementation of agro-technical measures, which aim: the soil aeration through loosening; the soil reaction correction and balancing of the ratio carbon/nitrogen C/N through the administration of organic and mineral fertilizers (Sabau and Sandor, 2014). It was possible to reduce the content of hydrocarbons markedly in oil-polluted soils, even without fertilizers and amendments, as a result of intensive loosening. This indicates a sufficiently large potential of sod-podzolic soils for self-recovery and

high effectiveness of loosening in the first period after pollution (Lednev, 2007). Migratory capacity of heavy metals depends on cumulative properties of soil, chemical pollutants and landscape environment (Breus *et al*., 2001; Dybin, 2001). Pollutants coming from the atmosphere tend to concentrate in the soil horizons containing humus. However, in soil with low buffering capacity and under the influence of physic-chemical, biological and other processes heavy metals (HMs) are transferred from exchange-absorbed state into the soil solution and with downdrafts can be moved into the underlying layers. The objective of research– to evaluate the influence of agrochemical rehabilitation on the heavy metal migration to the water.

MATERIAL AND METHODS

Long-term stationary experiments were conducted in lysimeters of experimental design of Russian Institute of Hydraulic Engineering and Land Reclamation (VNIIGiM) with pristine soil profile. The area of stationary soil lysimeters was equal to 0. 78 and 1. 17 m. Within the research program there were carried out experiments to study the following fertilizer systems: organic (cattle manure), organomineral and mineral. High doses of double superphosphate were used periodically and annually in rotational cropping. The allowance of 100 t ha^{-} (table 1) was accepted for podzolic heavy loam chernozem.

Table 1. Scheme of establishment and implementation of the field lysimeter experiment

Variants	Names of variants and the fertilizer application system in the crop rotation link
I	Control
II	Cattle manure 100 t ha^{-} – Periodic application
III	Cattle manure 100 t ha^{-} – Periodic application, $N_{60-90}P_{60}K_{60-120}$ – Annually, depending on a culture
IV	Application of phosphorus, once in 2 years in a dose of 120 $kgha^{-}$ andannual application of $N_{60-90}K_{60-120}$
V	Application of phosphorus, once in 4 years 240 kg ha^{-} and annual application of $N_{60-90}K_{60-120}$
VI	Annual application of an elevated dose of phosphorus (120 kg ha^{-})and $N_{60-90}K_{60-120}$

The research was conducted in the period between 2006 and 2008. Barley of Nevskiy variety, fodder beet Eckendorf yellow, oat – Horizon.

In the experiment an elevated level of soil contamination was modelled based on the geochemical background of the region: Cu – 90, Zn – 110, Pb – 40, Cd – 0. 6 mg kg^{-}. For this purpose, chemically pure salts Zn $(CH_3COO)_2x2H_2O$, $CuSO_4x5H_2O$, $Pb(CH_3COO)_2$, $CdSO_4$were used, taking into account background levels of gross forms of heavy metals in the soil.

RESULTS AND DISCUSSION

In our conditions pollution of the soil with heavy metals was conducted in autumn of the year 2004 by means of water-soluble salts. It is known that due to sorption processes when salt is applied to the soil HMs are absorbed by soil colloids, which are presented in the soil as mineral, organic and organomineral compounds. Due to polyfunctionality of soil as a sorbent, its sorption capacity is not the same for different HMs and their cations. And processes of internal diffusion of molecules and ions may limit the speed of sorption. It may be also limited by the dissolution rate of soil compounds involved in the subsidence of contaminants. Consequently, when contaminants are released into the soil it takes a long time to establish sorption equilibrium.

There are two periods in the water regime of chernozem: 1. Draining - covers all summer and the first half of autumn, when water is rapidly consumed by plants and evaporates because ascending currents prevail over descending ones; 2. Soaking - starts from the second half of autumn, is interrupted by frosts and continues in spring, because of snowmelt waters and spring rainfall. Mainly the depth of rainfall, its distribution over time and its temperature determines these factors. Summer rainfall moistures only the topsoil. Moisture in the subsurface of chernozems is created by precipitation during the cold period (Elpatyevskiy, 1993). Therefore, precipitation of the late period creates additional moisture, which contributes to pollution of intrasoil waters with heavy metals.

The studies of Meshchersky Branch of VNIIGiM (Mazhayskiy et al.,2000) carried out at the ecological testing area “Meshchera” (Russia) showed the following concentration ranges of HMs in the water infiltrated through sod-podzolic soil: Pb - from 0. 33 to 0. 80 mg $L \cdot 10^{-}$; Cd from 0. 6 to 0. 11 mg $L \cdot 10^{-}$; Zn from 0. 16 to 2. 37 mg $L \cdot 10^{-}$; Cu from 0. 9 to 0. 21 mg $L \cdot 10^{-}$.

The research conducted within a lysimetric experiment aimed at studying the chemical composition of intrasoil water has shown that contaminated black soil has a high absorption capacity for HMs (Chernikova, 2010). The bulk of HMs brought about in a form of water-soluble salts, was adsorbed and converted by soil colloids of podzolized chernozem into relatively stable compositions. Contaminated soil of the first variant during the years of research increased migration of Cu and Cd to the intrasoil waters, whereas Pb and Zn demonstrated a better resistance to washing out in comparison to natural soils (table 2, table 3). The studied fertilizer systems in varying degrees increased the release of Pb and decreased migration of Cd to the infiltration waters. Their contamination with Pb increased by 1. 6% and equalled 89%, but concentration of Cd lowered by 20% and equalled 53%, except from the variant for which increased doses of phosphate were used (variant VI) (table 2, table 3). In this variant we registered increased leaching of Cd to the infiltration waters.

Table 2. Influence of agrochemical rehabilitation on the migration of Zn and Cu to the intrasoil waters (mg L·10^{-})

Variant	Zn			Cu		
	2006	2007	Average	2006	2007	Average
I	0. 48	1. 28	0. 89	0. 12	0. 36	0. 24
II	0. 51	2. 37	1. 44	0. 21	0. 19	0. 20
III	2. 08	0. 37	1. 23	0. 23	0. 25	0. 24
IV	0. 40	1. 32	0. 86	0. 12	0. 11	0. 12
V	0. 41	0. 11	0. 26	0. 17	0. 10	0. 14
VI	0. 51	0. 96	0. 78	1. 6	0. 11	0. 86

In the waters infiltrated through the profile of chernozem organic and organic-mineral fertilizers increased concentration of Zn by 62% and 38% respectively. Content of this element decreased by 3. 4% and equalled 40. 8% under the influence of mineral systems.

Table 3. Influence of agrochemical rehabilitation on the migration of Pb and Cd to the intrasoil waters (mg L·10^{-})

Variant	Pb			Cd		
	2006	2007	Average	2006	2007	Average
I	0. 70	0. 58	0. 64	0. 15	0. 14	0. 15
II	0. 62	0. 99	0. 85	0. 06	0. 08	0. 07
III	1. 62	0. 80	1. 21	0. 07	0. 13	0. 10
IV	0. 88	0. 80	0. 84	0. 05	0. 19	0. 12
V	0. 74	0. 56	0. 65	0. 03	0. 20	0. 12
VI	0. 82	1. 19	1. 01	0. 08	0. 29	0. 18

Under the influence of annual dose of phosphorus (P120) copper like lead and cadmium substantially migrated to infiltration waters. Other mineral fertilizer system (variants IV and V) approximately halved concentration of copper in intrasoil waters. With the organic system a decrease of copper in the waters was less (17%), and with the organic-mineral system concentration has not changed compared to Variant I (control).

Microcosm experiments detected the microbial community modifications with greater precision in the short-term, while field experiments showed that the biological effects of Cu contamination may be overcome or hidden by pedo-climatic variations (Ranjard et al., 2006).

(Rusu et al., 2004) the experimental data confirm the positive significance of the treatments effects of reaction correction in interaction with organic or complex mineral fertilization as well as the interaction of the zeolitic tuffs and bentonite with fertilizers. These agrochemical measures aim to acidity neutralization, decrease of the heavy metals mobility activity, calcium and primary macro elements contribution and in the last but least, restoration of the soil buffering capacity (Mohammad, 2015). The results showed the effect of water temperature on the explosive extraction/degradation and redistribution of the HMs in the soil.

CONCLUSION

Results of the analytical research showed that organic and organic-mineral systems where phosphates were used in the average volume of 60 kg of P_2O_5per hectare a year, reduced intake of cadmium in the subsurface water. Mineral systems also impeded migration of zinc and copper to the ground water. On the contrary, high doses of superphosphate in the fertilizer system increased the leaching of Cd, Pband Cu to the infiltration waters. The bulk of HMs brought about in a form of water-soluble salts, wasadsorbedand converted by soil colloids of podzolized chernozem into relatively stable compositions. The studied fertilizer systems in varying degrees increased the release of Pb and decreased migration of Cd to the infiltration waters: contamination with Pb incre-ased by 1. 6% and equalled 89%, but theconcentration of Cd lowered by 20% and equalled 53%, except fromthe variant for which increased doses of phosphate were used. In the waters infiltrated through the profile of chernozem organic and organic-mineral fertilizers increased aconcentration of Zn by 62% and 38% respectively. The content of this element decreased by 3. 4% and equalled 40. 8% under the influence of mineral systems. Under the influence of the annual dose of phosphorus (P120) copper like lead and cadmium substantially migrated to infiltration waters. Other mineral fertilizer system approximately halved concentra-tion of copper inintrasoilwaters. With the organic system a decrease of copper in the waters was less (17%), and with the organic-mineral system concentration has not changed compared to control.

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ADAPTIVE CAPACITY OF SOME LAVENDER AND LAVANDIN CULTIVARS*IN VITRO* AND *IN SITU*

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ABSTRACT

Lavandula angustifolia Mill. and (*Lavandulaxintermedia*EmericexLoisel) are promising fragrant plants with medicinal, aromatic and ornamental properties. Since the collection plantations of these crops are very damaged with viral pathogens and there is lack of seed propagation in valuable cultivars 'Belyanka', 'Record' (lavender) and 'Rabat', 'Snezhnyi Bars' (lavandin), were introduced *in vitro*. Chemotherapy was used for cleaning up. Regenerants were cultured (4-5 months) on MS medium with 0. 3 mg L^- Kinetin, 0. 025 mg L^- NAA and 0. 25 mg L^- GA_3 at 25±1°C under 16-h photoperiod. Intact plants were studied during the growing season. In order to reveal plants` biotechnological and genetic capacity some biochemical stress indicators, indexes of photosynthetic activity and water regime were identified. Under the open field cultivation, tested plants were rich in ascorbic acid, phenolic compounds, and redox enzymes (catalase, polyphenol oxidase, superoxide dismutase) were active. Leaf tissue hydration was 56-62%, with greater part of bound water. Photosynthetic activity was reduced only in the samples with visible damages with viral pathogens. In plants cultured *in vitro,* amount of ascorbic acid and phenolic compounds were lower, so as enzymatic activity and proline concentration were higher than in intact plants. The rate of hydration was high (70-77%), with the same trend of water fractional composition. Photosynthetic activity and vitality index indicated no photoinhibition. It was found out the lavandin cultivars had better capacity for a wide use under various culture conditions.

Keywords: *Lavandula sp, tissue culture, biochemical indicators, photosynthetic activity, water regime.*

INTRODUCTION

Lavender (*Lavandula* L.) is a valuable essential oil, aromatic, decorative and medicinal culture. The main cultivated species are *Lavandula angustifolia* Mill. and lavandin (*L. hybrida* Rev. or *Lavandulaxintermedia*EmericexLoisel). Lavender and Lavandule plants contain valuable essential oil, which is used in medicine,

perfume, cosmetics and food industry (Libus et al., 2004). Phenolic compounds, which have a wide range of physiological activities, were also identified in plant feedstock (Torras-Claveria et al., 2007).

Medical feedstock is the entire aboveground mass of plants. Traditionally, these crops are propagated by seeds (*L. angustifolia*) or vegetative (*L. angustifolia* and *L. hybrida*). In recent years, high degree of viral pathogens damages was noticed for cultivated plants. High quality planting material may be obtained by cleaning up *in vitro*. Besides, biotechnological methods enable to produce a large number of genetically homogeneous virus-free plants (Mitrofanova et al, 2014; Gonçalves and Romano, 2013). Plants damaged with viral pathogens are exposed to stress. One of the earliest responses to the stress effect is active forms of oxygen (AFO) formation and they violate many processes in the cell and its structure. To prevent such violations antioxidant systems including low molecular weight protective compounds and specific antioxidant enzymes are present in cells. The study of the antioxidant systems functioning is important for predicting plant adaptive capacity (Mittler, 2002; Mullineaux and Baker, 2010). The sensitive parameter for changes in plant functional state is also photosynthetic activity and the viability index (Byron et al, 2000; Stirbet and Govindjee, 2011).

Thus, the aim of the work was to determine physiological and biochemical changes in vegetative organs of valuable lavender and Lavandula cultivars in plants with symptoms of viral diseases and after cleaning up *in vitro*.

MATERIAL AND METHODS

The objects of this study were valuable cultivars of lavender (Belyanka, Record) and lavandula (Rabat, Snezhnyi Bars) from the collection of Nikita Botanical Gardens. The researches of intact plants were carried out during the phenophase "technical maturity" (the second and third decade of July 2015). For the studies plants without visual symptoms of viral diseases and damaged ones were selected. Apical meristems of axillary buds were introduced *in vitro*. Chemotherapy *in vitro* was used for cleaning up.

Regenerants were cultured for 4-5 months on the modified MS medium with 0. 3 mg l^{-} Kinetin, 0. 025 mg l^{-} NAA and 0, 25 mg l^{-} GA_3. Explants in the culture vessels were kept in a growth chamber at 25 ± 1°C under 16-h photoperiod supplied by cool-white fluorescent lamps giving 37. 5 μmol m^{-} s^{-}. The callus formed 2-5 microshoots, 23-82 mm height and each microshoot produced from 10 to 26 lanceolate leaves, 9-15 mm length.

Biochemical parameters were determined by standard methods: the content of proline due to modified procedure by Chinarda using ninhydrin reagent (Andryushchenko et al.,1981), the amount of phenolic substances by photometric method using Folin-Ciocalteu reagent (Gerzhikova, 2002), ascorbic acid by iodometric titration (Richter, 1999). The activity of catalase (EC 1. 11. 1. 6) was determined titrimetrically (Voskresenskayaetal.,2006), polyphenol oxidase (EC 1. 14. 18. 1) - in the presence of catechol and p-phenylenediamine (Ermakov, 1987), superoxide dismutase (EC 1. 15. 1. 1) – due to quercetin oxidation reaction

(Kostyuk et al., 1990). Such physiological criteria characterizing the water regime as the total water content in leaves, water fractional composition and the water deficit under the cultivation in the field were used (Ross, 2012). Weather data of the study period are presented according to the observations of agrometeorological station "Nikita Garden": the average air temperature in the period of water deficit studies was 22. 2˚C, the maximum air temperature – 32. 4˚C, minimum relative humidity – 39%. The basic amount of precipitation fell in the beginning of July; their sum for the month was 15. 6 mm or 50% of the norm. In late July, stocks of productive soil moisture at the depth of the lavender root layer (0-20 cm) were 40 mm. The parameters of photosynthetic activity were measured with a portable fluorometer "Florotest" (Ukraine, 2010). During the experiments the following indexes of Kautsky fluorescence induction kinetics were registrated: the initial fluorescence (F_0), the maximum (Fm) and stationary (Fst) fluorescence values after dark adaptation. Viability index and photosynthetic activity were calculated (Bajron et al., 2000).

RESULTS AND DISCUSSION

To evaluate the adaptive capacity of plants requires data about protective compounds of various chemical origin are needed. Proline is among those compounds and it is a source of energy, carbon and nitrogen under the lack of resources and reduce of synthesis enzyme activity caused by stress (Kavi Kishor et al., 2005). Other proline functions including osmoregulation and participate in gene expression were found out (Lyers and Caplan, 1998).

In the conditions of the open field growing for lavender and Lavandula plants withoutvisual symptoms of viral diseases proline content was the highest and it decreased slightly (to 29%) in the plants with symptoms of viral diseases (Table 1). Perhaps, proline reduction in the plants with viral symptoms was due to proline synthesis slowdown in response to stress and its consumption for engagement with surface hydrophilic residues of proteins to increase their solubility and protection from denaturation. In regenerants under *in vitro* conditions, proline concentration was higher than in intact plants, despite the high water content in tissues. This suggests that free proline can influence the processes of cell growth and differentiation in Lavender and Lavandula.

Phenolic substances are also among the main plant protective compounds. They are involved in the basic processes of plant cell activity: photosynthesis, respiration, as well as stress protection (Zaprometov, 1993). Plant response to viral infection is the increase of phenolic compounds content (Dikilitas et al., 2011).

Lavender and Lavandula intact plants without visual viral diseases symptoms accumulated maximum amount of phenolic compounds. Together with viral diseases symptoms appearance phenolics content reduced (to 14-31%), which is probably corresponds to the specific reactions of these cultures for viral infection, i. e. preferred accumulation of protective compounds of the other chemical origin. Antioxidant protection of that type plants can be carried out due to the presence of high-molecular antioxidants and volatile compounds.

Considering that influence of adverse factors on plant resulted in oxidative stress ascorbic acid is under the interest as a protective compound. It is the most common antioxidant in plants. It is known that this compound is a regulator of cell growth and cofactor for many enzymes and it is involved in photosynthesis, respiration and growth of plants (Smirnoff, 2000).

In intact plants without visual viral diseases symptoms concentration of ascorbic acid was the highest and it slightly decreased (by 15-29%) together with viral diseases symptoms appearance. The exception was Lavandula cultivar Snezhnyi Bars, in which the changes in ascorbic acid content under the appearance of viral diseases symptoms did not demonstrate significant difference.

In plants under controlled conditions *in vitro* concentration of phenolic compounds and ascorbic acid were lower than in intact plants, due to high water content in tissues and absence of stress factors.

Table 1. Biochemical indexes of lavender and Lavandula cultivars

Cultivars		Proline, μg g^{-}	Ascorbic acid, mg 100 g^{-}	Phenols, mg 100 g^{-}
Belyanka	*in situ*	7. 69±0. 23	20. 06±0. 58	1033±26
	*in situ**	5. 49±0. 16	18. 04±0. 52	785±23
	in vitro	8. 24±0. 24	5. 61±0. 16	645±17
Record	*in situ*	12. 95±0. 37	18. 92±0. 54	1181±31
	*in situ**	10. 99±0. 32	15. 05±0. 44	816±24
	in vitro	33. 75±0. 99	5. 94±0. 17	490±14
Rabat	*in situ*	6. 67±0. 20	19. 14±0. 55	1305±34
	*in situ**	5. 49±0. 14	15. 40±0. 45	1119±30
	in vitro	35. 72±1. 04	4. 95±0. 13	668±19
Snezhnyi Bars	*in situ*	21. 59±0. 63	14. 96±0. 44	1492±40
	*in situ**	18. 05±0. 53	15. 62±0. 46	1165±32
	in vitro	35. 32±1. 05	5. 98±0. 17	777±22

* - plants with visual symptoms of viral diseases

The results presented in Table 2 demonstrate that in the studies of redox enzymes in and lavender and Lavandula cultivars it was determined that plants without visual symptoms of viral diseases are characterized by maximum values of catalase, polyphenol oxidase and superoxide dismutase activities.

In plants with symptoms of viral infection activity of all studied enzymes decreased: catalase activity by 10-56%, SOD activity - by 5-26%, PPO activity - by 22-60, suppose that is associated with reduced antioxidant status of plants due to excessive accumulation of the active forms of oxygen. The greatest decrease in catalase and PPO activity together with poor changes of SOD activity was observed in infected Lavandula plants cultivar Rabat.

