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THE INFLUENCE OF DIFFERENT PURITY OF NATURAL ALFALFA SEEDS ON THE PROCESSING EFFICIENCY

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ABSTRACT

This paper presents the results of the seed processing of ten lots of natural alfalfa seed with different purity (from 68.0% to 86.5%). The test was carried out at the seed processing center of the Institute for forage crops Kruševac-Serbia. Seed losses, processing output, seed yield and quality of the processed seed were investigated. It is important that the difference between the amounts of pure seed from laboratory assessment and the actual amount after processing, are low. The purity of natural alfalfa seed depends on the crop condition and the harvest process. In the seed processing of small-grained leguminous plants, the processing output of seed is directly dependent on the percentage of weed species and other species in the natural seed. Seeds of quarantine weeds of dodder and curly dock are a particularly big problem in alfalfa seeds. In the case of high-purity seeds with low quarantine weeds share, processing output are high. By the legal procedure on the seed quality, the content of pure seed, inert materials, weeds and other species in the processed seeds is defined. The efficiency of the alfalfa seed processing depends on the initial purity of the seed, as well as the applied technical and technological process of seed processing. Based on the obtained results, it is possible to optimally adjust and select the appropriate equipment for the processing of alfalfa seed, depending on the quantity and type of weeds and other ingredients in the natural alfalfa seeds.

Keywords*: alfalfa, purity, processing, seed, weeds.*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is often called the "Queen of forage crops". It is characterized by extreme flexibility. Alfalfa is a plant with an efficient source of nitrogen and high production of biomass (Barnes et al., 1988). In the region of Southeast Europe alfalfa is the most important perennial forage legume. In this region fodder is mainly dried after cutting and hay is used for animal nutrition (Knežević et al., 2014). Alfalfa requires neutral soil. In animal feed alfalfa can be used fresh or conserved as hay, haylage, silage, flour, pellets, and paste (Jakšić et al., 2013; Jakšić et al., 2017). Alfalfa is one of the best and most important forage species according the vitamin and mineral content (large amounts of pro-vitamin A, vitamins B_1, B_2, C, D, E, K). There is about 20% crude proteins in dry matter of alfalfa (Vučković, 1999). Except to the forage, alfalfa is also used for the seed production. By cultivating alfalfa seed, with the appropriate technology it is possible to achieve high yields and very good financial effects (Đokić at al., 2015).

Serbia's climate is judged to be moderately favorable for the production of seed legumes. The average yield of alfalfa and red clover seeds is about 250 kg ha^{-1} (Karagić et al., 2010). In the modern production of plant species, one of the prerequisites for achieving high and stable yields, as well as for approaching the realization of maximum genetic yield potentials, is the use of high quality seeds (Mladenov and Milošević, 2011). The purity of natural alfalfa seeds is different, which significantly affects the seed losses during the seed processing. The task of seed processing is to prepare the quality seed for sowing, with satisfied germination and sprouting (Đokić et al., 2012; Đokić et al., 2016). Seed processing is based on the physical characteristics of the seed and therefore it is necessary to carefully analyze all seed features and to do the appropriate adjustment of the equipment (Babić and Babić, 1998; Black et al., 2006; Copeland et al., 2004; Đokić, 2010; Đokić and Stanisavljević, 2012).

In the seed crop of alfalfa, the most damaging quarantine weeds are dodder and curly dock. The dodder (*Cuscuta* spp.) can cause enormous damage especially if no suppression is done. It is one of the most dangerous and economically most damaging weeds (Čuturilo and Nikolić, 1986; Đukić at al., 2004; Miladinović, 2001).

The Law on Seeds of the Republic of Serbia defines the conditions and manner of production, processing, use, trade, import and testing of the quality of seeds of agricultural plants (Official Gazette of the Republic of Serbia, 45, 2005). The Rulebook on the Quality of Seeds of Agricultural Plants (Official Gazette of the SFRY, 47/1987), which is harmonized with international seed regulations (ISTA, 1999) defined the quality standard for the quality of seeds of alfalfa. According to this Rule, the alfalfa seed (*Medicago sativa* L.) must have a minimum seed purity of 95%, with 2% of seeds of other species, weeds not more than 0.5% (including dodder and curly dock), and up to 2.5% inert materials.

The aim of this study was to analyze the influence of different purity of alfalfa seed on the efficiency of seed processing. On the basis of the obtained results, it is possible to optimize the selection of the appropriate alfalfa seed processing equipment, depending on the quantity and type of weeds and other ingredients in the natural seed of the alfalfa.

MATERIAL AND METHODS

The experiment was carried out at the processing center of the Institute for Forage Crops in Kruševac-Serbia. In three replications the ten lots of natural alfalfa seed with different purity was processed. The purity of natural alfalfa seed ranged from a minimum of 68% to a maximum of 86.5%. The processing equipment was Danish manufactures of Kongskilde and Damas, and magnetic separator type 4 of the German manufacturer Emceka Gompper.

To clean alfalfa seed favorable combination schedule screens on the machine for fine cleaning of seed was established. In the upper shaker shoe were located sieves and sieve with round holes of the following diameters: 2.75 mm; 2.5 mm; 2.25 mm; 2.0 mm; 2.0 mm and 1.9 mm. At the bottom of the shaker shoe was in the sieve with longitudinal-cut openings width: 1.3 mm; 1.2 mm; 1.1 mm; 0.6 mm; 0.5 mm and 0.5 mm.