Table 2. Redox enzymes activity in lavender and lavandin cultivars

Cultivars		Catalase activity, g $O_2 g^{-} min^{-}$	SOD activity, a. u g^{-}	PPO activity, a. u. $g^{-} \cdot s^{-}$
Record	*in situ*	18. 13±0. 45	13. 60±0. 33	0. 628±0. 016
	*in situ**	15. 45±0. 39	11. 28±0. 27	0. 472±0. 012
	in vitro	6. 80±0. 16	6. 12±0. 20	0. 101±0. 003
Belyanka	*in situ*	30. 68±0. 87	12. 98±0. 32	0. 524±0. 13
	*in situ**	25. 50±0. 64	10. 83±0. 27	0. 378±0. 009
	in vitro	7. 65±0. 19	5. 62±0. 14	0. 103±0. 002
Snezhnyi Bars	*in situ*	36. 97±0. 92	14. 82±0. 38	0, 377±0. 008
	*in situ**	36. 12±0. 90	10. 83±0. 26	0, 294±0. 007
	in vitro	2. 98±0. 08	10. 48±0. 28	0. 124±0. 004
Rabat	*in situ*	31. 45±0. 77	12. 55±0. 32	0. 600±0. 015
	*in situ**	13. 74±0. 43	11. 93±0. 30	0. 238±0. 007
	in vitro	3. 68±0. 09	12. 43±0. 31	0. 112±0. 003

Under *in vitro* culture catalase activity in Lavender cultivars was higher than that in Lavandula ones. At the same time, SOD and PPO activity in lavender *in vitro* had lower values than the in Lavandula. Comparative analysis of enzymes activity in intact plants without viral diseases symptoms and plants cleaned up *in vitro* demonstrated that minimum values of catalase and polyphenol oxidase activity are characteristics of Lavender and Lavandula cultivars grown *in vitro*. Reduction of enzyme activity corresponds to the high water content in tissues, low content of ascorbic acid and phenolic compounds, as well as the absence of stress factors. SOD activity in Lavandula cultivars *in vitro* was at the same level with the healthy plants grown *in situ*, and in Lavender cultivars *in vitro* it was 50% lower than in healthy intact plants.

Table 3. Indexes of the water regime and the relative quantum efficiency of photosystem-2 in Lavender and Lavandula cultivars

Indexes		Cultivars			
		Belyanka	Record	Rabat	Snezhnyi Bars
Total water content, %	*in situ*	61. 1 ± 3. 0	57. 9 ± 2. 5	56. 3 ± 4. 8	62. 3 ± 2. 1
	*in situ**	59. 1 ± 2. 9	58. 1 ± 2. 8	70. 8 ± 1. 9	58. 2 ± 2. 5
	in vitro	76. 1 ± 3. 3	72. 3 ± 2. 9	77. 0 ± 2. 5	74. 4 ± 3. 2
Bound water fraction, % of total water content	*in situ*	78. 3 ± 4. 9	90. 6 ± 3. 5	82. 1 ± 4. 3	93. 2 ± 1. 3
	*in situ**	84. 9 ± 2. 3	87. 4 ± 1. 9	79. 3 ± 5. 0	86. 5 ± 4. 3
	in vitro	69. 5 ± 4. 1	58. 1 ± 2. 2	68. 3 ± 4. 8	49. 4 ± 6. 1
Plant-water deficit, %	*in situ*	26. 9 ± 1. 4	24. 8 ± 2. 9	23. 1 ± 2. 9	29. 1 ± 1. 2
	*in situ**	23. 5 ± 2. 9	38. 2 ± 4. 1	26. 4 ± 1. 4	25. 3± 1. 8
Relative photosynthetic activity$(F_m-F_{st})/ F_m$	*in situ*	0. 68 ± 0. 09	0. 70 ± 0. 05	0. 75 ± 0. 10	0. 71 ± 0. 05
	*in situ**	0. 62 ± 0. 07	0. 52 ± 0. 08	0. 73 ± 0. 12	0. 68 ± 0. 02
	in vitro	0. 28±0. 10	0. 45±0. 05	0. 55±0. 08	0. 45±0. 09
Viability index F_m/F_{st}	*in situ*	2. 61 ± 0. 50	2. 51 ±0. 61	3. 18 ± 0. 52	2. 94 ± 0. 70
	*in situ**	1. 94 ± 0. 31	1. 82 ± 0. 29	2. 73 ± 0. 68	2. 85 ± 0. 32
	in vitro	1. 41±0. 03	1. 71±0. 12	2. 36±0. 37	2. 00±0. 36

Under the open field growth conditions water content in leaves was 56-62% (Table 3) and the part of bound water was from 79 to 93% of the total water content. After a long period (18 days) period without precipitation total water content in vegetative organs decreased, while the part of bound water increased. The maximum water-holding capacity was characteristic of the vegetative organs tissue in the cultivars Snezhnyi Bars and Record due to the fraction of bound water. Studied parameters did not reveal any significant differences between the plants with visual symptoms of viral diseases and ones without symptoms.

The level of leaf-water deficit was 23-29% in the cultivars Rabat, Belyanka and Snezhnyi Bars. The maximum (38%) leaf-water deficit was noticed in Record cultivar plants with the symptoms of viral pathogens damages. We did not notice significant differences between the affected and unaffected plants in other studied cultivars. Changes in the water regime and the degree of cultivar susceptibility affected largely on the photosynthetic activity of Lavender cultivars. They demonstrated decrease of the relative quantum efficiency of photosystem 2, photochemistry and efficiency of the energy capture with open reaction centers. Viability index was in the normal, but it was lower in Lavender cultivars. Photoinhibition expressed in plants with visual symptoms of viral diseases. The observed effect of changes in the photosynthetic apparatus activity together with visual damages of vegetative organs in the studied plants are in agreement with the conclusions by S. Nogues and L. Alegre (2002).

Leaf water content in *in vitro* regenerants was higher in Lavandula plants (74-77%) and no significant differences in this parameter was not revealed between the cultivars. However, the lowest variability of water content in regenerants during the culture and maximum ratio bound/free water fraction let us to select the cultivars Rabat and Record. The high photosynthetic activity of leaves in regenerated plantlets was found. Viability index was normal for plants grown under controlled conditions *in vitro* and the same was in plants grown at a relatively heterotrophic nutrition type. According to these indexes, better functional state was characteristic of Lavandula cultivars.

Functional state indexes of the studied plants *in vitro* demonstrated no photoinhibition and normal activity of photosystems at both light harvesting complexes and at the time of electron donors' oxidation in the reaction center of photosystem 2.

CONCLUSIONS

It was found that in the open field growth conditions content of proline, phenolic compounds, ascorbic acid and the activity of catalase, superoxide dismutase and polyphenol oxidase were maximum in Lavender and Lavandula symptomless and they decreased in plants with symptoms of viral diseases. According to those parameters, no significant differences between Lavender and Lavandula cultivars were revealed. Changes in the water regime of the cultivars and the degree of their susceptibility affected largely on the photosynthetic activity in Lavender cultivars.

In plants under *in vitro* conditions proline concentration was higher and the content of phenolic compounds, ascorbic acid and the enzymatic activity was lower than in intact plants. Photosynthetic activity and vitality index demonstrated no photoinhibition.

It was revealed that studied Lavandula cultivars have greater plasticity and capacity for growing *in vitro* and *in situ*.

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MODELLING RESERVOIR SEDIMENTATION AT BIN EL OUIDANE DAM, MOROCCO

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ABSTRACT

This study was conducted in the Oued El Abid watershed upstream of the Bin El Ouidane dam, in Tadla-Azilal province (Morocco) to quantify the dam siltation rates. To assess the annual soil erosion and the sediment yield the universal soil loss equation (USLE) was used. A geographic information system (GIS) was used to generate and integrate maps of the USLE factors. A spatial distribution of soil erosion in the Oued El Abid watershed was obtained. The soil erosion was determined for each rural commune in order to identify the soil erosion hotspot and estimate the amount of soil that has been transported downstream (Bin El Ouidane Dam). Soil erosion ranged from very limited values for flat and well covered areas to over 2100 t /ha/y in mountainous areas with sparse vegetation. The total annual soil loss within the watershed is estimated at 19. 6 million tons per year. An equation of sediment delivery ratio (SDR) based on river gradient was calculated. It was found that the value of *SDR* at the outlet of the watershed Oued El Abid was 0. 65 with a sediment yield of 12. 74 million tons per year which affect the durability of the dam.

Keywords: *soil, erosion, USLE, GIS, SDR.*

INTRODUCTION

Soil erosion is a complex dynamic process by which productive soil particles are detached, transported and accumulated in a distant place. This results in exposure of subsurface soil and sedimentation in reservoirs(Jain et al., 2001). In Morocco, the dam siltation retained annually reduces the storage capacity of 75 million m, which represents 0. 005% of the annual water mobilization with a total shortfall of 1 billion dollars per year. Out of the total area of 710 850 Km² of Morocco, it is estimated that about 150 000 km is affected by serious water and wind erosion (Namr and Mrabet, 2004).

The entire downstream area of High Atlas mountain chainis affected by a serious problem of soil erosion. All rivers flowing through this region transport a heavy load of sediment which is then trapped in several reservoirs. In this respect, this study aims at highlighting the relationships between the biophysical and hydrological conditions that control the erosion processes in Oued El Abid watershed upstream of the Bin El Ouidane dam which knew a shortage in its capacity of 274. 5 million m between 1954 and 2008 with an average shortfall of 5 million m per year.

However, it is difficult to model soil erosion because of the complexity of the interactions of factors that influence the erosion process (Lufafa et al., 2003). Many soil erosion models have been developed, ranging from simple empirical equations, such as the Universal Soil Loss Equation (USLE) (Wischmeier and Smith, 1978) and its revised version, RUSLE (Renard et al., 1997), to more sophisticated models, such as the Water Erosion Prediction Project (WEPP) (Flanagan et al., 2007)and EUROSEM (Morgan et al., 1998). The latter may be functionally more powerful than the empirical models, but those models often need lots of data and are computationally intensive to use in many circumstances, particularly with respect to modelling soil erosion in medium- and large-scale watersheds (Wang et al.,2009). On the contrary, the USLE has been extensively applied all over the world at many scales mainly due to the simplicity of the model formulation and the possibility to estimate the input parameters with limited input data(Wang et al., 2009).

The USLE provide an estimation of the sediment mobilized by surface runoff, but is not able to model whether the sediment will be exported out of the catchment or re-deposited as colluvium or alluvium within the catchment (Hui et al.,2010). In fact, the sediment delivery ratio (SDR) was introduced to estimate the sediment yield in Bin El Ouidane reservoir based on the slop of drainage line extracted from the Data Elevation Model using Arc Hydro tools which is a geospatial and temporal data model for water resources designed to operate within ArcGIS (Maidment, 2002).

MATERIALS AND METHODS

Study area

The watershed of Oued El Abid with an area of 7686 km is the upper part of the great Oum Er Rabia drainage basin of 50, 000 km (see Figure 1. A). The Oued El Abid watershed is located upstream of the Bin El Ouidane dam in the region of Tadla Azilal between the High Atlas and the plain of Tadla. The main water course is the Oued El Abid, one of the most important water resources of Morocco which is using for irrigation and hydropower. The watershed stretches over three different provinces and is divided into 27 administrative rural communities (see Figure 1. B).

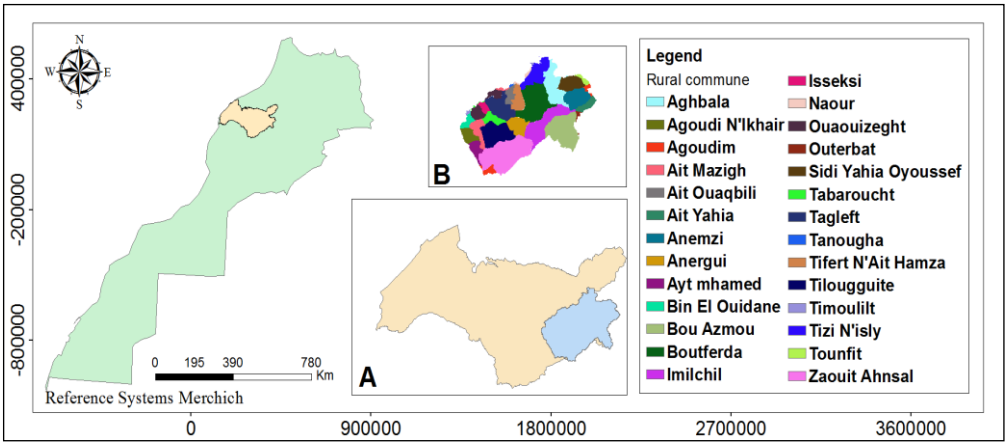


Figure 1. Localisation of study area

Methodological approach

The general methodology (see Figure 2) involves the use of the USLE in a GIS environment. The individual raster layers were calculated for each USLE factor and processed in a GIS. The product of those factors has given the annual loss of soil in the entire watershed.

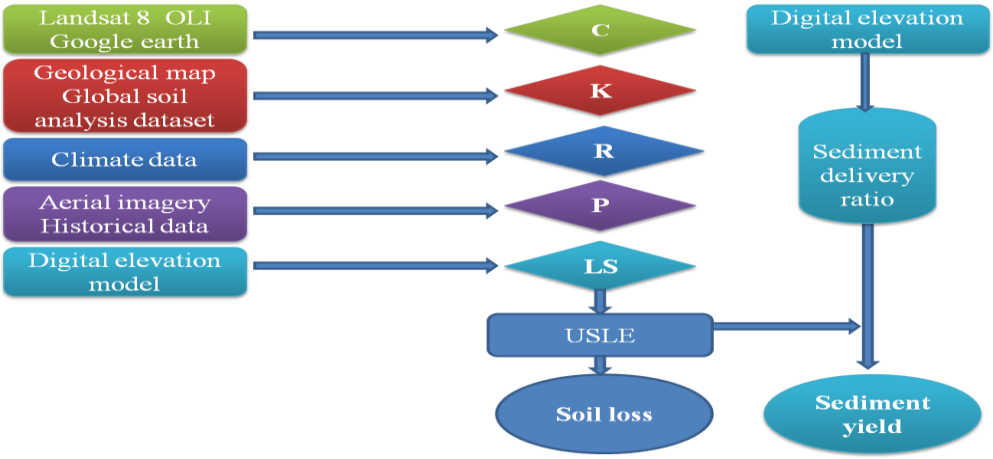


Figure 2. Schematic of the approach used

The sediment yield was calculated using the sediment delivery ratio (SDR) based on river gradient extracted from ASTER satellite imagery using arc hydro tools (Maidment, 2002).

Application of the Universal Soil Loss Equation (USLE)

The proposed method is based on the universal soil loss equation(Wischmeier and Smith, 1978). This equation provides the average annual erosion for a long period of time based on the slope of a field, data of rainfall, cropping system and management practice. Five key factors are used to calculate soil loss at a given location. Each factor is a numerical estimate of a particular component which affects the severity of soil erosion at that location.

A = R * K * LS * C * P

A: expresses the potential average annual soil loss in (Tonne / ha / y).

R: corresponds to the Rainfall erosivity factor.

K: is the soil erodibility factor.

LS: is the length factor and slope gradient.

C: corresponds to the land use factor.

P: is the conservation practice factor.

1. Topographic Factor LS

The LS factor was calculated using the following equation (see Equation 1) (Bizuwerk et al.,2003; Stone and Hilborn, 2012):

$$LS = [0.065 + 0.0456(Slope) + 0.0065(Slope)^2](\frac{Slopelength}{22.1})^{0.5} \quad (1)$$

Where:

Slope = slope steepness in %

Slope length = (flow accumulation * cell resolution) in m (Van Remortel et al. 2004)

The slope was extracted from SRTM with 30 m resolution using arc hydro extension.

2. Rainfall Erosivity Factor R

To estimate this factor we have used the equation provided by (Arnoldus, 1977)(see Equation 2)which was used by FAO in Morocco to develop an iso-erodent (Hui et al.,2010)The equation expressed as follow (see Equation 2):

$$R = 0.\times F \cdot \quad (2)$$

F is the Fournier index modified expressed as follow (see Equation 3):

$$F = \sum_{i=1}^{12} \frac{ri}{P} \quad (3)$$

Wherer_i is the precipitation in the month i and *P* is the annual precipitation.

3. Soil Erodibility Factor K

Erodibility (K) is a function of the organic material and texture of soil, the permeability and the profile structure. K is a measure of the susceptibility of soil particles to detachment and transport by rainfall and runoff (Wischmeier and Smith, 1978). It varies from 0. 70 for the most fragile soils to 0. 01 on the most stable soils (El Garouani et al.,2008).

Our results are obtained by using soil analysis of 54 soil samplesand aggregate data of soil analysis provided by FAO in their harmonized database of the World Soil

(Dewitte et al.,2013) and Sheet information of OMAFRA based on USLE (Stone and Hilborn, 2012).

4. Vegetation Cover Factor C

The land cover map is obtained from the recent (2013/2014) Landsat 8 OLI satellite image with 30m resolution using the supervised classification. The use of Google earth image has brought more details especially for forests classes. The kappa coefficient calculated from confusion matrix is used to determine the accuracy of the supervised classification(Ruiz-Luna and Berlanga-Robles, 2003).

5. Soil Conservation Factor P

The erosion control practice factor is defined as the ratio of soil loss with a given surface condition to soil loss with up-and-down-hill plowing. It varies between 1 in a soil without erosion control practice and 0. 1 when on a slight slope, we practice tied ridging (Roose, 1996).

Sediment Delivery Ratio Module

The sediment delivery ratio is affected by many highly variable physical characteristics of a watershed. It varies with the drainage area, slope, relief-length ratio, runoff-rainfall factors, land use/land cover and sediment particle size, etc . The empirical equations relating SDR with one or morefactors are still useful tools to estimate SDR (Ouyang and Bartholic, 1997).

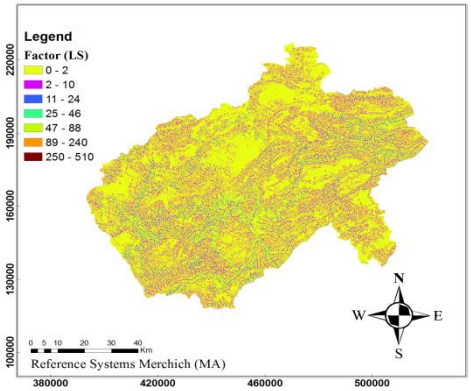
(Jimmy R. Williams, 1972) used slope of the main stream channel to predict sediment delivery ratio.

The model is written as follow (see Equation 4):

$SDR = 0.\times SLP^{0.0}$ (4)

SLP: is slope of drainage line in degree.

RESULTS AND DISCUSSION



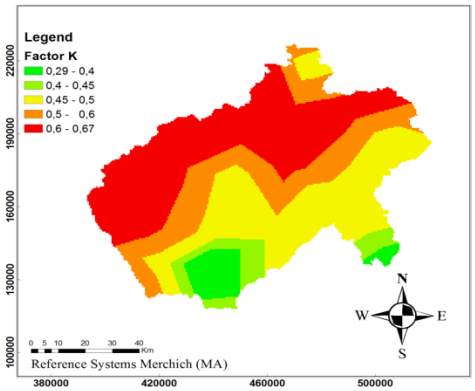
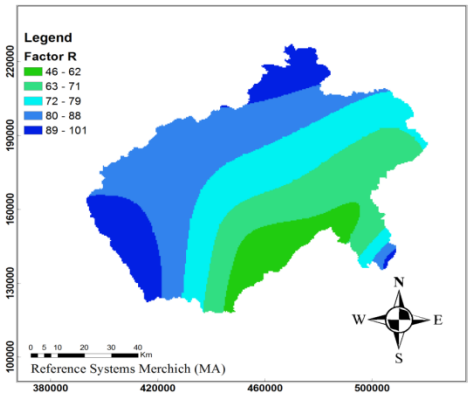


Fig. 3 Spatial distribution of LS factor**Fig. 4**Spatial distribution of K factor



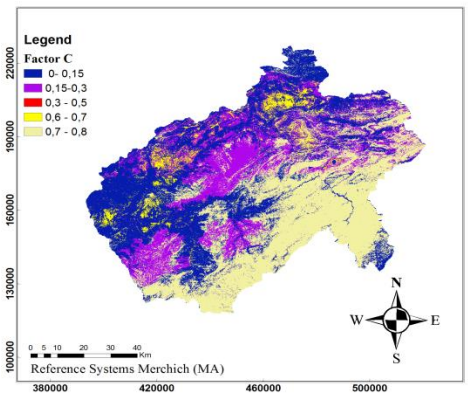
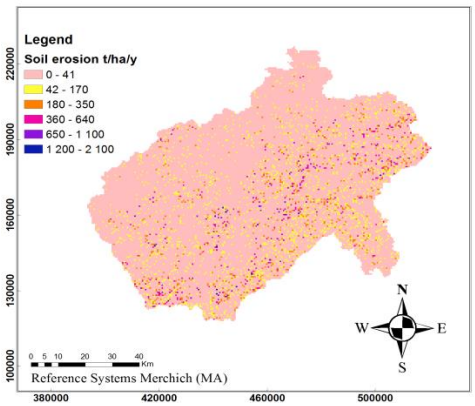


Fig . 5 Spatial distribution of R factor**Fig . 6**Spatial distribution of C factor



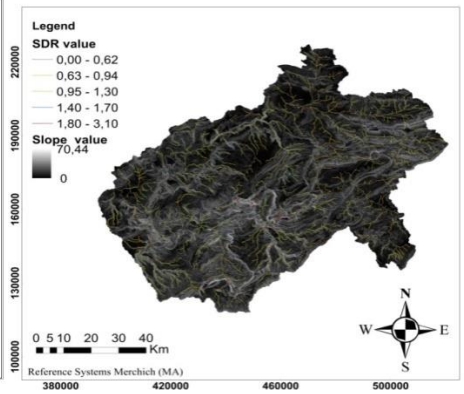


Fig. 7 Spatial distribution of soil loss**Fig. 8**Sediment delivery ratio

The inspection of the property shows the absence of erosion control practice, thus a value of "1" is attributed to P factor.

After we combine all these five factors (see Figure 3; 4; 5; 6) we got the soil loss in t/ha/year (see Figure 7).
The results show that the commune called *Agoudim* is the most degraded with 77, 66 t/ha/y (see Table 1).

Tab. 1 Soil loss in each commune

Commune	Area (ha)	Min (t/ha/y)	Max (t/ha/y)	Mean (t/ha/y)
Aghbala	44895, 74	0	977, 55	18, 59
Tizi N'isly	34590, 77	0	515, 62	7, 38
Timoulilt	1633, 71	0	101, 88	2, 39
Naour	2576, 24	0	161, 47	6, 88
Ait Ouaqbili	10524, 89	0	195, 00	4, 71
Tounfit	6723, 37	0	405, 93	34, 88
Tanougha	2293, 48	0	275, 38	7, 72
S. Y. Oyoussef	24411, 47	0	1069, 95	17, 82
Ouaouizeght	20201, 51	0	977, 07	13, 53
Tabaroucht	13666, 65	0	814, 63	20, 52
T. N. Hamza	19667, 41	0	548, 89	6, 93
Anemzi	31951, 69	0	1065, 81	43, 32
B. El Ouidane	8545, 59	0	149, 58	2, 46
Ait Yahia	10713, 40	0	1067, 44	64, 89
Isseksi	6346, 35	0	206, 92	9, 84
Tagleft	38455, 14	0	573, 29	12, 78
Outerbat	1759, 39	0	1144, 26	53, 73
Boutferda	73045, 91	0	1032, 11	24, 56
Anergui	22872, 01	0	1306, 91	24, 00
A. N'lkhair	14703, 43	0	669, 56	12, 71
Tilougguite	46843, 63	0	1196, 28	24, 88
Ait Mazigh	18567, 80	0	1028, 00	16, 59
Imilchil	55923, 32	0	2100, 00	37, 92
Bou Azmou	67453, 58	0	802, 15	31, 81
Ayt mhamed	13383, 90	0	889, 08	19, 88
Agoudim	6566, 28	0	1338, 53	77, 66
Zaouit Ahnsal	98745, 50	0	1611, 09	26, 28

The SDR value at the outlet of Oued El Abid watershed is estimated at 0. 65(see Figure 8) then the sediment yield was found to be 12. 74 million t/a.
The analysis of soil samples showed a mean soil density of 2. 45 t/ m, thus the sediment yield is estimated at5. 2Mm/year.

The results presented suggest that taking the sediment delivery ratio into account greatly reduces the total sediment output compared to what is calculated from the USLE.

In the North-Morocco many studies performed by (Dahman, 1994; Rahhou, 1999; Al Karkouri, 2003; Aroussi et al., 2011) had focused on erosion phenomenon, but they had not addressed the sedimentation delivery phenomenon. (El Garouani et al. 2008)have used a sedimentation model based on the revised universal sol loss equation and the spatial variability of the field to estimate the amount of soil load for the Tlala watershed outlet. Recently (El Gaatib and Erraji, 2014) did a study in the Oued el Beht watershed based on the USLE model. They coupled rainfall and flow rate in order to estimate the transport of sediment to the El Kansra dam.

(Lahlou, 1982) used practical methods (bathymetry, topography survey, turbidimetry measurement,emptying an filing of dam) in order to estimate the silting rate in 27 dams across Morocco,the annual silting rate was estimated to be between 1, 35 and 10 million m. As regard the Bin El Ouidane dam the author used the curve extrapolation of specific degradation of watershed surface based on measurements from watersheds. The annual silting rate was estimated to be 1, 5 million m.

The bathymetric measurement between the years 1953 and 2008 showed a sediment yield rate of 5 million m/year.

The differences between the result obtained by the dual focus on erosion and deposition and this given by the specific degradation(Lahlou, 1982) are due to the global warm which was expected to lead to a more vigorous hydrological cycle, includingmoretotal rainfall and more frequent high intensity rainfall events.

The new proposed method in Morocco gives results are acceptable compare to those given by validation methods that are more accurate and expensive with a slight overestimation does not exceed 0. 04% maximum. This method is also applicable on all watersheds regardless the superficies, then it can be considered technically a reliable method and deserves to be applied instead of the commonly used.

Indeed, the confidence for the factors values cannot be defined by lack of validation on the parcels for calibration. Custom field will be the subject of our next step in our project.

CONCLUSIONS

Taking into account the variability of erosion and deposition processes at the same time resulted in reducing the estimated soil erosion values calculated by the USLE model. Although the results obtained by this study are questionable because of probability of error in the data used and the limits of the USLE model when applied to the large watershed, this method provides an important support to decision makers and planners to simulate scenarios for the evolution of the region and plan interventions against erosion. It also helps to monitor the impact of land use and development on the quality of soil resources.

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CONDITIONS OF AGRICULTURAL LAND PRICES DEVELOPMENT IN POLAND

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ABSTRACT

The aim of the paper has been to diagnose factors determining agricultural land prices, classifying them and determining which of them plays the most important role. The considerations were based on selected subject literature, the results of researches conducted by other authors, as well as analysis of statistical data from the Central Statistical Office of Poland and Eurostat. Factors determining agricultural land prices in Poland can be divided into two groups: 2) Market: supply and demand for agricultural land allotted to agricultural production; supply and demand for agricultural land that can be, in compliance with the binding law, allotted to purposes other than agricultural; simultaneous functioning of two market segments - private (on which a majority of land trading is conducted among farmers) as well as the state market segment – the Stock of Agricultural Property of the State Treasury managed, on behalf of the owner - the State Treasury, by the state institution - the Agricultural Property Agency; profitability of agricultural production. 2) Non-market: historical conditions – dominance of private property in Polish agriculture, both in the market economy and in the previous economic system; tradition, culture - passing farms from generation to generation; Poland's accession to the European Union and covering Polish farmers with common agricultural policy instruments; ending the transitional period of purchasing Polish agricultural properties by foreigners on 1 May 2016. After this period, the hitherto limitations (special permits) for foreigners in purchasing agricultural properties in Poland were lifted.

Keywords: *Agricultural Property Agency (APA), price of agricultural land, agricultural land market.*

INTRODUCTION

Economists consider land, next to capital and labour, as a classical production factor. They underline that land is a limited and immobile asset, which significantly affects the specification of the land market. Furthermore, they also draw attention to its basic function, that is, food production (Marks-Bielska, 2014a; Milczarek-Andrzejewska and Zawalińska, 2015). The key determinant of the market mechanism, including the scale of trading in agricultural properties, consists in the price of this stock. At first, agricultural land prices were significantly diversified depending on the quality thereof. Empirical studies prove that currently, the quality

of land determines agricultural land prices to a negligible extent. These results from the possibility to conduct agrotechnical treatments improving soil properties as well as purchasing properties for purposes not related with direct agricultural production. Currently, the determinants of agricultural land prices are as follows: agricultural raw materials' prices, productivity of land, level and character of agricultural subsidies, as well as designating production areas (Dige 2010).

In the case of transactions covering whole farms, the unit price per ha of property decreases together with the size of the subject of trade. Land prices are also affected by: the proximity of the purchased plot with previously owned lands, and then soil valuation class, the possibility of using the property alternatively, the type of agricultural land, and the supply of land in a given area. Land prices are also influenced by the profitability of the agricultural sector. A deteriorating income situation frequently causes postponing the decision on selling land by small farms, and the increasing profitability of agriculture causes an increase in the demand for land, which contributes to an increase in the price thereof (Majchrzak, 2015).

Each plot of land, if it only has any value from the economic point of view, has its price. Not because the input for production thereof increased, but because it fulfils specific functions and constitutes a source of future benefits – it generates income.

Prices of natural resources, including land, developing in free markets and resulting from demand-supply relations constitute an indispensable condition of rational economy (Maśniak, 2013). The price of land in the developed market economy plays a significant role, since it constitutes an element of investment profitability in agriculture, a basis for the financing of any activities in a farm with outside capital (in particular in the case of bank loans, where it constitutes grounds for mortgage debt insurance), as well as the primary and most stable component of a farming family's assets (i. e. property, capital). Moreover, as a component of assets it is the object of inter-generational transfers of goods or market turnover (sale) (Klank, 2008).

We deal with land price primarily when there are natural or legal persons who express their willingness to purchase it. This price is determined by, for example, comparing its value to the value of other land, the price of which is known. Land price can also be determined on the grounds of the land rent it gives. In another approach, the land price constitutes the cost that must be incurred by a person who does not have land and wants to utilise it by purchasing or leasing it.

Land prices are very diverse, according to the usefulness of particular land plots, soil quality and their natural fertility, the situation in the market, the condition of the environment, etc. Irrespective of spatial diversity, prices also change over time (Maśniak, 2013; Marks-Bielska and Lizińska, 2015).

Agricultural land price in the market economy is decided not only by its supply and demand. It results from specific, aforementioned features of this stock, and covering the land market with state interventionism. Legal provisions concern, e. g. land allocation due to its quality (the best, fully agriculturally valuable land cannot be allocated to afforestation or to construction – this status cannot be changed in the case of every agricultural land plot).

In Poland, due to the characteristics of the ownership in agriculture before the system transformation which started in this economic sector at the beginning of the 90s[1], two simultaneously functioning and intertwined agricultural land markets (private and state) developed (Marks-Bielska, 2014a).
The aim of the paper has been to diagnose the factors determining agricultural land prices, classifying them, and determining which of them plays the most important role.

MATERIALS AND METHODS

Achieving the aim required reviewing the subject literature, referring to the results of relevant researches conducted by other authors as well as the analysis of secondary data. Statistical data from EUROSTAT, the General Statistical Office of Poland (GUS), the Agricultural Property Agency (APA), the Ministry of the Interior, and reports of the Institute of Agricultural and Food Economics – National Research Institute (IAFE-NRI)*Market of Agricultural Land…* 2005-2015 by Sikorska (ed.) were used. The report has been prepared under the implementation of Task 2, within Topic II, entitled: ″Social polarisation versus economic stability in the development processes of agriculture and rural areas″ as part of the Multiannual Programme of IAFE-NRI on ″The Polish and the EU agricultures 2020+. Challenges, chances, threats, proposals″.

RESULTS AND DISCUSSION

The land market in Poland can be conventionally divided into two segments – the private market (called neighbourly trade between private entities) and the state market (among others, state land included in the Stock of the Agricultural Property of the State Treasury managed by the state institution – the Agricultural Property Agency)[2]. These markets are intertwined.

[1]In Poland, in contrast with the majority of Central and Eastern Europe countries, before the start of system transformation, the ownership structure in agriculture was characterised by a dominance of private farms (individual and cooperative), which constituted 99. 9% of farms, having 75. 7% of agricultural land, utilising 81. 1% of the total amount of agricultural land being utilised by the total number of farms. In 1990, state farms utilised 18. 7% of the whole of agricultural land (18. 9% of farms being used) and generated 77. 3% of global, 76. 3% of final, and 73. 1% of commodity agricultural production.

[2]After 1989, during system transformation, ownership transformation processes took place in Polish agriculture, as a result of which, among others, state farms were to be privatised. Pursuant to the Act of 19. 10. 1991 on the management of the agricultural property of the State Treasury (Journal of Laws of 1991, no. 107, item 465, as amended), state farms were eliminated and included in the Stock of the Agricultural Property of the Treasury. Furthermore, lands from the State Land Fund (SLF) and the ones purchased by the Agricultural Property Agency (APA) on the private market were also included in the Stock. In Poland, this Stock is managed by the state institution appointed on the grounds of the aforementioned Act – the Agricultural Property Agency (APA), until 2003 known as the Agricultural Property Agency of the State Treasury. As of entering into force the act on agricultural system development (2003), the activities of the Agricultural Property Agency refer not only to the state agricultural property market, but also to monitoring the transactions of transferring the ownership of agricultural property on the private market (Marks-Bielska, 2013).

Currently, the trade in agricultural properties in Poland is regulated by, among others, the act on agricultural system development (Journal of Laws of 2003, no. 64, item 592). The intention of the adopted provisions was to accelerate the land concentration process and to improve the agrarian structure (Marks-Bielska and Babuchowska, 2013).

In the private market the supply of land intended for sale is limited, both by the benefits resulting from features thereof (among others, receiving subsidies from EU funds, the possibility of cheap insurance provided by the Agricultural Social Insurance Fund (ASIF)[3], as well as the date of ending the period limiting the purchasing of land by foreigners – 1 May 2016, since in Poland, a negotiated 12-year, so-called, transitional period regarding a lack of possibilities of purchasing Polish land by foreigners without a permit from the Minister of the Interior was binding) (Sikorska, 2013; Marks-Bielska, 2014b).

The characteristic phenomenon in the agricultural land market comprises the difference in prices received by the Agricultural Property Agency (lower prices) and in the trade among farmers[4]. Land is sold by this state institution predominantly in the territory of northern and western Poland, where state land supply remains higher and, at the same time, the demand is lower, especially from individual farmers.

The price of the property of the Stock of the Agricultural Property of the State Treasury is set at an amount not lower than the value of this property, which is determined by an independent appraiser[5] selected through tender, on the grounds of local market research. The price of the agricultural land can also be set at an amount not lower than the total value of the land determined with a stipulation of the estimated rates of one hectare and the price of 100 kg of rye, in compliance with the provisions on agricultural tax and the values of these land components indicated by an appraiser. In practice, the valuation with estimated rates is used relatively rarely (Sikorska, 2013).

The starting price of properties sold through tender is set at an amount not lower than the value of a given property. Furthermore, costs incurred preparing the property for sale (e. g. costs related with collecting necessary documentation,

[3]Owners (users) of a farm can buy insurance in ASIF. This insurance is cheaper than insurance in the Social Insurance Fund (ZUS), covering social groups other than farmers. As a result, sometimes farm owners conducting additional (apart from agricultural) or basic (other than agricultural) economic activity benefit from insurance in ASIF.

[4]However, it should be underlined that this comparison has a limited cognitive value, as prices in the neighbourhood market refer to arable land, and in reference to the Treasury land, agricultural lands (arable land, grassland and waste lands) are taken into consideration. These are not identical categories.

[5]Methods from a comparative approach (comparison in the pairs method and the average price adjustment method) are most frequently used in the valuation of non-developed agricultural land included in the Stock of the Agricultural Property Agency. Both methods consider property features to have a significant impact on the value of agricultural property (Kurowska et al., 2014).

valuation costs, surveying works costs) are also taken into consideration (Kryszk et al. 2014).

The dynamics of land prices in 2014 in comparison with 2004 in Poland were higher in the state market (730. 00%) than in the private market (598. 65%). This may have resulted from boosting the sale of state lands and reluctance to divest land, which, apart from incomes from production, can also generate other incomes (e. g. related with common agricultural policy instruments), as well as an expected increase in prices after ending the transitional periodof the purchasing of agricultural properties by foreigners. An amendment to the act on agricultural system development(passed by the Seym of the Republic of Poland on 5 August 2015, signed by the President – 27. 08 2015, entered into force – 1 May 2016) is also of importance, as pursuant to this act it has been forbidden in Poland to sell agricultural land from the Stock of the Agricultural Property of the State Treasury for five years since the effective date of this legal act.

In the years 2003-2014 the biggest boost in agricultural land prices, both on the private and the state markets, was observed in 2004 in comparison with 2003 (on the private market 138. 61%, and on the state market – with the contribution of the Agricultural Property Agency – 150. 54%), as well as in 2008 in comparison with 2007. However, in this case the discrepancies between the private and the state markets were smaller than earlier (136. 89% on the private market and 138. 06 – on the state market) (fig. 1). Poland's accession to the EU (1 May 2004) and covering Polish agriculture with common agricultural policy instruments contributed, among others, to the increase in land prices in 2004.

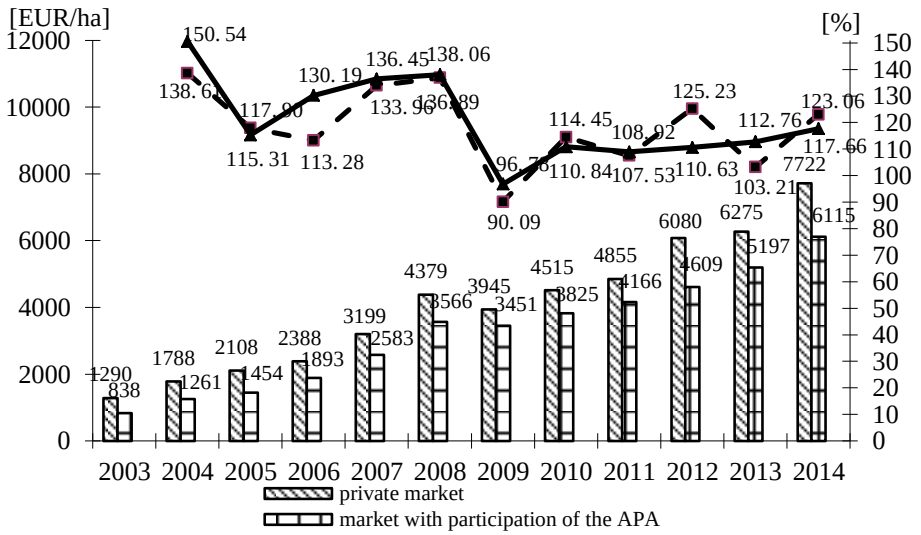

Fig. 1. Agricultural land prices on private and state markets (with the contribution of the Agricultural Property Agency – APA) and dynamics of prices on these markets [previous year = 100%]

**Source: authors' own elaboration on the grounds of GUS and EUROSTAT data.*

Results of the analysis conducted by Zawalińska (2011) showed that the common agricultural policy (joint pillars I and II) increases average prices of agricultural land by approximately 27%, in comparison with the situation that would exist if such a policy was non-existent. Pillar I causes an increase by approximately 21%, and pillar II by approximately 6%. Therefore, the impact of pillar I on the increase in the land prices is definitely higher than the one of pillar II. On one hand, it results from a difference in the form of granted support, and on the other, from the difference in the amount of support between pillars. In the case of pillar I, direct payments are higher and are granted in a form directly related with land, i. e. area subsidies per hectare within a uniform and supplementary area payment. In the case of pillar II, funds are paid in the form of different economic instruments, frequently not directly related with land, such as: investment subsidies (e. g. on modernisation activities), covering costs (e. g. of training, functioning of a production group), income transfers (e. g. structural rents) and only a part of activities is executed in a form of area payments (for areas of less favoured areas) (Milczarek-Andrzejewska and Zawalińska, 2015).