In order to analyze the content of foreign matter in the seed, a laboratory lamp with light and precision electronic scale was used. Samples for analysis were weighing 5 g and 50 g. Measurement of the mass of the processed seed was carried out using electronic weighing range of up to 300 kg. In each of the repetitions by laboratory analysis, the following parameters were measured: quantity of pure seed (%), seed of other species (%), inert matter (%), weed (%), and amount of processed seed (kg). Processing output (%) and seed losses on processing equipment (%) were determined by calculation.

The obtained results were analyzed by variance analysis (ANOVA), and the significance of the mean difference was tested with the Tukey test. The statistical program Minitab 16.1.0 (statistics software package) was used for data processing.

RESULTS AND DISCUSSION

The purity of the natural seed of the alfalfa of all ten seed lots is shown in Table 1. The presence of other plant species has not been established by analysis of the samples. The investigated natural alfalfa seed had a purity ranging from the lowest values of 68.0% in the seed lot I to the highest purity of 86.5% for the seed lot II. Seed lots II, VII, VIII, and X had higher purity of natural seed than lot I (Table 1). Inert substances are present in the form of harvest residues (stems, leaf, and other legumes), soil, sickly grain and damaged seed. The content of inert materials was from 12% for seeds of lot II to a maximum content of 27.2% for seed lot I. The presence of inert materials in alfalfa seed does not pose a greater problem in the process of finishing, except when there is a greater share of incomplete pods (Đokić, 2010). The content of weeds in the native alfalfa seed was 1.0% in the seed lot VII to 8.0% in lot VIII. From weeds in natural seed of alfalfa by analysis of samples, seeds of dodder were found in all seed lots, except for the lots I and VIII, where the seeds of dodder were not found. The largest number of dodder was 20 seeds (in a 5 g sample) in the lot II. The smallest number of dodder was 2 seeds (in a 5 g sample) in the lots IV and IX. In the analysis of the weed seed of the lot VII, seed of sorghum was found, and in the lot VIII seed of green foxtail was found.

Table 1. The average purity of natural alfalfa seeds

Lot	I	II	III	IV	V	VI	VII	VIII	IX	X
Seed structure	%	%	%	%	%	%	%	%	%	%
Pure seed	68.0 b	86.5 a	76.0 ab	75.5 ab	72.0 ab	72.5 ab	86.0 a	78.0 a	76.0 ab	81.0 a
Other species	-	-	-	-	-	-	-	-	-	-
Inert matter	27.2 a	12.0 b	18.5 ab	23.7 ab	23.4 ab	26.0 a	13.0 b	14.0 b	18.8 ab	17 ab
Weed	4.8 b	1.5 c dodder 20/5 g	5.5 b dodder 6/5 g	0.8 c dodder 2/5 g	4.6 b dodder 16/5 g	1.5 c dodder 5/5 g	1.0 c dodder 10/5 g	8.0 a	5.2 b dodder 2/5 g	2 bc dodder 6/5 g
Total	100	100	100	100	100	100	100	100	100	100

Tukey test statistical significance levels: $p \leq 0.05$, differences in row marked in small letters a, b, c...

The natural seed is transported from the receiving bunker to the fine-cleaning machine type Alfa-4 by a system of bucket and belt conveyors. The alfalfa seed is transported to a large bunker for seed reception after processing on a fine-cleaning machine with bucket conveyors. From the hopper the seed is inserted into a mixer where, at a certain time and in a certain proportion, it is mixed with steel powder and water. Nutra Fine RS steel powder was used for mixing alfalfa seed. Magnetic separator serves for the separation of weed species, especially the seeds of the dodder. The purity of alfalfa seed after processing and passing through the equipment is shown in Table 2.

Table 2. The average purity of processed alfalfa seeds

Lot	I	II	III	IV	V	VI	VII	VIII	IX	X
Seed structure	%	%	%	%	%	%	%	%	%	%
Pure seed	98.2 ab	99.6 a	99.0 a	97.4 b	98.2 ab	97.4 b	98.4 ab	97.8 b	97.6 ab	98.2 ab
Other species	-	-	-	-	-	-	-	-	-	
Inert matter	1.8 b	0.4 c	1.0 bc	2.6 a	1.0 bc	2.6 a	1.6 b	1.8 b	2.4 a	1.8 b
Weed	-	-	-	-	0.8	-	-	0.4	-	-
Total	100	100	100	100	100	100	100	100	100	100

Tukey test statistical significance levels: $p \leq 0.05$, differences in row marked in small letters a, b, c...

After the seed processing the purity of all ten seed lots was high and ranged from the lowest values of 97.4% in the seeds of the lots IV and VI to the highest values of 99.6% in the lot II. Seed lots II and III also achieved a statistically significant ($p \leq 0.05$) purity compared with lots V and VIII (Tab. 2). The content of inert matters ranged from the lowest value of 1% for seeds lot V, up to a maximum of 2.6% for seed lots IV and VI. A detailed analysis of the processed seeds in the lot I founded 2 seeds of orchard grass (*Dactylis glomerata* L.) in 50 g sample. In the seed lot II (50 g sample), there were 3 seeds of pigweed (*Amaranthus retroflexus*).