Due to the fact that the value of land in Poland is lower than in EU-15 states, it can be expected that (after ending the transitional period in the purchasing of agricultural land by foreigners) the availability of land for community citizens will contribute to increasing the price of land to the average price binding in the EU. The increase in the price of this basic production factor in agriculture that is intrinsically limited will also be affected by the forecasted increase in the demand for food (Marks-Bielska, 2014b).

In Poland, the private-family form of farm ownership dominates, which is expressed in, among others, passing on farms by way of donation, and a low tendency of sale, in particular in the case of gifted or inherited agricultural land. Intentions with regard to owned land concentrate around passing it to the next generation in the family. These facts strongly suggest the strong impact of informal institutions on the agricultural land market in Poland. This state of affairs is also affected by the historically based attitude of Polish farmers to land as a cultural and symbolic value.

A land purchaser, depending on his or her needs and preferences (e. g. land quality, location, future intention – cultivation, investment, construction) or expected increase in land prices (e. g. 2004 – Poland accession to EU, 2016 – the end of the transitional period for the purchasing of land properties by foreigners) is willing to allot a given amount to purchase a particular agricultural land plot. The price level, both in trade among farmers as well as the price of the land of the State Treasury managed by the Agricultural Property Agency, is primarily developed by the market, but it also depends on the instruments of the common agricultural policy and the national policy concerning this production factor.

The agricultural land market reflects the general macro-economic situation and structural changes in agriculture. The accession of Poland to the European Union and, therefore, the appearance of various forms of financial aid, including direct subsidies, the value of which depends on the size of a farm, contributed to an

increase in interest in purchasing agricultural land. Thus, the accession to the European Union was a special period in land price development in Poland determining this process.

The price of land can be decided by the type of rent expected by the owner or future buyer. Land rent is usually defined as extraordinary revenues constituting a difference between increased costs resulting from particular land features. These costs can also be related to agrotechnical procedures, conducted or intended investments, or other technical, economic and legal activities.

Agricultural land prices depend on many factors, among others, demand, the economic situation of farmers, the profitability of agricultural production, location, the size of plots, and the possibility of changing their use.

Agricultural land management is a significant component of agricultural policy as well as the development of rural areas. Despite the fact that a vast majority of the aforementioned stock in Poland is privately owned (predominantly by individual farmers), more and more often they become private-public good. The aims, directions and methods of managing agricultural land stocks are more and more frequently set not only from the point of view of land as a basic production factor in agriculture, but also as main values important for the whole society (natural, cultural and aesthetic values, etc.). The problem consists in combining an improvement in the effective use of agricultural land as a basic agricultural production factor, and land as a carrier of goods for the whole society. The success of this conception depends on higher remuneration for the execution of social aims. However, in order to effectively execute tasks within the use of stocks of agricultural land resulting from multifunctional agriculture, it is necessary to improve the education and skills of farmers in the scope of agricultural production, as well as regarding the execution of the aims of non-production farms (Marks-Bielska and Žukovskis, 2011).

Demand limitations in agricultural land trade will be influenced by decreasing stocks of land owned by the State Treasury. Purchase and sale transactions in this market segment will cover land purchase by previous leaseholders (this will be possible five years after the amendment of the act on agricultural system development entering into force, as of 1 May 2016), which, as an effect, will not significantly influence changes in agrarian structure.

Land in the economic dimension is a natural factor of a country's wealth, a production factor that is unique in location and structural meaning, a fixed and immovable asset, an object of value, which significantly influences legal provisions regulating trade in agricultural land. The aim of legislated legal acts in this scope is to, predominantly, conduct a relevant policy aimed at developing agrarian relations in a given country, in particular, desirable area structure.

An important component of these regulations is the supervision of purchase and sale transactions in which foreigners also participate. Factors that can limit their participation in the agricultural property market consist in provisions regarding, among others, the period and place of residence of a potential buyer of agricultural land, or his or her professional experience related to agriculture.

Factors determining agricultural land prices in Poland can be divided into two groups:

1. Market:
- supply and demand for agricultural land allocated to agricultural production;
- supply and demand for agricultural land that can be, in compliance with the binding law, allotted to purposes other than agricultural ones;
- simultaneous functioning of two market segments - private (on which a majority of agricultural land trading is conducted among farmers), as well as the state land market – the Stock of Agricultural Property of the State Treasury managed on behalf of the owner - the State Treasury, by a state institution – the Agricultural Property Agency;
- profitability of agricultural production.

2. Non-market:
- historical conditions - dominance of private property in Polish agriculture, both in the market economy and in the previous economic system;
- tradition, culture - passing farms from generation to generation;
- Poland's accession to the European Union and covering Polish farmers with common agricultural policy instruments;
- ending the transitional period of the purchasing of Polish agricultural properties by foreigners on 1 May 2016. After this period, the hitherto limitations (special permits) for foreigners in purchasing agricultural properties in Poland were lifted.

CONCLUSION

Identifying which factors are the most important and which are less important in Polish conditions is very difficult and requires in-depth research. Due to the specification of land (non-enlargeable, immovable, limited good), the State's intervention in the agricultural land market is necessary. Agricultural land is a multifunctional good constituting not only a basic factor of agricultural production, but also a natural, market and capital good (agricultural land price has been dynamically increasing); therefore, it is necessary to protect this stock and manage it reasonably.

On the grounds of the recent amendment of the act on agricultural system development that entered into force on 1 May 2016, in Poland state intervention in the agricultural land market is higher than previously. The introduced changes, concerning, among others: impeding the sale of properties included in the Stock of the Agricultural Property of the State Treasury for a period of 5 years from the entering into force of the aforementioned act, preferring leasing as a form of utilising the agricultural lands of the State Treasury, the pre-emptive right vested in the Agricultural Property Agency regarding both transactions in the private market (individual farmers), as well as stocks and shares in the commercial companies being the owners of agricultural properties (the pre-emptive right is also vested in the lessee being an individual farmer) were maintained in order to eliminate the possibility of speculative purchases of land. A ban was introduced on selling or

giving agricultural property into the possession of other entity without court consent for 10 years since the day of purchasing it (with the exception of the Agricultural Property Agency and local government units). Agricultural land can be purchased by individual farmers[6].

Upon the consent of the President of the Agricultural Property Agency expressed in an administrative decision, which is appealable to the Minister of Agriculture and Rural Development, agricultural properties can also be purchased by entities giving a guarantee of due performance of agricultural activity. The consent to purchase agricultural property can also be obtained by a natural person intending to start a family farm. In the case of a lack of consent to purchase agricultural property, the Agricultural Property Agency, upon the seller's application, is obliged to purchase this property at the market price.

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[6]Individual farmer – a person having agricultural qualifications, personally running a farm of an area not larger than 300 ha (family farm) and residing on this farm for at least several years, who is subject to farmers' social insurance in its full scope if the farm area exceeds 20 ha of agricultural land.

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THE EFFECT OF APPLIED CROSSBREEDING ON INCREASING OF MILK PRODUCTION IN SHEEP

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ABSTRACT

The aim of the research was to identify new methods to increase milk production in sheep. An evaluation of milk production at Țigaie local breed, in comparison with half-breed females from crossbreeding of local breed sheep with Awassi rams (Aw x Ti) and with Lacaune (La x Ti) was conducted. Estimation of milk production was done according to given by International Committee for Animal Recording (AT method) and statistical analysis was performed using the Restricted Maximum Likelihood method. Mean milk production in Aw x Tiewes was 29, 28% higher than in local breed Țigaie, and 32. 20% higher than in La x Ti ewes. Differences of milk yield found in three groups of animals were very significant ($p<0.001$). Based on obtained results it could be concluded that crossbreeding of Țigaie with Awassi breed rams had a better effect on increasing of milk production than crossbreeding with Lacaune breed rams.

Key words: *Țigaie sheep, milk yield, crossbreeding.*

INTRODUCTION

In the year 2014, Romania was situated in second place in Europe in sheep milk production (MADR, 2015). This placement, after Greece, is due to the attention that is offered to milk production of most sheep breeders in this country. The support of this production is due to a high demand, from both national and international market, for traditional products made of sheep milk, but also for other sorts of products, fresh or seasoned. Taking into account these trends, since 2008 in Romania have been applied various technical measures to support a further growth for this product. The action plan had a positive effect and, thus, currently in Romania, there are produced more than 18000 tons of sheep milk per year (INSSE, 2014). After 2012, given the high demand of the local and international markets towards quality cheeses, in Romania, the sheep and goat breeders organize producers' groups with the purpose of obtaining high-quality cheeses, especially

those with Protected Designation of Origin and of Protected Geographical Indication (Pascal, 2015; Gîlcă et al., 2009).

Because the local breeds of sheep, which own the share in live stock, are not characterized by productive high performance, in many holdings in which they grow sheep for milk, have been commissioned various works of improvement. In this process of improvement were also included the main local breeds, namely the Tigaie and Turcana, which together own 74% of the total number of sheep from Romania, but both have average milk production of 140 l (Pascal, 2014; Padeanu 2010; Tafta, 1996). To speed up the improvement process were developed more crossbreeding schemes that use genitors belonging to breeds with superior performance which have proven that alleviates significantly the main characters of the production specific to sheep.

MATERIAL AND METHODS

The main objective of the research was to evaluate the effect due to the possibility of improvement of crossing local Tigaie sheep with Lacaune and Awassirams. In this regard, have been set up in batches that included lactating females and which belonged to the following working groups: a control group (M) consisting of sheep that belonged to local race Țigaie and two other lots L1 and L2 consisting of crossbreed females F1, within the first lactation, resulting from crossing local sheep breed with Awassi rams (L1) and with Lacaune rams (L2). Each formed batch included 25 females belonging to that group.

The performance evaluation for milk production resulted in the analyzed lactation was based on the application of successive periodic inspections, and using for the lactation period the Nica method and for the milking period was exclusively applied the method AT_4in compliance with the technical specifications suggested by International Committee for Animal Recording.

Estimation of the average total production of milk was carried out using the Fleischmann method.

$$\text{Milk yield [kg]} = \mathrm{L}_1.\mathrm{int}_1 + \sum_{i=2an} \left(\frac{\mathrm{L}_i + \mathrm{L}_{i-1}}{2}.\mathrm{int}_i\right) + \mathrm{L}_n.14$$

where:

L_1 = milk yield of the 1st monthly test;

L_i = milk yield of the i^{th} monthly test (i = 1, . . .,n);

L_n = milk yield of the last test;

int_1 = number of days from kidding to 1st monthly test;

int_i= number of days between monthly tests (i-1) and i (i = 1, . . .,n);

n = total number of monthly test for a specific animal.

Data were statistically evaluated with the algorithm REML (REstricted Maximum Likelihood), which provides the achievements of the statistical parametric estimators within the normal range.

RESULTS AND DISCUSSION

To meet the real requirements of the market, from 2007, in Romania there have been started several activities to improve milk production in sheep. One of the routes concerned was represented by the crossings applied between local ewes and rams belonging to breeds that have the quality to improve the characters on which milk production relies on (Calin, 2010). Based on these principles, introduction into the productive circuit of some hybrid female populations was a technical action that highly contributed to the increased total milk yield obtained from this species.

The researches were conducted under an experimental plan in which have been included specific objectives to increase the productive performance in sheep. The option for an inclusion in the scheme of crossing local sheep breed Ţigaie was motivated by the fact that it currently holds roughly over 40% of the total number of sheep from Romania (Pascal et al. 2014) and is found in different farms both in the lowland and hilly areas, as well as in the depression, foothills and mountain areas.

The motivation of research was represented by initiating some actions in order to find some quick methods under which, by extension, to increase the total production of milk. Whereas the local breed Ţigaia emerged naturally from the wild form *Ovisvigneiarkar* (Drăgănescu, 1995) and is semi-late, the breed potential for milk production obtained during the interval of lactation is about 135 kg (Pascal 2007, 2015). That is why through the experimental protocol it was initiated a research that had intended to check new methods, more efficient, but also faster, to improve productive performance. In this case we opted out to apply improvement crossings between local Ţigaie sheep and male genitors belonging to Awassi and Lacaune breeds.

The usage of Awassi and Lacaune rams for crossbreeding was motivated by the result of previous studies carried out in Romania (Atanasiu et al.,2010, Călin et al.,2010, Pădeanu et al.,2010) and other countries (Espetin 1985, De la Fuente et al.,2006, Pacinovski et al.,2007). During the lactation subjected to the productive control, the three groups have benefited from similar conditions of maintenance and feeding. In table 1 there are presented the average total milk production obtained from each group of ewes.

Table 1. Total average milk production obtained in the controlled lactation (kg)

Specification		M	L1	L2
n		25	25	25
$\bar{x}$		101. 75	141. 72	97. 71
$\pm s_{\bar{x}}$		1. 29	1. 61	3. 57
V %		14. 43	13. 34	13. 09
s		14. 244	16. 23	14. 35
Limits	Minimum	74. 25	98. 50	86. 30
	Maximum	138. 5	187. 0	165. 5

Difference and its signification for the average total milk production

Batches	Difference ±	Statistical significance of the difference
M – L1	-39. 97	*** - F (403. 6698) > Fα (11. 0849) for 0. 001
L1 – L2	+ 44. 01	*** - F (88. 2851) > Fα (11. 3487) for 0. 001
L2 - M	-4. 04	N. S. - F (0. 8958) < Fα (3. 9034) for 0. 05

Contrary to all expectations, after obtaining the result of data processing it was found that the lowest productive level in all 200 days of lactation was 97. 71 kg, and resulted after the control made to the half breeds females which formed group 2. The L1 group achieved superior performance to the other two groups. Thus, the average level of the total production of milk made by females of this group was higherby28. 20% compared to the performance of the M groupand by31. 05% than L2 group.

This indicates that local Ţigaie breed reacted better to the crossing with Awassi, substantiating features of a high degree of genetics combinability. The L2 group realized reduced performances because the females of this group were more sensitive to microclimate factors. Based on this observation isconclude thatLacaune breed for crossbreeding should be used only for some infusion crossings to improve the productive performance in certain herds of sheep.

Differences in absolute values are presented in table 2 and have been of 44. 01 kg of milk between groups L1 and L2, and were very significant (P< 0. 001).

The performance of this group was well below expectations, being lower for 31% in relation to production level achieved by L1 ewes and for 3. 97% compared with control group (fig. 1).

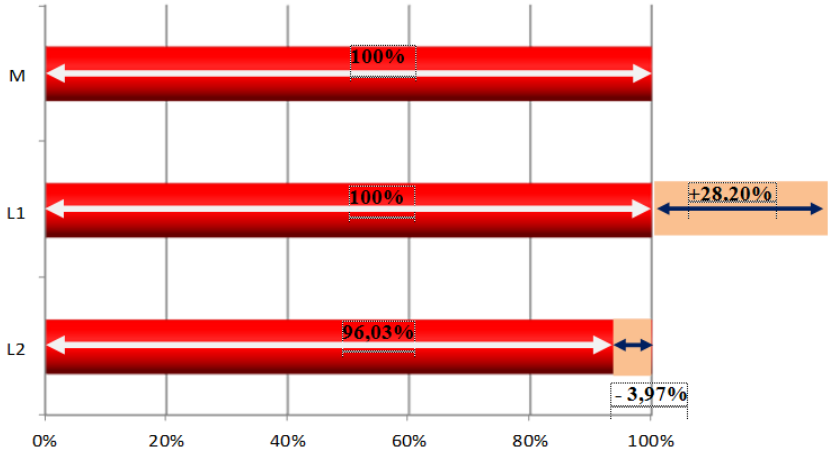

Figure 1. Graphical representation of the differences between groups for productive performance recorded in the controlled lactation

Average variability requires, as a necessity, further selection work and improvement of the three populations in increasing milk production.

The overall mean values assessed during the research for the total average production of milk obtained from Ţigaie sheep are close to the values mentioned by the specialized literature in Romania, quoted by various authors (Pascal, 2007; Taftă et al. 1996).

CONCLUSION

The use of crossbreeding to improve milk production in sheep has proven to be a good solution, which enables the growth performance of time for milk production in sheep, to be reduced considerably compared to the application of the enhanced performance of the pure breed.

Using the Awassi breed rams for crossbreeding was beneficial and has increased the average production of milk obtained in the 205 days of lactation for 28% compared to pure Ţigaie ewes.

Regarding the Lacaune breed, due to the fact that crossbred ewe shad a lower average milk production by about 4%, compared to pure-breed Ţigaie sheep, it is recommended that the rams of this breed to be used, in particular, at infusion crossings.

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CEREALS SEED LIFECYCLE

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ABSTRACT

Cereals seed lifecycle is considered as duration of a variety commercial use, or period of having margin from variety market introduction till its withdrawal from the market. In most of cases, it coincides with average period of variety listing. But available National Registers includes list of approved varieties, and don't keep tracks about withdrawal ones. The article goal was to check author's methodology of calculation of actual cereals seed lifecycle. National Registers of Commonwealth of Independent State (CIS) countries (Belarus, Kazakhstan, Russia, Ukraine) compare to German Bundesortenamt were used as data base. It was assumed thatnumber of varieties, been listing within five years period, is constant; therefore share of listed varieties in older groups shows probability of a variety listing within any group. It was found, that winter wheat seed commercialization period lasts about 24 years in Kazakhstan, 14 in Russia, 13 in Germany, 12in Ukraine and 9in Belarus. Similar trends are calculated in other cereals (spring oat, spring wheat, spring barley, and winter rye). The higher yields are, the shorter variety lifecycle, the better seed exchange rate (certified seeds share), and even return of breeding investments. Seed lifecycle is under influence of such factors as average crop multiplication rate, seeding rate, crop margin, yields, operational risks, acreage, etc. Markets of high marginal crops with shorter seed lifecycle are attractive for international seed companies expansion.

Keywords: *CIS, national register, cereals, listing, seed lifecycle.*

INTRODUCTION

The seeds business is reasonable to explore from the standpoint of marketing, i. e. as the lifecycle length of a product. The actual length of a product, or seed lifecycle is considered as duration of its commercial use, or period of having margin from a product market introduction (a variety registration) till its withdrawal from the market. Actually, it is length of listing an average variety period.

The shorter is lifecycle of a product, the faster market growth is, especially in the conditions of high competition on the seeds market, high input technologies, and profitable crop production. New generations of varieties with better adding value (greater yield potential, better quality and adaptation) are able to increase the profitability of the whole value chain from breeder till processor.

Fairness of the variety registration, patent protection and complicated certification have a direct impact on the pace of varieties flow in the National register and the duration of their lifecycle. The CIS countries and the EU ones have common approach to the protection of intellectual property of seeds due to UPOV rules, and the National registers populating schemes.
Comparison of lifecycle duration between field crops explains some details of crop competitiveness, attractiveness of markets for seed and other commercial products.