In the lot III, there were 2 seeds of curly dock (*Rumex* spp.), 9 seeds of chamomile (*Matricaria chamomilla*), 1 seed of green foxtail (*Setaria* spp.), and 1 seed of Sudanese grass (*Sorghum sudanense* Pers.). In the lot IV, there were 4 seeds of curly dock (*Rumex* spp.). The sample of seed lot VI had 3 seeds of red clover (*Trifolium pratense* L.), 3 seeds of pigweed (*Amaranthus retroflexus*) and 2 seeds of foxtail (*Setaria* spp.). Part VII was in the sample with 1 wild carrot seed (*Daucus* spp.), 5 seeds of foxtail (*Setaria* spp.), 1 seed of pigweed (*Amaranthus retroflexus*) and 3 seeds of red clover (*Trifolium pratense* L.). In the seed sample of lot X (50 g sample) there were 1 seeds of curly dock (*Rumex* spp.) and sorghum (*Sorghum* spp.) in traces.
At the end of the seed processing the amount of processed seed was measured. The quantities of natural seed of the alfalfa of all ten lots at the beginning of the seed processing and the amount of processed seeds at the end of the seed processing are shown in Table 3. Also, processing output and seed losses are shown in Table 3. Amount of the natural and recovered seed of lot X was significantly higher ($p \leq 0.05$) than in the other lots. Processing output in the lot VII was significantly higher than in the other lots ($p \leq 0.05$), but not statistically significant than in the lots II and X. Seed losses were significantly higher for lots V and IX (Table 3).

Table 3. Amounts of proceseed alfalfa seeds on processing machines

Seed structure	Lot									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Natural seed (kg)	794 e	1260 de	990 ef	1412 d	660 e	1107 e	2025 bc	1900 c	2250 b	8000 a
Processed seed (kg)	520 bc	900 b	690 bc	970 b	343 c	760 bc	1633 b	1300 b	1300 b	5896 a
Processing output (%)	65.5 b	71.4 ab	69.7 b	68.7 b	51.9 b	68.6 b	80.6 a	68.4 b	57.7 ab	73.7 ab
Losses (%)	3.7 d	17.4 b	8.3 ce	9.0 dc	27.8 a	5.3 dc	6.2 dc	12.3 c	23.9 a	9.0 dc

Tukey test statistical significance levels: $p \leq 0.05$, differences in row marked in small letters a, b, c...

By analyzing the results obtained after the alfalfa seed processing it was noted that the processing output was different. It ranged from at least 51.9% of seed lot V, while the highest was in seed lot VII (80.6%). Seed lot VII also had a high initial purity of natural seed of 80.0%. Seed losses were also varied and ranged from the lowest loss of 3.7% for seeds of lot I to the highest seed losses lot V (27.3%). High seed losses were also in the seed lot IX (23.9%).

CONCLUSION

By seed processing on a magnetic separator, using steel powder and water, high purity and quality seed was obtained. After the seed processing of ten lots of natural alfalfa seed, seed purity was high and ranged from 97.4% in the seeds of the IV and VI lots to 99.6% in the seeds of lot II. The content of inert substances was from the lowest value of 1% for seeds of lot V, up to a maximum of 2.6% for seeds of lots IV and VI. Seed losses were varied and ranged from the lowest losses of 3.7% for seeds of lot I to the largest losses in lot V and amounted to 27.3%. Significant seed losses were also found in lot IX and amounted to 23.9%. Processing output had the lowest value in the seed lot V (51.9%), up to the largest in seed lot VII (80.6%).

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RAGWEED AND MUGWORT POLLEN (ASTERACEAE FAMILY) - MONITORING AND COMPARATIVE ANALYSIS OF SEASONAL DYNAMICS DURING 2011-2017

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ABSTRACT

In addition to appearing in crops, weeds may pose a risk to human health, indirectly due to the widespread use of herbicides and directly, because they are source of pollen that in susceptible people can cause allergic reactions. Among the weed species, the main allergens are the species of the botanical family: Asteraceae, Amaranthaceae, Urticaceae, Euphorbiaceae and Plantaginaceae. Asteraceae family includes 1,100 genera and 20,000 species, which is one of the largest flowering plants. However, in our area as a potential allergen the most important is Ambrosia and Artemisia pollen. Sampling of ragweed and mugwort pollen during the pollination period 2012-2017 was conducted in urban part of Banja Luka in PI AIRS, BL with Hirst sampler using the method defined by the International Association for Aerobiology (IAA). The first mugwort pollen grains in 2011 were recorded at the end of May, during 2012 and 2016 in the first decade of June, and from 2013 to 2015, as well in 2017 in the second decade of June. Mugwort pollination period lasted for an average of 55 days and it was characterized by low (1-10 p/m^3) to moderate (11-50 p/m^3) concentrations. The ragweed period pollination during the seven-year monitoring lasted, on average, about 115 days. High concentrations (51-500 p/m^3) were recorded between the second decade of August to the third decade of September, while very high concentrations (>501 p/m^3) were recorded only in 2011. On an annual basis not only the season of ragweed pollination lasted longer, but the results of the monitoring and comparative analysis showed significantly higher % share of ragweed pollen within the weed species in the family Asteraceae in the city of Banja Luka.