MATERIALS AND METHODS

Updated data of the National Register of Belarus (BY), Germany (DE), Kazakhstan (KZ), Russian Federation (RU), and Ukraine(UA) of cereals in 2015 [National Register BY, 2015; Beschreibende Sortenliste. 2015; National Register KZ, 2015; National Register RU, 2015; National Register UA, 2015], been interpreted with the author's method of variety life cycle calculation (Goncharov, 2013) served as source for initial data. Also, official statistics of Russia and USDA, results of author's market researches, and market assumptions are used.

RESULTS AND DISCUSSION

The National Register of breeding achievements admitted for use contains the list of registered varieties in the year of its publication. New registered varieties entered there, leaving ones from the market are excluded.
There were 296 winter wheat varieties from by 60 breeders are listed in the National Register of Russia in 2015. Direct count of varieties listed annually shows a dynamic range, which is insufficient for a variety lifecycle calculation (Figure 1). It is possible to count the number of varieties listed for example by 2001 in updated National Register, but it is unknown number of withdrawn ones, as tracks are not available.

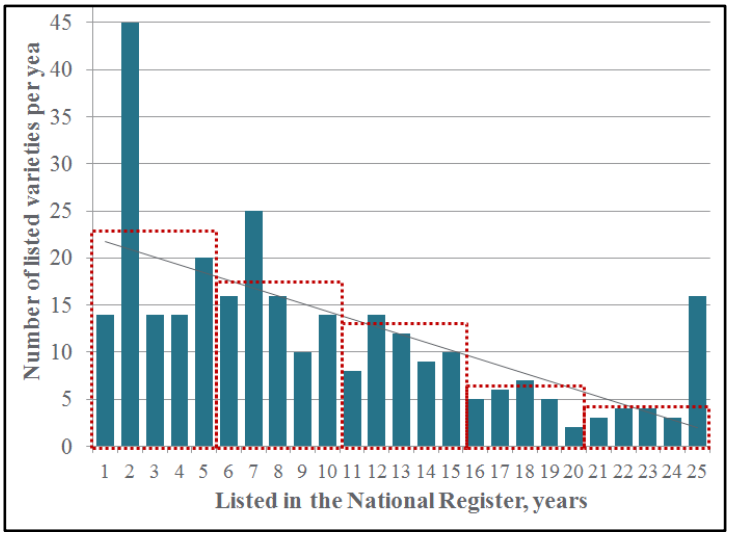

Fig. 1. Dynamic row of winter wheat varieties number with period of their listing

The oldest variety is Bezostaya 1, listed in 1959, Mironovskaya 808 (1963), and Mironovskaya Yubilejnaya (1970) were commercialized for 57, 52, and 46 years correspondingly. But they are exceptionally old; the majority of varieties listed much shorter. That period of time depends on legal patent expiration.

Let's make the assumption that, the number of varieties, listed within five years, is constant, and combines varieties over the past 30 years, released during the five-year period of time in the group. There are 36% listed varieties as "new" ones; they are up to 5 years old; 27% are commercialized within 6-10 years, 18% are used within 11-15 years and so on (Table 1).

Table 1. Calculation of a variety life cycle on winter wheat example, based on the National Register of Russian Federation, 2015

Period of varieties registration, years	Varieties number per group	Share of every group, %	Relative share compare to group≤5 years	Populating in the group for a variety, years
≤5	107	36%	100%	5. 0
6-10	81	27%	76%	3. 8
11-15	53	18%	50%	2. 5
16-20	25	8%	23%	1. 2
20-25	17	6%	16%	0. 8
≥26	13	4%	12%	0. 6
Total	296	100%	-	13. 8

It is logically, that all varieties not older 5 years will be listed in the National Register all 5 years long period. The varieties of the group 6-10 years old will be listed all the 5-years long period with probability 76%, or their input in life cycle is 3. 8 years (5 years x 76%)(Figure 2). The varieties from the group 11-15 years old will be listed all 4 years period with probability 50%, or their input in life cycle is 2. 5 years. Adding all probabilities will receive average life cycle of winter wheat variety in Russia (14 years). Similar procedures with other cereals varieties listed in the National registers demonstrate wide range of their commercial cerealslifecycles (Figure 3) in Russian Federation.

Spring oat is a crop with the longest variety life cycle (25 years) in Russia compare with spring durum wheat for example. Oatacreagedomains in ornamental regions with low-input conditions (Middle Volga, Western and Eastern Siberia). Climate conditions have stronger effect on crop performance there, compare to genetics. Winter durum wheat is comparatively new crop at the introduction stage of the grain market without significant acreage. Therefore, winter durum varieties have been listed only last 15 years only in this country.

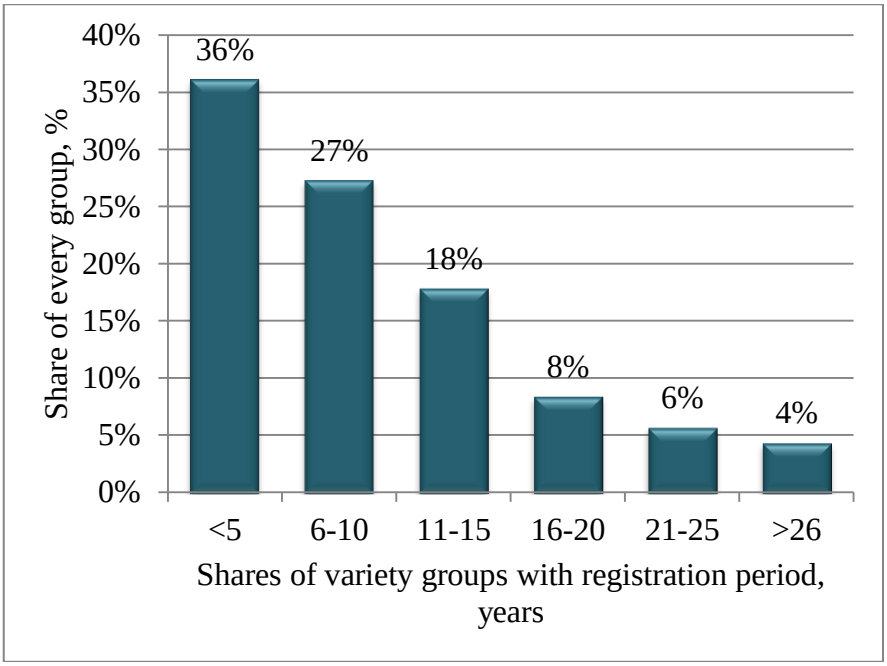

Fig. 2. Groups of winter wheat varieties with different period of their listing in the National Register

Acreage per crop, and acreage per variety in particular are other reasons for difference in lifecycles. Calculated by us cereal seed lifecycles are close to the results of Dr. Khalipsky assessment, who has reported about "length of registration period" such crops as winter rye (22. 6 years), oat (18. 1), common wheat (13. 6), and barley (12. 9) in Siberian part of country (Khalipsky, 2009).

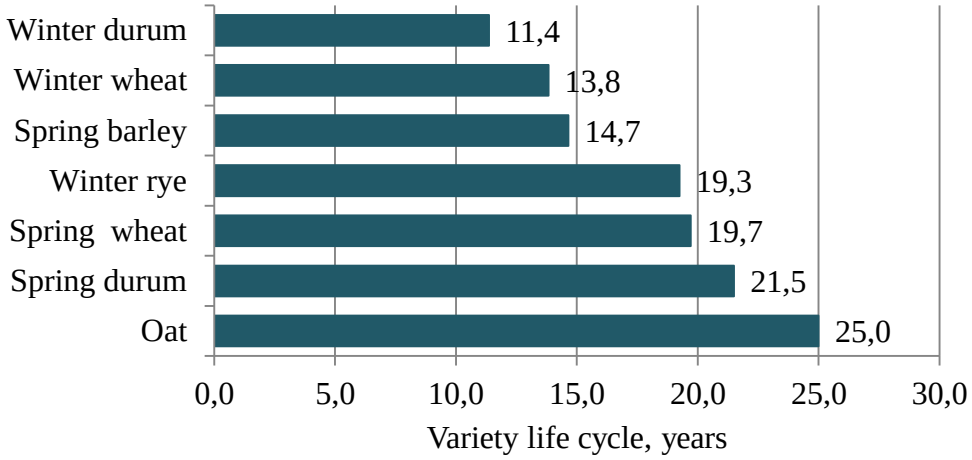

Fig. 3. Variety life cycle of cereals varieties in Russian Federation, 2015.

Calculated cereals seed lifecycle in other countries based on their national registers, showed, that periods of variety commercialization are longer in Russia and Kazakhstan, compare to Ukraine, Belorussia, and Germany (Figure 4). Surprisingly, seed lifecycles in last three countries are similar by duration, what's contradicts common opinion about faster seed commercialization rate in EU. For example, spring barley lifecycles have the same duration there (about 10 years) in DE, BY, and UA. There is no winter rye acreage in Kazakhstan, that's why no records there.

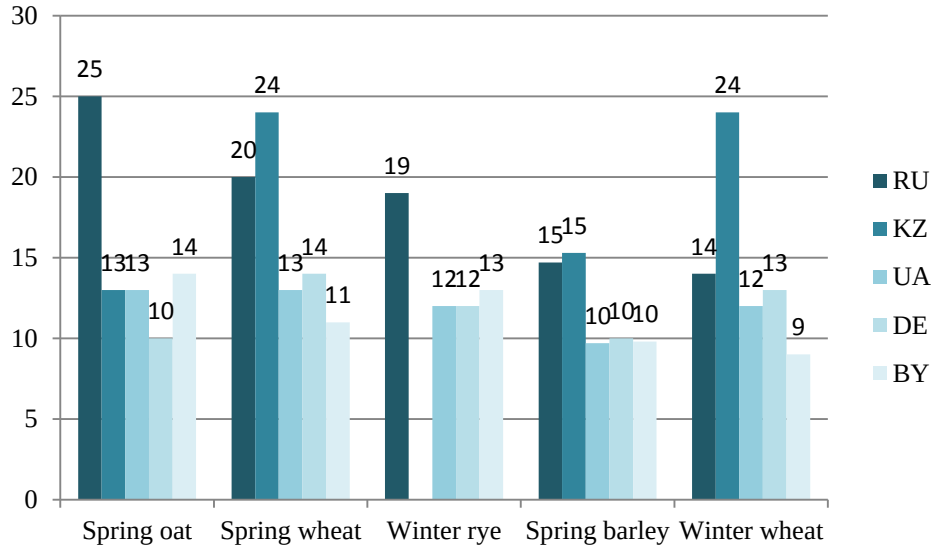

Fig. 4. Seed life cycles in CIS and Germany, 2015.

Spring wheat lifecycle in Kazakhstan lasts 24 years, what is more, thenin Russian Federation (20 years), in Germany (14 years), Ukraine and Belarus (13 and 11 years correspondingly). But spring wheat acreage in Russia and Kazakhstan both is about 24 million hectares, what is insignificantly more compare to Germany, Ukraine and Belarus all together. There is no actual spring wheat breeding in Germany, but only facultative one, as it is insured crop to mitigate risks of winter kill. In the Republic of Kazakhstan variety of winter wheat on the average "lives" for 24 years, since there are about planted 200 thousand hectares of the crop only. Winter wheat lifecycle in Russia lasts 14 years, what is close to the performance of Germany (13 years) with acreage of 3. 2 million ha, Ukraine (12 years) with 8 million hectares and Belarus (9 years) with 0. 5 million ha.

Malting barley market has strong impact on reduction of seed lifecycle. Downstream demand of malting barley commodity from approved varieties leads by malteries, and breweries.

Seed lifecycleis affected by a number of factors, among them the most important are crop input, seeds exchange rate, planting rate, seed prices, and generations offered for sale, crop margin, and others (Figure 5).

Markets of high marginal crops with shorter seed lifecycle are attractive for international seed companies expansion. German seed companies (KWS, Saaten-Union, DSV etc.) extend registration of their varieties to CIS countries. Cereals varieties flows come from Germany (country with advanced breeding programs) to CIS, but it is not happened in opposite direction (Figure 6).

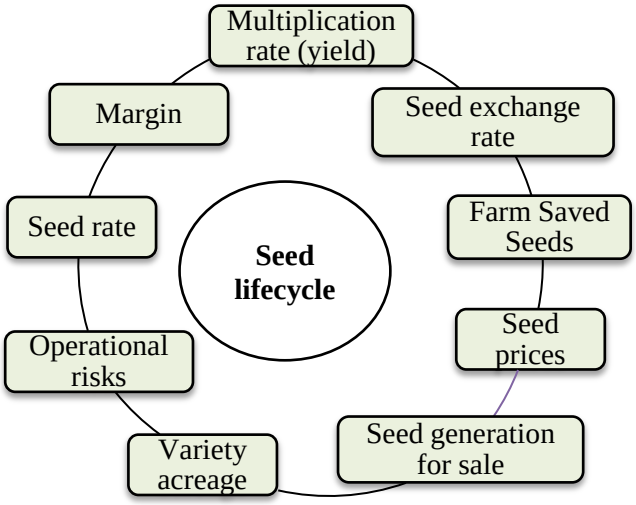

Fig. 5 Factors with the biggest affect to cereals seed lifecycle

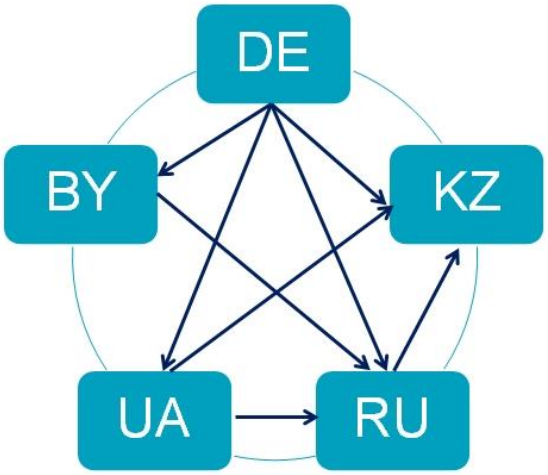

Fig. 6 Cereals seeds flow directions

In common cereals are losing competition with oil seeds, corn, sugar beet, and other crops with higher margin. But from point of crop ratio, cereals acreage will not be reduced radically, and will be stabilized, especially in “row materials zones” close to grain markets.

CONCLUSIONS

Cereals seed lifecycle takes from 9 years (winter wheat in BY) till 25 ones (spring oat in RU). It is shorter in Belorussia, Germany, Ukraine compare to Russia and Kazakhstan. Cereals with higher input (winter wheat and spring barley) have shorter seed lifecycle compare to winter rye, spring wheat, and spring oat.

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LEVEL OF LARVAL ATACK ON MAIZE ROOTS AS A CONSEQUENCE OF ARTIFICIAL INFESTATION WITH WESTERN CORN ROOTWORM EGGS

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ABSTRACT

The Western corn rootworm (WCR), *Diabrotica virgifera* sp. *virgifera* (Col.,Chrysomelidae), is an oligophagous pest native in America. WCR is a maize pest present in all regions of the Corn Belt. It is an invasive species which was, in Europe, first identified in Serbia, in 1992, near the Belgrade airport. The presence of this pest in maize field can cause losses and plant damages up to 100%. A field experiment was carried out in Bečej, Vojvodina province (Serbia), during 2014 and 2015. In the field, 96 plants (maize cultivar NS 640), arranged in 48 pairs were selected. Each pair consisted of one plant artificially infested with WCR eggs (D plant) and the control plant (C plant). In both years, the experiment in the field was regularly inspected, once a week. During each observation, the presence of "goose neck" symptoms was recorded, and the number of plants damaged by the most important stem boring and leaf feeding insects (*Ostrinia nubilalis*, *Helicoverpa armigera*, *H. zeae*, aphids, mites, cicadas, etc.) was counted. Root damages were assessed at the end of the experiment (September), according to Ostlie and Notzel (1987), on scale 1-6. Comparing the root damages on C and D plants, less root damages were established on C plants. Only six i. e. four D plants had healthy roots (rate 1) during 2014 and 2015, respectively. Between D plants in 2014, the most damaged were14 plants, with the rate 3 (least one root chewed to within 1½ inches (3. 8 cm) of the plant). In 2015, severe damages were registered on 18 plants, which were ranked as level 6 (with three or more nodes destroyed). Only two C plants during vegetation 2014-2015were registered with damages with rate 5 (two nodes destroyed) and rate 6 (three or more nodes destroyed), respectively.

Key words:*Maize, WCR, Artificial infestation, Root inspection, Damages.*

INTRODUCTION

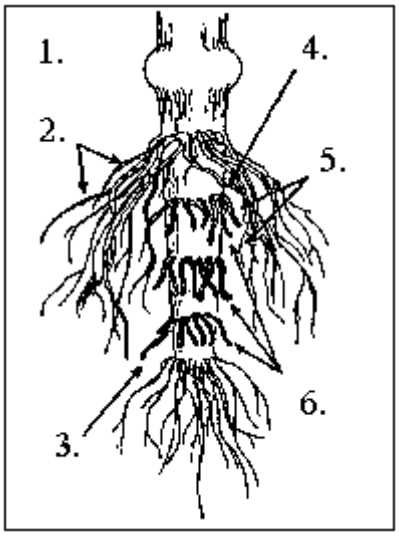
Western Corn Rootworm (WCR) *Diabrotica virgifera* sp. *virgifera* Le Conte (Col.,Chrysomelidae), is economically important Europeanmaize pest, native to America (Tollefson, 2007). It is an oligophagous pest, firstlyfound in Serbia (Europe) near the Belgrade airport, in the early '90 (Bača, 1993). After the Serbian findings, this pest spread to almost every maize field in Europe, causing serious damages on plants and reducing yield (Hummel *et al*., 2008). The WCR can spreadup to 100 km per year (Baufeld and Enzian, 2001). The pest has one generation per year (Bača, 1993) and severely damages roots and above-ground parts of maize (James *et al*., 2005; Ciobanu *et al*., 2009). Damages caused by WCR larvae feeding on the root are more serious than the once caused byadults, that feed on young leaves until the appearance of maize silk (Wesseler and Fall, 2010). Due tolarval damages ofthe root system, waterand mineral nutrientsuptake by maize plants is impeded (Chiang, 1973; Kahler *et al*., 1985). WCR larvae cause plant lodging after feeding on the nodal and lateral roots (Gavlovski *et al*.,1992) and also leadto yield losses (Tollefson, 2007). The appearance of a symptom called "goose neck" in the field indicates the presence of WCR (Wesseler and Fall, 2010). Well-developed root system, plant logging and the amount of secondary rootsare the indicators of maize tolerance to WCR (Gray *et al*., 1998). The robust root system represents a measure of high tolerance of maize to WCR roots injury that also occurs in the form of decreased lodging and increased root system size (Riedell and Evenson, 1993). Damages of WCR larvae are highly depended on environmental conditions, soil moisture, the type of soil andthe larval abundance in soil (Spike and Tollefson, 1989; Ciobanu, 2009).

MATERIAL AND METHODS

The field experiment was carried out in municipality of Bečej located in province of Vojvodina in the Northern Serbia, during 2014 and 2015. In 2014, the experiment lasted from 2nd June until the 19th September. In 2015, it was performed from May 30th to September 10th, with Serbian cultivar NS-640. The field chosen for the experiment represents a filed with the low natural WCR infestation. During the experiment, 96 maize plants were selected, labelled and arranged into pairs. The plants were in two rows with a space of 1 m, between the labelled plants. In each pair, one plant was artificially infested in the root zone withmL of WCR eggs in 0. 125% agar suspension (D plants). One mL of suspension contains 136 WCR eggs. The other plant from the pair was the control plant (C). In the root zone of C plant, the same amount of distilled water (4 mL) was injected.