Keywords: *pollen, ragweed, mugwort, seasonal dynamics, Banja Luka.*

INTRODUCTION

In addition to appearing in crops, weeds may pose a risk to human health, indirectly due to the widespread use of herbicides and directly, because source of pollen that in susceptible people can cause allergic reactions. Among the weed species, the main allergens are the species of the botanical family: Asteraceae, Amaranthaceae, Urticaceae, Euphorbiaceae and Plantaginaceae. Asteraceae family includes 1,100 genera and 20,000 species, which is one of the largest flowering plants (Gadermaier *et al*., 2004). However among species belonging to Asteraceae family, in our area as a potential allergen the most important is Ambrosia and Artemisia pollen. The genus Ambrosia belongs to the tribe Heliantheae in the Asteraceae family (= Compositae, daisy family). It consists of about 40 species, 22 of which occur naturally in North America. Today it is common in agricultural ecosystems, in urban-industrial ruderal sites and along roadsides (Lavoie *et al*., 2007; Otto *et al*., 2008). They are expanding their range in both their native areas and other parts of the world, including Europe, where the most abundant species in Europe is *Ambrosia artemisiifolia* L. (common ragweed) (EFSA, 2010). In addition, a number of authors Makovcová *et al*. (1998), Dahl *et al*. (1999), Rybnícek *et al*. (2000), Laaidi *et al*. (2003), Makra *et al*. (2004), Peternel *et al*. (2005) and Taramaracaz *et al*. (2005) stated Bosnia, amoung many countries, as one of the most contaminated place in Europe with *Ambrosia artemisiifolia* L., the highly allergenic plant, while Pušić *et al*. (2012) also state that ambrosia is very widespread and it is in expansion in the area of Banja Luka. Never the less, Trkulja *et al*. (2012) stated that ragweed presents one of the most invasive weed species in our area. However, one of the largest genera of the family Asteraceae is genus Artemisia (McArthur and Plummer, 1978; Valles and McArthur, 2001). Regarding to sensititvity to weed pollen, research in our area, as many in Europe, state ragweed pollen as main one (Wopfner *et al*., 2005). But, after ragweed pollen, the second most significant weed pollen is mugwort pollen (*Artemisia vulgaris*). Concidering that ragweed and mugworth have almost identical flowering season, clinical and serological studies indicate that the sensitivity to these two pollen often are associated (Asero *et al*., 2006). Considering the above mentioned, the main aim of this study was to analyze the seasonal dynamics of ragweed and mugwort pollen during seven year monitoring (2011-2017).

MATERIAL AND METHOD

Monitoring the concentration of ragweed and mugwort pollen during the pollination period (2011-2017) was conducted at the PI Agricultural Institute of Republic of Srpska, Banja Luka (N 44º47`41.0``, E 017º12`22.6``), by Hirst's type pollenometar (Hirst, 1952). Sampling of aero allergenic pollen was conducted in urban, industrial part of Banja Luka, using the method defined by the International Association for Aerobiology (IAA). The trap brand Burkard (Burkard Manufacturing Co., Uxbridge, Middlesex, England) is calibrated for sampling 10 liters of air/min through a orifice 14 x 2 mm diamtear, which always faces the wind direction and it is protected from direct rainfall. As air passing through the orifice, pollen grains are fixed on glass slides coated with silicone gel, which moves at the rate of 2 mm/h.

Visual identification, or qualitative and quntitative assessment, of sampled ragweed and mugwort pollen grains was carried out on a daily basis after 24 hours, based on the morphological characteristics under a light microscope Olympus BX51 at magnification x400, according the International Association for Aerobiology and converting the obtained results in the concentration of pollen grains per m^3 of air. Immediately prior to screening, microscopic slide with 24-hour segment, is prepared by placing polyvinyl alcohol substrate (Gelvatol), phenol, and glycerol, which allows color fuchsin staining of pollen grains and easy separation of the same from dust particles and fungy spores. After preparation and drying microscopic slide determining the number of pollen grains is carried out by the method of longitudinal lines in two-hour intervals and reviewing the 3 horizontal lines. At the end of the analysis, the obtained values are converted to daily concentrations determined by multiplying the number of pollen grains by a factor F, depending on the characteristics of the device for sampling, surface of 24 hour segment, characteristics of the microscope and the surface of the inspected sub-sample. Concentration of the pollen grains per m^3 of air is important for symptoms occurence of allergic reactions. Thus the monitoring results are presented to the public in the form of daily aero pallinological reports or so called "Pollen traffic light" on the official web site of City of Banja Luka (Table 1).

Tab. 1. Number of weed pollen grains in the air with the corresponding percentage of persons in whom is possible occurrence of symptoms of allergic reactions (Forsyth County Environmental Affairs Department Pollen Rating Scale, PRS)

Level of pollen	***Number of pollen grains /m³ air***	***Occurrence of symptoms of allergic reactions***
	Weeds	
Not present	0	No symptoms
Low	1-10	Only in extremely sensitive individuals
Moderate	11-50	In 50% of sensitive individuals
High	51-500	Almost all allergic people
Very high	>500	In all allergic people

RESULTS AND DISCUSSION

During the seven-year sampling (2011-2017) of aeroallergen ragweed and mugwort pollen in the city of Banja Luka dynamics, i.e. the beginning, duration and end of the pollination period with presenting daily low, moderate, high or very high concentrations (p/m^3) as well as a total weed pollen number on annual level (p/m3) was monitored. Annual total concentrations of mugwort pollen grains was 664 p/m^3 in 2011, 664 p/m^3 in 2012, 246 p/m^3 in 2013 and 2014, 273 p/m^3 in 2015, 302 p/m^3 in 2016 and 187 p/m^3 in 2017 (Figure 1). Annual total concentrations of *Ambrosia* pollen grains was 9587 p/m^3 in 2011, 8993 p/m^3 in 2012, 5004 p/m^3 in 2013, 4970 p/m^3 in 2014, 5478 p/m^3 in 2015, 5256 p/m^3 in 2016 and 6166 p/m^3 in 2017 (Figure 2). In addition, high concentrations in six years of monitoring (2012-2017) were recorded from third decade of August to the second decade of September; while in the same period very high concentrations (>501 p/m^3) were recorded only during 2011.

The first mugwort pollen grains in 2011 are recorded at the end of May, during 2012 and 2016 in the first decade of June, and from 2013 to 2015, as well in 2017 in the second decade of June. Mugwort pollination period lasted for an average of 55 days and it was characterized by low (1-10 p/m^3) to moderate (11-50 p/m^3) concentrations. The high concnetrations are recorded only in 2011 and 2012, and lasted for two days with peak of 84 p/m^3 in second decade of August. The maximum, i.e. moderate and high concentrations are recorded from first until the third decade of August.

The first ragweed pollen grains grains in 2011 are recorded in the second decade of July, during 2012 in the third decade of July, in 2013, 2014, 2016 and 2017 at the end of June and during 2015 at the begining of July. Low to moderate concentration are registred until the second decade of August to the third decade of September. During that period high concentrations (51-500 p/m^3) were recorded, while very high concentrations (>501 p/m^3) were recorded only in 2011. The ragweed period pollination during the seven-year monitoring lasted, on average, about 115 days (Figure 3-9).

On an annual basis not only the season of ragweed pollination lasted longer, but the results of the monitoring and comparative analysis showed significantly higher % share of ragweed pollen within the weed species in the family Asteraceae in the city of Banja Luka.

Thus, according to a annuall comparative analysis of recorded ragweed and mugwort pollen in the city of Banja Luka the participation of ragweed pollen was 94% in 2011, 93% in 2012 and 95% in 2013, 2014, 2015, 2016 and 97% in 2017.

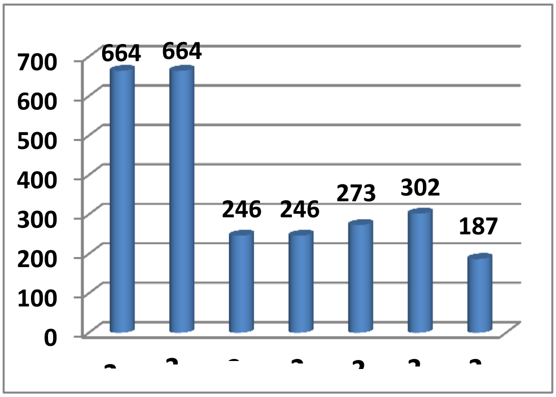

Figure 1. *Annual total concentrations of mugwort pollen grains in Banja Luka, 2011-2017*

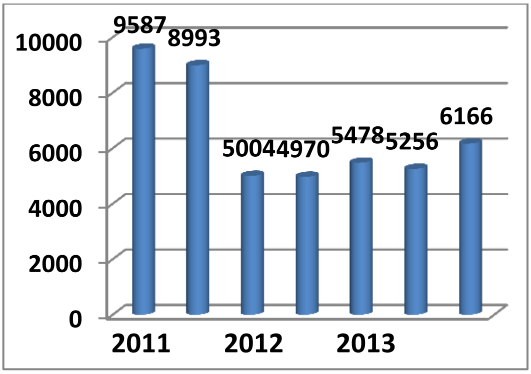

Figure 2. *Annual total concentrations of ragweed pollen grains in Banja Luka, 2011-2017.*