In the both years, the experiment in the field was regularly inspected, once a week. During each observation, the presence of "goose neck" symptoms was recorded, and the number of plants damaged by the most important stem boring and leaf feeding insects (*Ostrinia nubilalis*, *Helicoverpa armigera*, *H. zeae*, aphids, mites, cicadas) was counted.

During the last field inspection on September 19th, 2014 and September 10th 2015, the damages of maize roots, caused by WCR larvae were evaluated. The root inspection was conducted in the following way: all marked plants were dug out, and the soil was removed from roots and after, the roots were rinsed. After the



Root rating scale (Ostlie and Notzel, 1987)

1 - No feeding damage
2 - Visible feeding scars present
3 - At least one root chewed to within 1. 1/2 inches of plant
4 - One entire node of roots destroyed
5 - Two nodes destroyed
6 - Three or more nodes destroyed

preparation, root damage was ranked from 1 to 6, according to scale by Ostlie and Notzel (1987).

Picture 1. Root damage scale (Ostlie and Notzel, 1987)

The differences between damages on D and C plants, based on the rate were analyzed using non-parametric Mann-Whitney test (Z).

RESULTS AND DISCUSSION

The results in 2014 indicate that, based on the injury rate more, severe root damages were recorded onD plants, compared to the control (C)(Figure 1). From the total number of 48 D plants, only six (12. 5%), were with healthy root systems (rate 1). With visible rootdamages (rate 2) were two D plants or 4. 2 %. The most of damaged were 14 plants out of 48 D (37. 5%), rated as damage level 3 (at least one root chewed to within 3. 8 cm (1½ inches) of the plant). With one entire node destroyed (rate 4), ten plants were recorded, or 20. 8%. Eight plants (16. 7%) were rated as level 5 (with two nodes destroyed) and only seven D plants (14. 6%) had the highest root damages (rate 6).

On the other hand, from the total of 48 C plants, 46 plants (95. 8%) were with the healthy root system and only two (4. 2%) with two nodes destroyed, rate 5 (Figure 1).

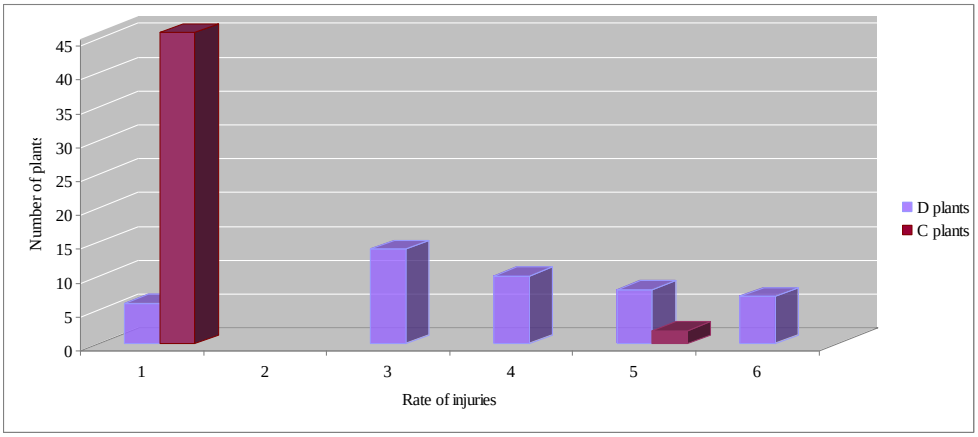


Figure 1. The level of root damages on D and C plants according to traditional scale (Ostlie and Notzel 1987) in 2014

In 2015, the number of D plants damaged by *H. armigera* was eleven and seven C plants, while the *O. nub*ilalis damages were registered on only one C plant. The damages from adult WCR were recorded on nine D plants and only on one C plant.
The obtained results in 2015 (Figure 2) were completely different compared to 2014. From a total of 48 C plants, 25 i. e. 52. 1% were with healthy root systems (rate 1). Visible damages causes by larvae (rate 2) were registered on three C plants (6. 25%). Rate 3 (with at least one root chewed to within 3. 8 cm (1½ inches)) were on nine plants or 18. 75%. Only one plant (2. 1%) was with one entire node destroyed, rate 4. Eight plants or 16. 7% were rated as the level of damage 5, and only two C plants (4. 2%) were with three or more nodes destroyed (rate 6).
The most of the infested D plants, 18 specimens (37. 5%) were with the highest root damages (rate 6). From the total number of infested plants, 11 plants (22. 9%) were with one entire node destroyed (rate 4). Seven plants (14. 6%) were with at least one root chewed to within 3. 8 cm (1½ inches) of the plant (rate 3). With visible damages caused by larvae (rate 2) wereeight plants or 16. 7%. Only fourD plants (8. 3%) from 48 plants were with healthy root system (rate 1). D plants with two nodes destroyed, rate five were not recorded in the experimental field (Figure 2).
The difference between the level of root damages on D and C plants was highly significant during the last observation ($Z=4.85^{**}$, $p<0.01$).

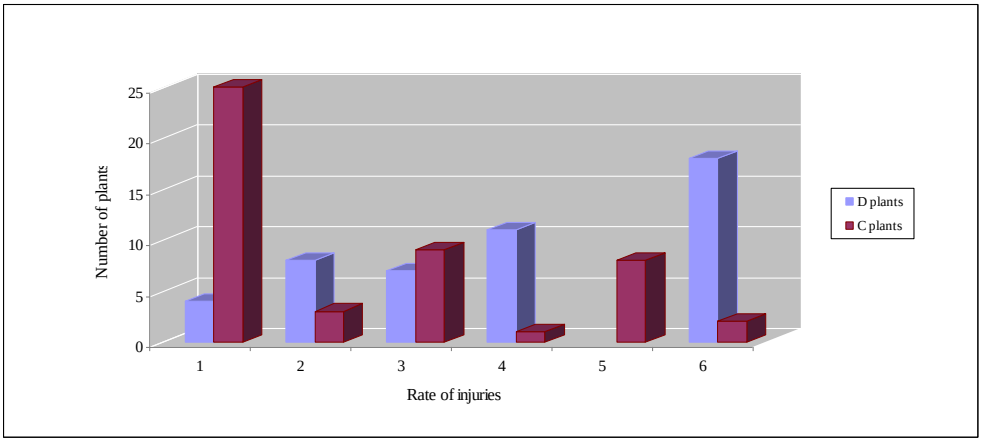


Figure 2. The level of root damages on D and C plants according to traditional scale (Ostlie and Notzel 1987) in 2015

During 2014 and 2015, WCR infestation caused damages on D plants, withthe different rate of root injury, 85% and 95. 7% respectively. These results show the higher level of damages on D plants in 2015 by10. 7%. From the total of 48 C plants in 2014 and 2015, the presence of WCR larvae caused different root injuries, 4. 2% and 47. 9% respectively. Larval presence in soil can cause different root damages as a consequence of different climatic condition or soil structure (Ciobanu, 2009). These results point to the higher level of damaged roots in 2015 on C plants (43. 7%).

In the field weekly observed the influence of WCR presence on appearance and damages of stem borers and leaf feeders. In 2014, the number of damaged D plants by *H. armigera* was 12, and of C was eight, while the damages from *O. nubilalis* were recorded on five D and four C plants. In 2015, the number of D plants damaged by *H. armigera* was11 and seven C plants, while the *O. nub*ilalis damages were registered on only one C plant. The damages from adult WCR were recorded on nine D plants and only on one C plant. It represents first parallel monitoring of presence stem borers, and leaf feeders in the WCR infested field.

During 2014 and 2015, the percent of C plants with healthy root system was 95. 8% and 52% respectively. These results show that the number of plants with no root injuries was by 43. 8% higher in 2014. Small larval mobility, different climatic conditions (vegetation period in 2015 was arid with a high number of tropic days) can be one of the reasons for the higher WCR population and increased damages on maize roots(Spike and Tollefson, 1989; Bača, 1998 Ciobanu, 2009).

According to the literature data, larval mobility is less than 50 cm (Bača, 1998), and these results also indicate that there is no rule of movement WCR larvae in the soil. The level of damages caused by the presence of WCR larvae in maize monoculture can increase the percent of lodged plants from 3% to 15% (Čamprag *et al*., 1997) and yield lossescaused by the lodging of plants, up to 75% (Bača *et al*., 1998). In their researches, Spike and Tollefson (1989) also point out that larval

presence in maize field leads to a decrease in yield. The same authors (1989) indicate that larvae causemore severe root injuries then adults on maize silk. Monoculture in maize field represents one of the main reasons for the increase in WCR population and contributes to bigger plant damages and root injuries (Sivcev *et al*., 2009; Chiang *et al*., 1969). The maize is a plant with a high ability to recover, and maize root tolerance is associated with its capability to grow new roots after larval injury (Gray *et al*., 1998).

In further research, the yield and the damages on maize plants, caused by larvae and adults of WCR will be registered.

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GERMINATION CHARACTERISTICS OF TEDERA (BITUMINARIA BITUMINOSA VAR. BITUMINOSA)

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ABSTRACT

This study was conducted to evaluate the germination response of tedera seeds (*Bituminaria bituminosa* var. *bituminosa* C. H Stirt) to water submersion t. Tedera is a perennial legume considered as a potential new pasture for Mediterranean, African and arid areas and traditionally used as forage for ruminants. It is characterized by deep-rootedness, drought tolerance, high protein content and nutritive value, offering elevate interest in animal nutrition. The experiment was undertaken from October 2015 with freshly harvested seeds collected by hand on natural populations from Gran Canaria, Canary Islands. Seed weight of tedera was compared using four replicates of 50 seeds. The seeds were divided into two groups (two replicates each group) and submerged on ambient temperature water during 16 or 24 hours each group. The mean seed weight was 23mg and it germinates in temperatures ranging from 12°C to 20°C. Tedera have no germination requirement for light. Tedera showed radicle protrusionfrom 4-8 cm with no significant difference between groups. However, the highest percentage of seed germination was achieved in the 24 hours group (65%) compared to the 16 hours group (27%), which showed a significant decrease ($p<0, 05$) in the percentage of seed germination. This study demonstrated that submersion during affected the *Bituminaria bituminosa* var. *bituminosa* seed germination and suggesting that tedera is a promising species for direct seeding. The results suggest that additional experiments are required for direct seeding.

Key words: *Bituminaria bituminosa*, seed germination, water submersion, direct seeding.

INTRODUCTION

Tedera (*Bituminaria bituminosa* L. C. H. Stirton) is a legume widely distributed in African and Mediterranean areas (Real et al., 2009) that can endure drastic conditions, contaminated and degraded soils. Tedera, traditionally used for feeding ruminants in the Canary Islands, is yet a promising legume as a feed for livestock (Sternberg et al., 2006). *Bituminaria bituminosa* L. presents 3 botanical varieties (*bituminosa*, *albomarginata* and *crassiuscula*). *Bituminaria bituminosa var.*

*bituminosa*has a wide adaptation across the Canary Islands and is the only one present in the Mediterranean basin. Characterized by deep-rootedness, drought tolerance, high protein and nutritive value, tedera offering elevates interest for low-rainfall or arid areas (Foster et al., 2015; Ventura et al., 2004; 2009; Pecetti et al., 2016). The domestication of *B. bituminosa* will require producing seed at the lowest cost. To achieve this objective, it is necessary to study on germination capacity of the seeds (Correal et al., 2008). Temperature and light are key factors that influence seed dormancy and germination. Temperature is the most important factor affecting the breaking of dormancy and seed germination (Baskin and Baskin, 1998; Cochrane and Probert, 2006; Mott, 1974), whereas light tends to stimulate germination (Vleeshouwers et al., 1995). Species such as lucerne must be sown at 5-10 mm for best establishment (Moore et al., 2006). The aim of this project was to study germination requirements of tedera seed and identify any potential constraints to its establishment. Such information is crucial to enable this new species to be sown commercially.

MATERIALS AND METHODS

Tedera seeds were collected by hand from natural populations of Gran Canaria, Canary Islands, on 12 October 2015. To investigate water submersion effect on tedera seed germination a completely randomized design with 2 replications was used. Studied factor included duration of water submersion: 16 and 24 hours. Tedera seeds were submerged on ambient temperature water during 16 and 24 hours. Then submerged seeds were germinated on filter paper moistened with distilled water in sterilized petri dishes. After 6 days, under the same conditions, temperature and UV protection, final germination percentage and germination rate were calculated. Seeds were classified as germinated if there was a protrusion of the radicle through the seed coat.

Statistical analysis

The germination was calculated according to the following formula;

Germination (%) = Number of germinating seeds/number of viable seeds initiated x 100

The data were tested at 5% level of significance to interpret the significant findings. Thedata were compoundedfor analysis of variance(ANOVA) by software StatPlus (AnalystSoft Inc.,2015). The percentage data fromthe different germination tests were arcsinetransformed.

RESULTS AND DISCUSSION

The widely populations produced a mean of 126, 9 flowers per plant with a mean of 19, 30 seeds per flower in autumn (Table 1). Thus, estimation seed production founded was 2442 seeds per plant (58, 78 g). The mean seed weight was 23, 83 mg, these results are similar to *var. albomarginata* (24, 7 mg) reported by Correal *et al.,*(2008). However, production founded are higher than the reported by Correal *et al.,*(2008), who showed that *var. bituminosa* produced 43g, *var. albomarginata* 50g and *var. crassiusscula* 38g per plant. According to Beard *et al.,*(2014), the results confirmed that tedera seed was large (Figure 1) and it could be easier to establish.

Figure 1. Flower, fruit, seed and germinate seeds of Tedera.

The effects of submersion during (16 or 24 hours) are summarized in Table 2. According with Bewley and Black (1994), germination was defined when the protrusion of the radical through the seed coat. Overall, the results presented showed that tedera was found to have no significant physical dormancy characteristics that restrict germination. Thus, seeds germinate without the presence of light or scarification.

The highest percentage of germination was achieved in the 24 hours water submersion (65%) compared to the 16 hours (27%), which showed a significant increase in the percentage of seed germination under water submersion.

Table 1. Description of sample populations;seeds per flower, number of flo-wers, number of produced seeds, weigh of seeds, seed size and seed produced (g per plant).

	Sample populations											
Parameters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**SEM**
Seeds per flower	22, 00	18, 00	22, 00	20, 00	18, 00	18, 00	19, 00	20, 00	18, 00	18, 00	19, 30	1, 64
Number of flowers	120, 00	98, 00	108, 00	134, 00	129, 00	156, 00	142, 00	128, 00	140, 00	114, 00	126, 90	17, 35
Seed produced	2640	1764	2376	2680	2322	2808	2698	2560	2520	2052	2442	324
Weight (mg)	22, 30	25, 00	22, 70	24, 60	23, 98	24, 14	24, 44	23, 90	24, 00	23, 20	23, 83	0, 85
Size (mm)	13, 50	18, 00	10, 00	15, 30	17, 00	16, 60	17, 20	15, 70	17, 80	14, 00	15, 51	2, 46
Seed produced (g)	58, 87	44, 10	32, 23	65, 93	55, 68	67, 79	65, 93	61, 18	60, 05	76, 06	58, 78	12, 56

Table 2. Mean values of seed weight, seed size, germination percentage and radicle protruding.

Parameters	submersionduring (16hrs)	(24hrs)	SEM	p-value
Weigh (mg)	23, 00	22, 80	0, 29	0, 75
Seed size (mm)	13, 50	13, 25	0, 18	0, 25
Germination (%)	27, 00	65	21, 97	<0, 01
Radicle protruding (cm)	6, 04	6, 44	0, 28	0, 29

This study demonstrated that submersion during affected the *Bituminaria bituminosa* var. *bituminosa* seed germination and suggesting that tedera is a promising species for direct seeding. According to Beard *et al.* (2014), the results indicated that tedera requires a high water content to germinate. The results supported the findings of Correal *et al*. (2008),who reported a germination rate without scarification of 55 and 70% for var. bituminosa from Mijas and Calnegre populations, respectively. Castello et al.,(2015) founded that var. albomarginata and crassiuscula with soaking in gibberellic acid and mechanical scarification resulted in high germination (79.7% and 84.3%, respectively) than the results showed in this study for var. bituminousa (65%) without scarification or soaking in gibberellic acid. However, the results showed that var. Bituminosa under 24 hours submersion (65%) has a higher germination rate than variety albomarginata unthreshed with beak intact (25%) reported by Beard et al. (2014).

CONCLUSION

Overall, this study demonstrated that submersion during affected the *Bituminaria bituminosa* var. *bituminosa* seed germination. The results presented that tedera was found to have no significant physical dormancy characteristics that restrict germination. Thus, seeds germinate without the presence of light or scarification. The highest percentage of germination was achieved under 24 hours water submersion (65%). Also, this research founded that var. *bituminosa* was a plant with highly prolific seed production with a mean of 2442 seeds per plant (58, 78 g). The results suggesting tedera as a promising specie for direct seeding and easier for establishment. The domestication of *B. bituminosa*var. *bituminosa* will require additional researchs for producing seed and direct seeding.

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SEQUENCE ANALYSIS OF INSECTICIDE RESISTANCE AND DETOXIFICATION RELATED GENES IN *Spodoptera littoralis*(LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) is a well-known as one of the most destructive agricultural lepidopterous pests. It is a true generalist species with a promiscuous feeding strategy which enables it to attack numerous economically important crops all year round including vegetables, ornamental plants, and cotton. Recently, chemical control has been commonly used to suppress populations of *S. littoralis*; however, a very large number of *insecticides have led*to the emergence of*resistance.* An extensive use of insecticides also has other side effects, including the elimination of non-targeted organisms, environmental damage and harm to human health. Genome-wide high-throughput technologies help developing resistance management strategies, especially identifying genetic mechanisms of resistance. The aim of the present study was to produce a de novo transcriptome for *S. littoralis* as a resource for current and future studies of this pest species by using next-generation sequencing. This resource was then used as a reference for identifying genes by encoding the target sites of insecticides currently in use for Egyptian cotton leaf worm control. To achieve this, a cDNA library was sequenced using 454 FLX Titanium Sequencing on the Roche platform which revealed good coverage of genes encoding insecticide target sites and detoxification enzymes using a manual annotation. Annotations of assembled sequences were carried out by BLASTx against NCBI non-redundant protein sequence databases using the software Blast2GO. The g*ene*sencoding enzymes*involved*in *insecticide* detoxification such as *Acetylcholinesterase, Cytochrome p450, Glutathione S-transferase* were characterized. Furthermore, a phylogenetic analyses based on three protein sequences were generated in order to give evolutionary insights into insecticide resistance gene families of *S. littoralis*.

Keywords: *Spodoptera littoralis, transcriptome, insecticide resistance, detoxification.*

INTRODUCTION

Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval), is a polyphagous pest that damages numerous economically substantial crops in countries around the Mediterranean Basin and in Southeast Asia (Guz *et al*., 2013). The use of insecticides to control this species cause important damage to ecosystems, development of resistance in agricultural pests and elimination of non-targeted organisms from nature. Insecticide resistance is a genetic change in response to selection through target site mutation, and also boosted metabolism by sequestering insect detoxification enzymes (Feyereisen, 1995; Karatolos *et al*., 2011).

Acetylcholinesterase (AChE), Cytochrome P450 (CYP) and Glutathione S-transferase (GST) are the key enzymes that are associated with insecticide resistance. AChE, is the primary enzyme terminates nerve impulses in insect central nervous system by hydrolysis of the neurotransmitter acetylcholine (Toutant, 1989). Inhibition of acetylcholinesterase causes death in insects. Two major insecticide families, organophospates and carbamates, have been developed to inhibit this enzyme in an irreversible way. CYPs are hemoprotein super families which are widely distributed in all aerobic organisms. Their main function is to oxidize various substrates and also to catalyze plenty of other reactions (Mansuy, 1998). Increased metabolic detoxification by CYP450 is one of the most common mechanisms of insecticide resistance primarily to pyrethroids (Scott, 1999). GSTs are a varied enzyme family which plays a central role in detoxification of xenobiotics and endogenous compounds (Salinas and Wong, 1999). GSTs have been implicated in resistance against organophosphorous in many insects (Dauterman, 1985; Oppernoorth, 1985). More recently, elevated levels of GST activity have also been reported in pyrethroid resistant insect species (Vontas *et al*., 2001, 2002; Ranson and Hemingway, 2005).