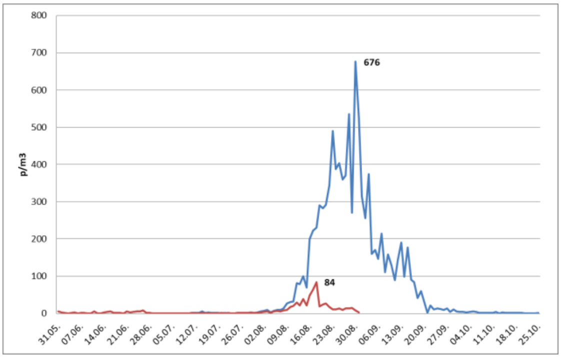

Figure 3. *Seasonal dynamics of mugwort and ragweed pollen in 2011*

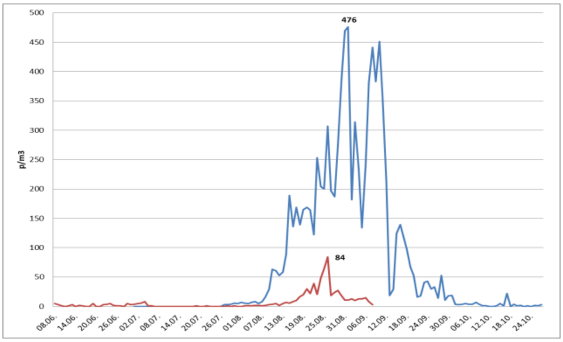

Figure 4. *Seasonal dynamics of mugwort and ragweed pollen in 2012*

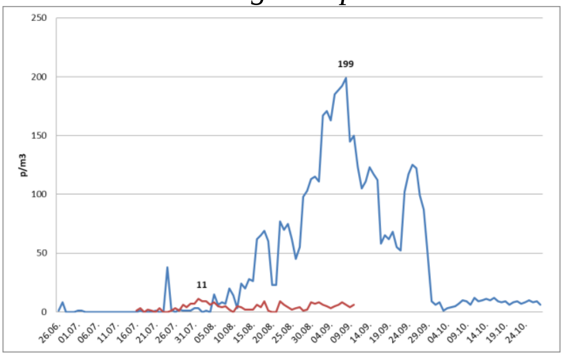

Figure 5. *Seasonal dynamics of mugwort and ragweed pollen in 2013*

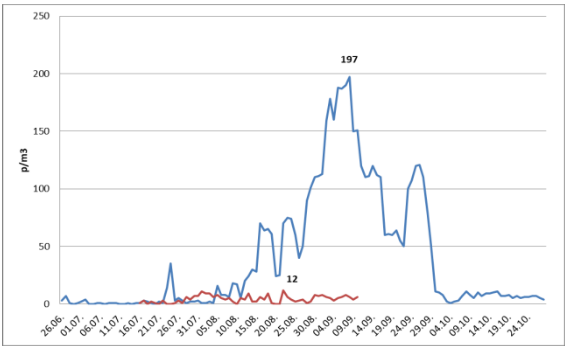

Figure 6. *Seasonal dynamics of mugwort and ragweed pollen in 2014*

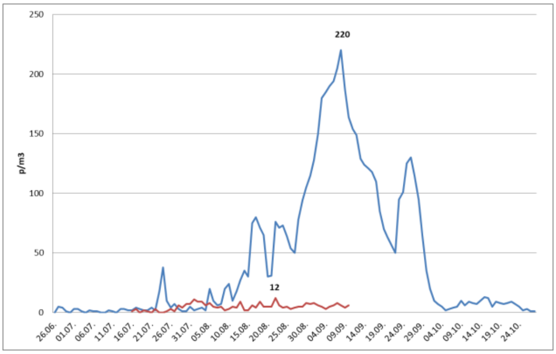

Figure 7. *Seasonal dynamics of mugwort and ragweed pollen in 2015*

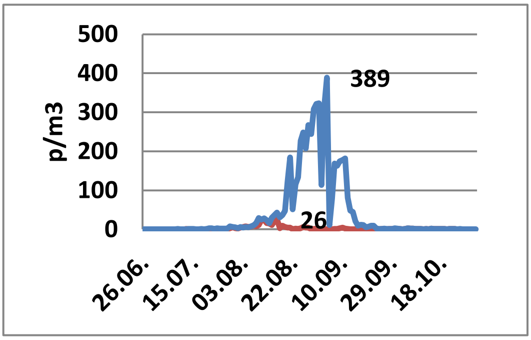

Figure 8. *Seasonal dynamics of mugwort and ragweed pollen in 2016*

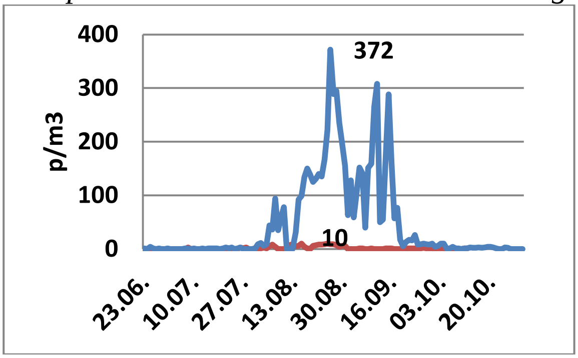

Figure 9. *Seasonal dynamics of mugwort and ragweed pollen in 2017*

CONCLUSION

On an annual basis the season of ragweed pollination lasted longer than mugwort pollination. Also significantly higher % share of ragweed pollen within the weed species in the family Asteraceae (>90%) is registered in the city of Banja Luka durin seven year monitoring. Never the less, previous results of the population analysis (late adolescence patients) by skin *prick* test to pollen from Clinical Center Banja Luka conducted in the ten-year period (2001-2010) shown that from the individual weed pollen in the total sample and by groups of respondents *prick* test is mainly positive to ragweed, but on the second place among weed pollen is mugwort pollen rated (Balaban *et* Balaban, 2012). According to Asero (2011) in the‘80s of the last century, before the sudden appearance of ragweed, mugwort sensitization was rarely observed; subsequently, the prevalence of mugwort sensitivity increased dramatically in parallel with the spread of ragweed. Thus, as well that ragweed is wide spread in our area, previous results from Clinical Center Banja Luka and conducted monitoring pointed out on *Ambrosia* and *Artemisia* pollen as a growing health and social problem in our country.

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NOBLE SUGARCANES AND MODERN CULTIVARS IN TAHITI RELATIVE TO ORGANIC RUM PRODUCTION: DESCRIPTION AND KEY CHARACTERISTICS

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ABSTRACT

Since the beginning of the twentieth century, various actions regarding sugarcane improvement were implemented. Researchers and breeders created new varieties for the sugar industry, more resistant to pests and diseases and more productive than noble sugarcanes. Today, modern cultivars are used for both sugar industry and distillery and noble sugarcanes are no more cultivated for this purpose. However, they could be cultivated in some particular contexts such as the organic cultivation, where the production costs are similar for both cane varieties. In Tahiti, a company decided in 2015 to produce organic rum from both noble sugarcanes and modern cultivars. A 2 835 m² experimentation was installed on a machineable land, representative to agriculture requirements. Six noble sugarcanes plus three modern cultivars all found locally (315 m² / variety) were tested. The agronomic yields reached were around 70 tons/ha for the best noble sugarcanes and around 100 tons/ha for the modern cultivars while at small-scale industrial processing, the noble sugarcanes present a greater juice extraction about 10 to 25% more than the modern varieties. In this situation, the organic cultivation of noble sugarcanes could be a valuable improvement regarding the whole agriculture and sugarcane industry in French Polynesia. In the future, we will also study the aromatic contents within the noble sugarcane plants and determine if it can improve the quality of the rum.

Keywords: *Noble sugarcane, sugarcane variety, organic cultivation, French Polynesia, Tahiti.*

INTRODUCTION

Sugarcane (Poaceae family) is considered native to New Guinea between 8 000 to 15 000 before Christ and it is recognized as *Saccharum officinarum* variety groups coming from *Saccharum robustum* (Artschwager & Brandes, 1958). According to these authors, the dispersal was done by human means to proximity islands and it was carried to Tahiti in French Polynesia by Polynesians people during their migrations between 500 and 1 100. At the same time, two groups of natural hybridized canes were observed in India and China as *Saccharum barberi* and *Saccharum sinense*, coming also from New Guinea (D'Hont et al., 2002). During the late 18th century, navigators such as Bougainville, Bligh or Cook contributed to disperse the Otahiti cane variety around the world for the sugar production. It was then worldwide cultivated until the years 1840 to 1890 where it was stopped and replaced by other varieties as the Cheribbon ones (Stevenson, 1965). Both of them were then replaced by the very first new varieties from new research centers in the 1890s because of the crop damages caused by pests and diseases. So the first breeding stations were built in Java, Barbade, Mauritius, Reunion, British Guiana, Queensland and Hawaii (Heinz, 1987). The first commercial intra-specific cross occurred between Otahiti and Cheribbon varieties at the Java station and at the same time, the variety H109 was created with different Otahiti canes (Lahaina) in Hawaii. In 1929, in the Java station, a major turning point was reached with the "nobilization" of some wild species such as *Saccharum spontaneum* crossed with *Saccharum officinarum* to create new varieties (inter-specific) with a new agronomic potential, the POJ 2878 being the first of all. It was the beginning of the expansion of the modern hybrid varieties (contrary to the noble *Saccharum officinarum*) we continue to use nowadays for both sugar and rum industry. For Stevenson (1965) and Heinz (1987), even if the Otahiti cane has never been outstanding as a breeding cane as opposed to the Cheribbon, breeding programs pedigrees show that it is a remote ancestor of POJ 2878 and other famous Javan and Hawaiian hybrids. Such an argument give the Otahiti cane a big interest to produce high value rum but today, in Tahiti, it is very difficult to recognize the varieties, even based on the old bibliography. Cuzent (1860) has made one of the best descriptions of the Tahitian canes but no collection was created and maintained until today (Vitrac *et al*., 2018). Moreover, we didn't found Otahiti canes in the following collection centers: USDA in the United States of America, CIRAD in Montpellier (southern France), CTCS in Guadeloupe (Caribbean's), eRcane (Reunion Island) and HARC (Hawaiian Agricultural Research Center). In 2016, HARC sent us some photos of the Lahaina variety they conserved but without any true identification corresponding to the Tahitian varieties. We decided then to collect study and cultivate them to see: (1) their morphology as nobles or hybrids varieties; (2) their agronomic behavior in terms of sensitivity to bioagressors (weeds, pests and diseases); (3) their industrial potential; and (4) their aromatic potential. The main differences between noble and hybrid sugarcanes of *Saccharum officinarum* being their morphology, yields (biomass and sugar), resistance to bioagressors (Stevenson, 1965) and machinability (Van Dillewijn, 1960). The sugarcane has only been developed from decades regarding sugar production (Fauconnier, 1991). For this reason, the stalks of hybrids are taller, containing a higher sucrose content and they are also thinner with a stronger skin than nobles varieties (Van Dillewijn, 1960). But no aromatic interest has motivated breeders and very few aromatic studies have been published or only in the sense of legislation (Boscolo *et al*., 2000; Cimino Duarte *et al*., 2017). Furthermore, many nonsucrose components like starch, ash, polysaccharides or organic acids are extracted from the canes inside the juice, and the difference in the nonsucrose components are often significantly influenced by the cane variety (Rein, 2017). The organic sugarcane has a strong economic potential in Tahiti and it was decided to establish one distillery in 2015 to produce organic rum. The following questions were then raised: if it is possible to cultivate some Otahiti canes, do they have some special key characteristics which should be valued for the rum industry? Do the hybrid varieties show some particularities? In such a context of a small-scale organic production, expensive hand labor is needed at every stages of the cultivation and transformation process (Vitrac *et al*., 2018). We finally supposed that among noble and hybrid varieties we could find some materials which can fit to biomass production and transformation requirements of such a context.