In this study, we determined cDNAs encoding Acetylcholinesterase (*SpliAChE-1*), Cytochrome p450 (*SpliCYP450*) and Glutathione S-transferase (*SpliGST*) found in the transcriptome analysis of *S. littoralis*. We also presented phylogenetic reconstruction and structural bioinformatics analyses for each putative protein and discussed their putative roles in insecticide resistance and detoxification mechanisms.

MATERIAL AND METHODS

S. littoralis culture was maintained on an artificial diet at 25 ± 1°C with 60% relative humidity and 16:8 h light-dark photoperiod (Sorour *et al.* 2011). Nervous system was dissected from last instar larvae using sterilized forceps under a dissection microscope in ice-cold phosphate buffered saline (pH 7. 4). Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Isolated RNA was treated with DNase-free (Ambion, Austin, TX, USA) using 1. 5 units/μg of total RNA. Quantification and integrity was assessed by using ethidium bromide-stained 1% agarose gel and Nanodrop ND-1000 spectrophotometry (Thermo Scientific, Waltham, MA, USA), with a cut-off value of 1. 8 for the A260: 280 ratio.

RNA was fragmented with a zinc chloride solution. Fragmented RNA was quantified using the Agilent 2100 Bioanalyzer system using PicoGreen dsDNA Assay Kit (Invitrogen). cDNA was synthesized using the cDNA Synthesis System Kit with random hexamer primers (Roche Applied Science, Indianapolis, IN, USA). The cDNA fragments were subjected to ligation to the sequencing adaptors provided with the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Science), and small fragments were removed with AMPure XP (Beckman Coulter, Fullerton, CA, USA). Sequencing was performed on a GS FLX platform with Titanium chemistry (Roche 454) using a Small region of a Pico Titer Plate (PTP) per library, following the manufacturer's instructions.

Annotations of assembled sequences were carried out by BLASTx against NCBI (National Center for Biotechnology Information) non-redundant protein sequence databases using the software Blast2GO (Conesa *et al.*,2005). The partial cDNA and deduced amino acid sequences were compared using the BLASTx tool and EXPASY. Sequences were aligned using the MUSCLE software. Phylogenetic trees were inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were performed with MEGA6 (Tamura *et al.*,2013).

RESULTS AND DISCUSSION

Nervous transcriptome analysis of *S. littoralis* led to the identification of putative SpliAChE-1, SpliCYP450 and SpliGST proteins. The length of the nucleotide sequence of *SpliAChE-1*, *SpliCYP450* and *SpliGST* cDNAs were 670 bp, 493 bp and 610 bp, respectively. These sequences contained putative open reading frames (ORF) of 390 bp encoding a 130 amino acid for SpliAChE, 492 bp encoding 164 aa for SpliCYP450 and 610 bp encoding 203 aa for SpliGST polypeptides. BLAST analysis showed that SpliAChE-1, SpliCYP450 and SpliGST have similarities with other known insect proteins. SpliCYP450 showed the highest homology to *Spodoptera litura* CYP450 with 99% identity while SpliAChE-1 showed 88% homology to *Helicoverpa armigera* and SpliGST showed 69% homology to *S. litura* GST.

The partial cDNA encoding *SpliAChE-1* includes abhydrolase domain in *S. littoralis* (Marchler-Bauer *et al.*,2015). Based on the alignment of amino acid sequences, phylogenetic trees were constructed for three putative proteins (Figure 1, 2, 3). The phylogenetic analysis of SpliAChE showed that SpliAChE was clustered together in Lepidopteran AChE clade (Figure 1). SpliAChE is phylogenetically close to *H. armigera* AChE which is also consistent with BLAST analysis.

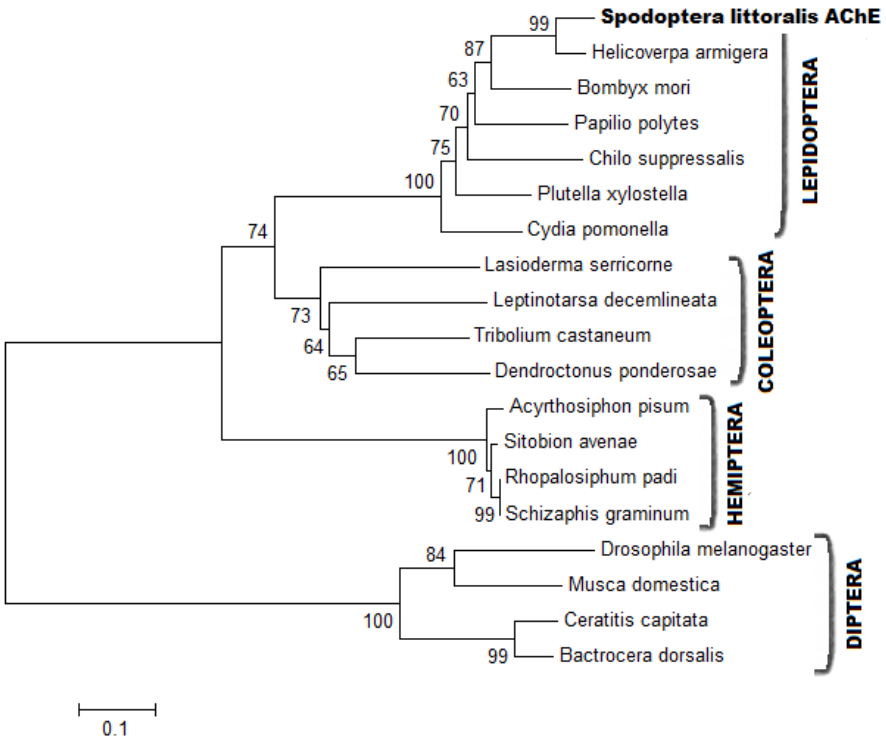

Figure 1. Phylogenetic analysis of putative AChE proteins from various insects. The species and GenBank accession numbers of the AChE sequences used to draw the phylogenetic tree are as follows: *Helicoverpa armigera* AAY59530. 1; *Bombyx mori* XP_012552768. 1; *Plutella xylostella* NP_001292470. 1; *Papilio polytes* XP_013148676. 1; *Cydia pomonella* ABB76667. 1; *Chilo suppressalis* AIY69049. 1; *Tribolium castaneum* NP_001280548. 1; *Lasioderma serricorne* ADA63843. 1; *Leptinotarsa decemlineata* AEI70751. 1; *Dendroctonus ponderosae* ENN79997. 1; *Sitobion avenae* AAV68493. 1; *Rhopalosiphum padi* AII01394. 1; *Acyrthosiphon pisum* XP_001948653. 1; *Drosophila melanogaster* AAL39345. 1; *Musca domestica* AAN06931. 1; *Ceratitis capitata* NP_001266363. 1; *Bactrocera dorsalis* AAO06900. 1.

The deduced sequence analysis of *SpliCYP450* includes specific P450 superfamily domain. Analysis reveals several conserved regions characteristic with other members of the P450 super family, such as the EXXR motif (EALR, position 387-390) located in the K helix, an oxygen-binding domain (proton transfer groove) ((A/G)Gx(E/D) T(T/S) as AGFET, position 329-333), and a microsomal cytochrome P450 motif PxxFxPE/DRF (PERF, position 444-452) (Werck-Reichhart and Feyereisen, 2000; Feyereisen, 2005) (Figure 2). Phylogenetic analysis of insect CYP450 showed that the SpliCYP450 was clustered closely with CYP450s from other members of Lepidoptera, including *S. litura*, *Spodoptera exigua, Mamestra brassicae, H. armigera* and *H. zea* (Figure 3).

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293 VAAGFATVEESAVGKKSADRVWSDEDLVAQAVLFFIAGFETVSTGMIFLLYELAVNPDVQ 352
353 ERLAQEIKEVDAKNGGKFDFNSIQNMVYMDMVVSEALRLWPPAVILDRMCTKDYNMGKPN 412
413 PKAEKDVILRKGTGVWIPAYAFHRDPQYFPNPDKFDPERFSEEN 456
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Figure 2. Deduced partial amino acid sequence of SpliCYP450. Putative conserved regions are shown with colorful frames.

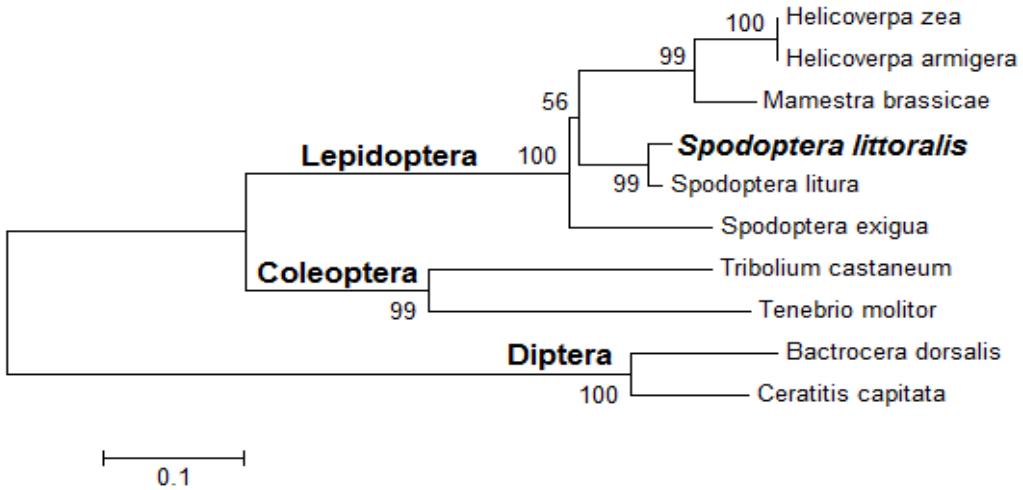

Figure 3. Phylogenetic analysis of putative CYP450 proteins from various insects. The species and GenBank accession numbers of the CYP450 sequences used to draw the phylogenetic tree are as follows: *Mamestra brassicae* AAR26518. 1; *Spodoptera litura* ACV88722. 1; *Spodoptera exigua* BAG71410. 1; *Helicoverpa zea* ABH09252. 1; *Helicoverpa armigera* AAV28704. 1; *Bactrocera dorsalis* AME15803. 1; *Ceratitis capitata* XP_012157753. 1; *Tribolium castaneum* NP_001034541. 1; *Tenebrio molitor* AKZ17702. 1.

Similar to other insect GSTs, SpliGST protein has a characteristic feature of glutathione binding region (G-site) and an electrophilic substrate binding region (H-site) conserved motifs that have an important role in GST activity (Deng *et al.* 2009). GSTs have been classified into seven main classes including delta, epsilon, omega, sigma, theta, zeta, and microsomal (Friedman, 2011). Among these delta and epsilon classes has been suggested to play significant roles in xenobiotic metabolism, particularly in insecticide detoxification (Ranson *et al.,* 2001, Ranson *et al.*, 2002, Meng *et al.*, 2015). For epsilon GSTs; there is a feature motif, RAVELTAK, found in *A. gambiae* (Ortelli *et al.*,2003). In epsilon SpliGST four amino acids were found conserved in this motif region (Arginine (R), Alanine (A), Leucine (L), Threonine (T)) (Figure 4). Based on the phylogenetic analysis and amino acid sequence comparison with epsilon GSTs from *Bombyx mori*, *Drosophila melanogaster*, *A. gambiae* and *Spodoptera litura* species, *SpliGSTs*were clustered into the Lepidopteran branch closely with epsilon-9 from *S. litura* (Figure 5).

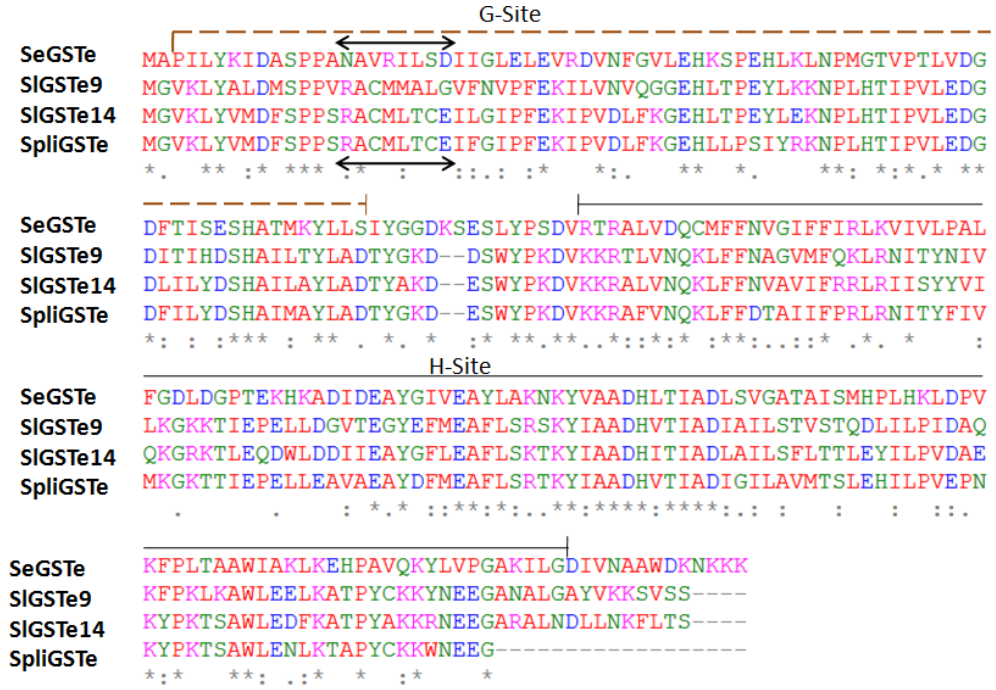

Figure 4. Multiple sequence alignment of SpliGST with other *Spodoptera* GSTs. The putative G-site and the H-site are indicated by dash and solid lines at the top of the panels, respectively. The specific motif of epsilon GST is implied with arrow. The accession numbers of the sequences used in the alignment are as follows: *S. exigua* (SeGSTe) AHB18378, *S. litura* (SlGSTe9) AIH07584, *S. litura* (SlGSTe14) AIH07589.

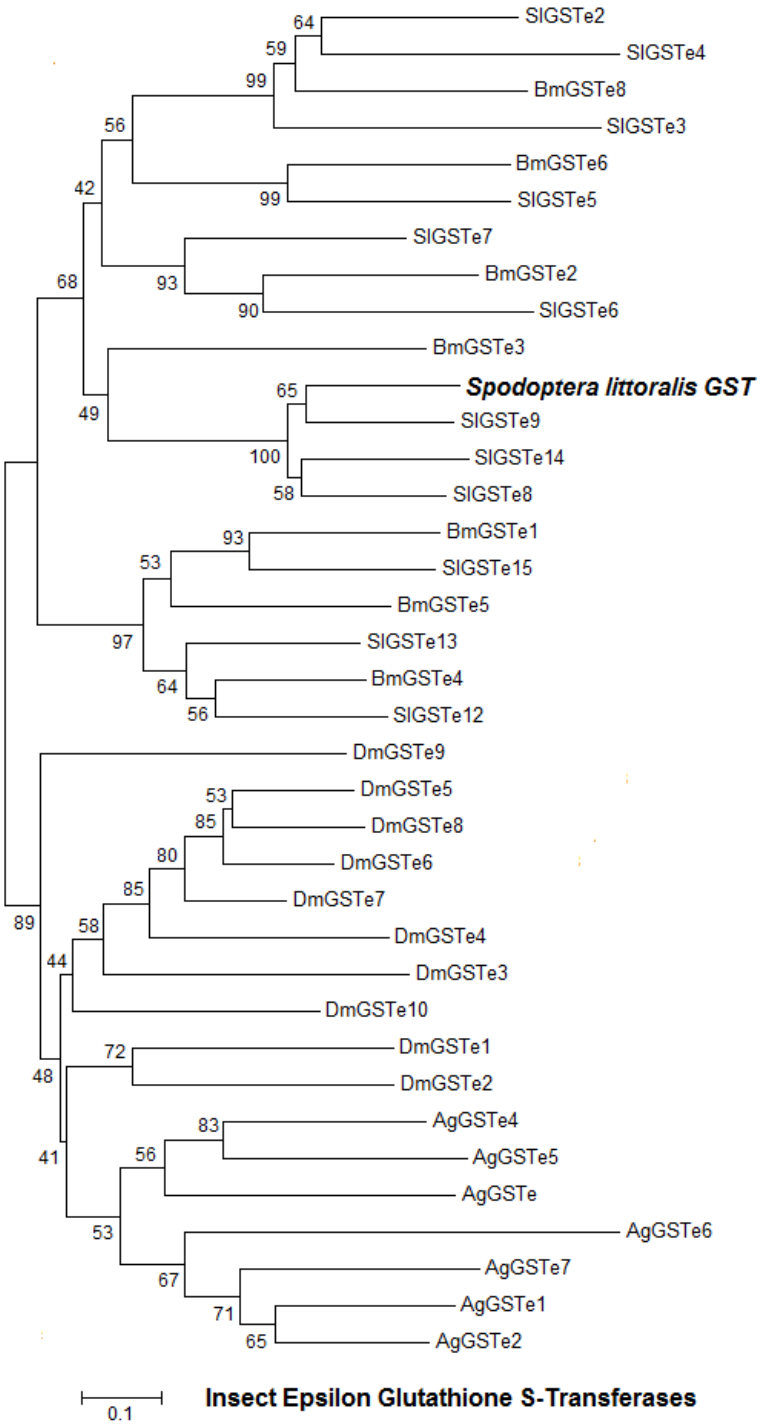
SlGSTe2
SlGSTe4
BmGSTe8
SlGSTe3
BmGSTe6
SlGSTe5
SlGSTe7
BmGSTe2
SlGSTe6
BmGSTe3
Spodoptera littoralis GST
SlGSTe9
SlGSTe14
SlGSTe8
BmGSTe1
SlGSTe15
BmGSTe5
SlGSTe13
BmGSTe4
SlGSTe12
DmGSTe9
DmGSTe5
DmGSTe8
DmGSTe6
DmGSTe7
DmGSTe4
DmGSTe3
DmGSTe10
DmGSTe1
DmGSTe2
AgGSTe4
AgGSTe5
AgGSTe
AgGSTe6
AgGSTe7
AgGSTe1
AgGSTe2
0.1
Insect Epsilon Glutathione S-Transferases

Figure 5. Phylogenetic analysis of putative GST proteins from various insects. The species and GenBank accession numbers of the GST sequences used to draw the phylogenetic tree are as follows: *Drosophila melanogaster* E1 (DmGST e1) NP_611323. 1; *D. melanogaster* E2 (DmGSTe2) NP_611324. 1; *D. melanogaster* E3 (DmGSTe3) NP_611325. 2; *D. melanogaster* E4 (DmGSTe4) NP_611326. 1; *D. melanogaster* E5 (DmGSTe5) NP_611327. 1; *D. melanogaster* E6 (DmGSTe6) NP_611328. 1; *D. melanogaster* E7 (DmGSTe7) NP_611329. 1; *D. melanogaster* E8 (DmGSTe8) NP_611330. 2; *D. melanogaster* E9 (DmGSTe9) NP_725784. 1; *D. melanogaster* E10 (DmGSTe10) NP_611322. 1; *Anopheles gambiae* E1 (AgGSTe1) XP_319969. 1; *A. gambiae* E2 (AgGSTe2) XP_319968. 3; *A. gambiae* E3 (AgGSTe3) XP_319972. 1; *A. gambiae* E4 (AgGSTe4) XP_319967. 1; *A. gambiae* E5 (AgGSTe5) XP_319966. 1; *A. gambiae* E6 (AgGSTe6) XP_001238234. 1; *A. gambiae* E7 (AgGSTe7) XP_319970. 3; *Bombyx mori* E1 (BmGSTe1) NP_001037197. 1; *B. mori* E2 (BmGSTe2) NP_001037420. 1; *B. mori* E3 (BmGSTe3) NP_001108466. 1; *B. mori* E4 (BmGSTe4) NP_001108460. 1; *B. mori* E5 (BmGSTe5) NP_001108464. 1; *B. mori* E6 (BmGSTe6) NP_001108465. 1; *B. mori* E8 (BmGSTe8)XP_004930497. 2; *Spodoptera litura* E2 (SlGSTe2) ACZ73898. 1; *S. litura* E3 (SlGSTe3) ACZ73899. 1; *S. litura* E4 (SlGSTe4) AIH07579. 1; *S. litura* E5 (SlGSTe5) AIH07580. 1; *S. litura* E6 (SlGSTe6) AIH07581. 1; *S. litura* E7 (SlGSTe7) AIH07582. 1; *S. litura* E8 (SlGSTe8) AIH07583. 1; *S. litura* E9 (SlGSTe9) AIH07584. 1; *S. litura* E12 (SlGSTe12) AIH07587. 1; *S. litura* E13 (SlGSTe13) AIH07588. 1; *S. litura* E14 (SlGSTe14) AIH07589. 1; *S. litura* E15 (SlGSTe15) AIH07590. 1.

CONCLUSIONS

In conclusion, we have identified three detoxification enzymes from *S. littoralis,* characterized the putative proteins and also reconstructed phylogenetic trees. The sequence data will provide a critical step toward a comprehensive functional characterization of resistance mechanism of the *S. littoralis*. Further functional analysis should be carried out to clarify insecticide detoxification mechanisms and to improve alternative insect pest management strategies against this hazardous pest.

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SEEKING AN OPTIMAL CLASSIFICATION SYSTEM FOR THE COMMERCIAL CATEGORIES OF OLIVE OILS

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ABSTRACT

The ultimate goal of food classifications systems (FCS) is to inform in an adequate way to the different agents of the market and mainly to consumers about the different categories, characteristics and quality of a food product. In particular, in this paper we focus on the olive oils classification systems. This system is formed by three product categories divided according to the objective quality of each one. Its main function is to help the consumers to distinguish and understand the distinctive characteristics of each category, motivating their learning to ensure that they can make informed decisions, avoiding confusion or mistakes. However, in many cases, the classification systems show important deficiencies that increase the confusion and hinder the consumer learning. For this reason, the objective of this work is to offer some orientations for the design of effective FCS. To analize the usefulness of diverse elements such as the length of the categories (short-long) and a series of visual signs (colours, numbers and images), in relation to the learning results, we conducted an experimental study with 840 spanish participants during November and December, 2014. The results allow us to provide a series of recommendations that can help to enhance the current classification system for olive oils.

Keywords: *food classification, food categorization, consumer orientation, agricultural policy.*

INTRODUCTION

Nowadays, consumers deal with a wide offer of products, varieties and quality levels in the market, making the choice processes relatively complex and requiring adequate levels of knowledge about the different options offered to consumers. However, reality shows that, in many cases, consumers are not able to choose in an adequate way; either due to a lack of informative campaigns, a consumer's lack of implication, the frequent changes that are produced in food classification systems (FCS) or the limitations of classification systems that are used. Indeed, problems such as the use of similar terms, ambiguous descriptions of the products included in

each category or the use of confusing or excessively technical descriptors are some of the factors that, instead of helping or facilitating the selection and purchase process, sometimes may confuse consumers and lead them to erroneous beliefs (Aydinoğlu and Krishna, 2011; Dörnyei and Gyulavári, 2015; Grunert et al., 2010; Hall and Osses, 2013; Mackey and Metz, 2009; Malam et al., 2009; Sharf et al., 2012). A typical case occurs in the olive oil sector, in which many consumers do not know the different types (and levels of quality) of olive oils and the characteristics of each level. The problem of the inadequacy of the categories of olive oils has been considered an important obstacle for the effective commercialization of the product, both in academic (Cabrera et al., 2015; Marano and López-Zafra, 2009, Marano et al., 2015; Navarro et al., 2010; Torres et al., 2012, 2015; Parras, 1996, 2000) and business levels[7]. Generally, it is argued that the similarity between the terms used and the ambiguity of the descriptors hinder an effective learning for the consumer. In addition, this problem aggravates since there is not only a lack of knowledge but confusion and erroneous beliefs as well. For instance, in the case of Spain, the top world olive oil producer and a country where this is one of the most emblematic products in the diet, over 60% of consumers think that "olive oil is pure olive juice, without manipulation", only 30% know that "olive oil is a mixture of virgin and refined olive oils, and over 70% think that "the main factor in differentiating between qualities is the acidity" (Torres et al., 2015). This could explain that the most consumed oil in Spain is olive oil (not virgin), in spite of its lower quality and healthiness and even though the difference in price is barely € 0. 3/litre according to the Ministry of Agriculture, Food and Environment Food Consumption Panel Data (2015). Furthermore, many producers face with difficulties to sell quality oils. In short, the official classification system does not incentive quality, production levels or consumption, in opposition to the guidelines of the Common Agricultural Policy and the efforts of the Spanish Government.

Our objective is to analyze to what extent does the current classification system limits or interferes in the effective commercialization of olive oils. Based on the idea that any food classification system must help consumers to distinguish between products, to facilitate the choice process, and to suggests (or invoke) them useful information, different options are compared in this study in order to determine (1) if the classification system really affects the consumer behaviour (and, therefore, to the commercialization of the oil) (2) which characteristics should a classification system have and (3) if this one is better than the current classification system.

MATERIALS AND METHODS

In order to address the objective of the present study, first we need to focus the framework, and to clearly establish some fundamental principles based on the present characteristics of agrifood purchases. Thus, within the scope of food

[7] Some producers of quality virgin olive oils think that "Premium" olive oils should be differentiated from the rest of extra virgin olive oils (a new category).

products, purchasing decisions tend to be routine (Schiffman & Kanuk, 2005), with a low level of involvement (Hamlin, 2010), and are characterised by a lack of cognitive processing of information[8], which leads consumers to simplify their decision and to misuse heuristics (Hamlin, 2010; Scheibehenne et al., 2007). Additionally, consumers are exposed to a great amount of information (Dunbar, 2010; Hall & Osses, 2013; Mackison et al., 2008), which they must process and make decisions in crowded places, such as supermarkets or hypermarkets, where it is difficult to reflect upon the information the product bears. Furthermore, consumers are also pressured by the time limit to process all this information (Loebnitz et al., 2015; Pieniak et al., 2007; Reutskaja et al., 2011; Suri & Monroe, 2003). Thus, this environment encourages the use of simplification mechanisms by the consumer, who, rather than reflecting upon the information provided, resorts to visual elements that act as heuristics to associate certain attributes to the product. That is, images or symbols are used to simplify the decision-making process, provided that these signs have previously been learned (Hoek, Roling, & Holdsworth, 2013; Sütterlin & Siegrist, 2015). Considering that olive oil is usually purchased on a self-service basis, an experiment has been suggested that intends to emulate the basic mental processes attached to shopping at a supermarket centered on the recognition of the product exposed. In this sense, three recognition indicators of the categories have been used to compare the classification systems. In particular, to design proposal of categories. the length (number of words of the categories, distinguishing between short and long) and the type of sign (colours, images and numbers) are the variables used. In relation to the length it is necessary to take into account that the labelling espace is limited and, therefore, introducing more text implies a smaller size of the font and consequently, less probability of perception (although it is more informative); in relation to the signs, the numbers have the advantage of producing an intuitive hierarchy, and have been used previously in the systems of classification (Cronley et al., 2005; Gunasti & Ross, 2010; Yan & Duclos, 2013). Moreover, the colours are easily identifiable and are also frequently used (Becker et al., 2015; Drescher et al., 2014; Olstad et al., 2015; Siegrist et al., 2015), similarly to images (McQuarrie & Mick, 2003; Schlosser, 2006; Schmitt et al., 1993). Considering the combinations of two variables, a balanced experiment was performed with six different classifications, plus a control group which receives the current system that is used in products (treatment 7). The seven treatments were randomly administered to an online panel sample of 840 subjects, at a rate of 120 per treatment. The interviewees were residents of the following Spanish areas: Santa Cruz de Tenerife, Cádiz, Barcelona, Madrid, Almería, Islas Baleares, Burgos, A Coruña, Guipúzcoa, Huesca, Navarra, Asturias, Las Palmas, Pontevedra, Cantabria, Tarragona, Valladolid, Vizcaya and Zaragoza. Likewise, in each treatment the composition of the sample was equal, in relation to the variables of age, level of studies and sex. The field work was performed between November 26th and December 4th 2014.

[8] With the exception of wine (Hamlin, 2010).

Through an online questionnaire composed by successive pages, in which participants could not go back, the subjects were submitted for one minute to the information of the treatment of their condition, which appeared reflected in the computer screen (Table 1), referred to the category used and the information associated with each category. After a few questions, recognition was measured through two suggested recall questions. All the categories of all treatments were displayed on the computer screen and it was requested to remember which of them they had viewed and to indicate an order of quality (from highest to lowest). In both cases, the number of successes of each interviewee was calculated. Finally, previous knowledge about olive oils was also measured through an 11-item scale.

Table 1. Information presented to each experimental group on the computer screen

Treatment 1(n=120) (short-images)	
Category	Category description
Olive oil 	Maximum quality oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil 	Olive oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil 	Olive oil obtained through a mixture of oils subjected to a chemical rebore treatment and oils obtained from pure olive juice.
Treatment 2 (n=120) (short-numbers)	
Category	Category description
Olive oil 1[a]	Maximum quality oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil 2[a]	Olive oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil 3[a]	Olive oil obtained through a mixture of oils subjected to a chemical rebore treatment and oils obtained from pure olive juice.
Treatment 3 (n=120) (short-colors)	
Category	Category description
Olive oil 	Maximum quality oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil 	Olive oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.

Olive oil	Olive oil obtained through a mixture of oils subjected to a chemical rebore treatment and oils obtained from pure olive juice.
Treatment 4 (n=120) (long-images)	
Category	Category description
Olive oil superior quality natural juice 	Maximum quality oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil natural juice 	Olive oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil natural juice mixed with rectified olive oils 	Olive oil obtained through a mixture of oils subjected to a chemical rebore treatment and oils obtained from pure olive juice.
Treatment 5 (n=120) (long-numbers)	
Category	Category description
Olive oil superior quality natural juice 1st	Maximum quality oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil natural juice 2nd	Olive oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil natural juice mixed with rectified olive oils 3rd	Olive oil obtained through a mixture of oils subjected to a chemical rebore treatment and oils obtained from pure olive juice.
Treatment 6 (n=120) (long-colors)	
Category	Category description
Olive oil superior qua-lity natural juice 	Maximum quality oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil natural juice 	Olive oil obtained from pure olive juice and only through mechanical procedures, which preserve all the flavour, odour and properties of the natural fruit.
Olive oil natural juice mixed with rectified olive oils	Olive oil obtained through a mixture of oils subjected to a chemical rebore treatment and oils obtained from pure olive juice.
Treatment 7 (n=120) (current classification)	
Category	Category description
Extra virgin olive oil	Maximum quality olive oil obtained directly from olives and only through mechanical procedures.
Virgin olive oil	Olive oil obtained directly from olives and only through mechanical procedures.
Olive oil contains-exclusively refined olive oils and virgin olive oils	Oil containing exclusively olive oils which have been exposed to a refining treatment and oils obtained directly from olives.

RESULTS AND DISCUSSION

Selection of the most adequate system.

A MANCOVA with length and sign independent variables was performed. As dependent variables we considered the two related to the suggested recall or recognition (expressed in number of hits over 3) and previous oils knowledge have been introduced as a co-variable. In a first phase, the hypothesis of homogeneity of slope, including the interaction between the co-variable and the treatment in the model has been corroborated. Since the interaction is not significant (the lowest level corresponds to Roy's larger root, with 0, 362) the model has been suppressed. In tables 1, 2 and 3 the MANCOVA, ANCOVA and means of each treatment for each dependant variable are displayed.

Table 1. MANCOVAeffect of the use of signs and length of categories

Effects		**Value**	**F**	**P**
Length	Pillai's Trace	. 018	6. 570	. 001
	Wilk's Lambda	. 982	6. 570	. 001
	Hotelling's Trace	. 018	6. 570	. 001
	Roy's largest Root	. 018	6. 570	. 001
Type of sign	Pillai's Trace	. 065	11. 975	. 000
	Wilk's Lambda	. 935	12. 134	. 000
	Hotelling's Trace	. 069	12. 293	. 000
	Roy's largest Root	. 066	23. 762	. 000
Length*type of sign	Pillai's Trace	. 011	1. 907	. 107
	Wilk's Lambda	. 989	1. 907	. 107
	Hotelling's Trace	. 011	1. 907	. 107
	Roy's largest Root	. 009	3. 215	. 041
Previous knowledge	Pillai's Trace	. 009	3. 291	. 038
	Wilk's Lambda	. 991	3. 291	. 038
	Hotelling's Trace	. 009	3. 291	. 038
	Roy's largest Root	. 009	3. 291	. 038

Table 2. ANCOVA effect of the use of signs and length of categories over the dependant variables

Origin	**Dependent variable**	**Type III sum of squares**	**F**	**P**
Length	Recognition	4. 482	3. 895	. 049
	Order or Hierarchy	11. 231	9. 919	. 002
Type of sign	Recognition	51. 007	22. 165	. 000
	Order or Hierarchy	30. 071	13. 279	. 000
Length*type of sign	Recognition	5. 384	2. 340	. 097
	Order or Hierarchy	2. 619	1. 157	. 315
Previous knowledge	Recognition	7. 282	6. 589	. 010
	Order or Hierarchy	5. 993	5. 293	. 022

Table 3. Marginal Means (number of right answers)

Length			**Sign**	
Recognition	Short	. 861	Image	1. 278
	Long	1. 019	Number	. 628
			Colour	. 913
Order or Hierarchy	Short	. 594	Image	. 992
	Long	. 844	Number	. 502
			Colour	. 663

The results show an additive and meaningful effect of length and symbol in the recognition of categories and order of quality. Also, the inclusion of the co-variable in the model is correct, that is, the recognition is affected by the previous knowledge. Considering the marginal means estimated, in general, the long systems have a higher hit rate than the short systems wheras regarding symbols, the image has a higher level of success than the number or the colour. From the two explicative variables, the type of symbol image has a greater effect than length either for the case of recognition of categories (eta2=. 058 and eta2=. 005 respectively) and the case of quality order or hierarchy (eta2=. 036 and eta2=. 014 respectively). In sum, the most appropriate system for recognition purposes, is the one includinglong categories and images (in this case olives) (treatment 4).

Comparison of the proposed classification with the current classification system.

To analyse if the proposed system is better than the current one, a MANCOVA – tables 4, 5, 6 and 7- has been performed in which the selected treatment (4) is compared with the current classification (7), considering, the previous knowledge as a co-variable. After accepting the homogeneity hypothesis of slope, the interaction between the co-variable and the treatment has been suppressed.

Table 4. MANCOVA effect of the interaction between the co-variable and the type of system (4&7)

Effects		**Value**	**F**	**P**
Treatments 4&7* Previous knowledge	Pillai's Trace	. 005	. 573	. 564
	Wilk's Lambda	. 995	. 573	. 564
	Hotelling's Trace	. 005	. 573	. 564
	Roy's largest Root	. 005	. 573	. 564

Table 5. MANCOVA effect of the type of system

Effects		**Value**	**F**	**P**
Previous knowledge	Pillai's Trace	. 005	. 540	. 584
	Wilk's Lambda	. 995	. 540	. 584
	Hotelling's Trace	. 005	. 540	. 584
	Roy's largest Root	. 005	. 540	. 584
Treatments 4&7	Pillai's Trace	. 030	3. 616	. 028
	Wilk's Lambda	. 970	3. 616	. 028
	Hotelling's Trace	. 031	3. 616	. 028
	Roy's largest Root	. 031	3. 616	. 028

Table 6. ANCOVA effect of the type of system over the dependant variables

Origin	**Dependent variable**	**Type III sum of squares**	**F**	**P**
Previous knowledge	Recognition	. 989	. 921	. 338
	Order or Hierarchy	. 471	. 380	. 538
Treatments 4&7	Recognition	4. 808	4. 477	. 035
	Order or Hierarchy	1. 143	. 922	. 338

Table 7. Estimation of the values

Dependent variable	**Values**	**B**	**Typ. Err**	**t**	**P**
Recognition	Intersection	1. 379	. 127	10. 865	. 000
	Previous knowledge	. 017	. 017	. 960	. 338
	(Treatment 4&7=4. 00)	. 283	. 134	2. 116	. 035
	(Treatment 4&7=5. 00)	0			
Order or Hierarchy	Intersection	1. 164	. 136	8. 533	. 000
	Previous knowledge	. 011	. 019	. 617	. 538
	(Treatment 4&7=4. 00)	. 138	. 144	. 960	. 338
	(Treatment 4&7=5. 00)	0			

The results show that, for recognition, the current classification is higher than the proposed; but when determining the order of quality within each system of categories, there are no differences between both systems.

CONCLUSIONS

In light of these results, we can conclude that the use of one system of categories has important implications on consumer's behavior and, consequently, in the commercialization of oils in the market. In this sense, before developing or modifying a classification system, it is very important to plan and carry out studies to determine the adequacy of the FCS, since its development may affect the whole sector.

Secondly, in the specific case of olive oils, and under the hypothesis of using a system that have an impact on the visual recognition of its categories, and specially of a hierarchical organization of their quality levels, a system with images (in this case olives) seems to be superior to a system based in colours or numbers. Additionally, a minor superiority of long systems (with more terms than shorter systems) is appreciated.

Finally, it remains open the question of whether the recommendations made to develop a classification system on olive oils, from the point of view of consumer recognition activity, would result in a better system than the current one. According to our results and considering that the proposed system has only been exposed during one minute, whereas the current one has already been several years in the market and it has been object of news, publicity, kitchen shows, public administration informative actions, interprofesional of the olive oils, etc (as well as the same minute), they are what it might be expected: better recognition levels. In other words, people bring it to mind and recognize the olive oil terms: virgin, extra virgin; but its knowledge does not go any further than that. Surprisingly, it gets the same hits than the system of signs (only exposed during a minute) when organizing the quality of categories hierarchically, proving the deficiency of the current system.

In this context it is possible to conclude that the lack of knowledge and confusion regarding olive oils, in part due to the shortcomings of the current classification. Therefore, we defend the need to make some modifications in this system in order to make it much more intuitive, evocative of the quality and transparent towards consumers. The ideal system would be one that allows consumers to learn easily the qualitative differences between the categories and to choose accordingly which one they consume.

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The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used, etc.

- RESULTS and **DISCUSSION**

Results and Discussion should be combined into a single section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

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.

The International System of Units (SI) should be used.

- 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.

- REFERENCES (LITERATURE)

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. Please see "INSTRUCTIONS FOR AUTHORS" / Bibliographic style on the web page of the Journal for more details: http://www.agrofor.rs.ba/page.php?id=8

Short communication should include the following sections: Title, Abstract, Key words, Main text, Acknowledgments, References, Tables and Figures with captions.

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