MATERIAL AND METHODS

Under organic standards, a field of about 1 ha was planted in a machineable context harvesting in private gardens 3,125 tons in 2015. A plot of about 2 835 m² was set up to evaluate seven varieties (315m² each) in 2015, and nine in 2016. Eight varieties were found around the island of Tahiti, and one was located in Taha'a in the same archipelago (Society Islands). The first canes were harvested 12 months later (December 2015) and first ratoon 24 months later (December 2016). A specific design using stripes was organized with one variety per stripe (composed of three rows) perpendicularly to a slope of about 3%. Before planting, original vegetation composed mostly of ferns was cut. Organic fertilization was then applied to correct the desaturated soil by spreading vinasse from the distillery (20t/ha), composted equine manure (5t/ha) and dolomite (2 t/ha). Minimum soil tillage was conducted (15cm deep) before creating furrows. Manual planting was done in paired rows with a distance between the plants of about 50cm and 1,6m (interrows), representing 20 000 cutting stalks / ha. Weed removing was conducted manually in the row and using a 4WD micro-tractor of about 16 horsepower (1,1m width) with a rotative disposer for the inter-row. Yields were estimated on 3 x 100 kg of fresh full hand-harvested canes by stripe, to get a range of data in this agricultural context. These canes were crushed one time (hand feeding three rolls 1t/h crusher), and the amount of juice was weighted for each sample of 100kg (giving us the crushing yield) and also for each whole stripe plot about 315m². We then deducted the production of sugarcane biomass per stripe. Regarding rat control, we counted and weighed the total amount of stalks damaged just after the shooting occurred. Finally, we added the weight of sugarcane produced and the weight of stalks damaged to get the global amount of sugarcane produced per stripe to calculate the total yield in tons/ha. The cultivation calendar for agricultural operations in the years of 2015 and 2016 is shown in table 1.

Table 1. Monthly cultural operations from plantation to the first ratoon.

	jan	feb	march	april	may	june	july	aug	sept	oct	nov
2015	G+H1	G+H1	G+H1		G+H1	R	G+H1		H2+R		H2+S
2016	G+H1		G+H1			B	G+H1 R- B	B	H2+R	H2+S	

G: rotative disposer; R: rat treatment (Brodifacoum 0,005%)
S: straw removing; B: Brix degree during growth
H1: hand hoeing before cane inter-row closure
H2: hand hoeing after cane inter-row closure

Regarding sampling / morphology, 30 canes of each variety were sampled regularly among the stripes one week before harvest in 2015 and 2016. Following UPOV (in french: Union Internationale pour la Protection des Obtentions Végétales) reference (2005), we measured the height (H), the diameter at H/2, the internodes and the tillering. We also measured the Brix degree (at the soil level, H/2 and H) with a portable ATAGO® refractometer. All of these measures being principal components of the aboveground biomass and alcohol amounts. We finally fermented (zymaflores® bio yeast 100 g / 1 000 l; pH = 3,5; 26 – 29 °C) and distillated (100 l pot still copper alambic) the sugarcane juice for each whole 315m² stripe to get the yield in Pure Alcohol hector-Liter (PAhL) / hectare (ha) with no repetition. The maturation and dilution process to get rums at 50° alc/vol. was done in stainless steel tanks as the following: (1) 10 minutes/day of aeration (by pumping and drenching over the rum inside the tank) during 1 month; (2) same aeration and adding of ceramic filtered water (0,1µm) to reduce alc/vol. rate about 5° every two days, 3 times a week. First hedonic sensorial analysis was done by an expert jury.

RESULTS AND DISCUSSION

Among the nine varieties cultivated, aboveground biomass yields ranged from 34,7 to 111,1 ton/ha in 2015, and from 34,9 to 92,8 t/ha in 2016. In the communication "organic sugarcane cultivation in Tahiti" (Vitrac *et al.*, 2018) 2 groups were identified based on their yields: the varieties with yields of more than 70 t/ha (3 varieties) and those with less (6 varieties). This result is reinforced regarding Brix degrees and showing the Otahiti canes (the noble *Saccharum officinarum*) to constitute the group with the lower yields and the lower Brix. Moreover, for this group, we observed the highest rat attacks in 2015 while treatments and maintenance of the field was correctly done and also in 2016. It is probably due to the thickness of the cane stalks which might be thinner than those not attacked, which is a characteristic of the noble sugarcanes (Van Dillewijn, 1960). The morphological sampling (Table 2) also showed a lower height, internodes and tillering and a higher diameter for these canes. It is then probable that this sugarcane group is representative to noble varieties. However, we didn't found any information about such measurements to confirm the Otahiti group in the literature (Cuzent, 1860).

Table 2. Morphological characteristics for the nine varieties after plantation and 1st ratoon. *Saccharum officinarum* are: RBV (Rouge à Bandes Vertes); JRP (Jaune à Rayures Pourpres) ; VE (Verte) ; 3C (3 Couleurs) ; PO (Pourpre) ; VBP (Verte à Bandes Pourpres). Modern varieties are : RRV (Rouge à Reflets Verts) ; JR (Jaune Roseau) ; BL (Blanche).

	varieties:		RBV	JRP	VE	3C	PO	VBP	S. off.	RRV	JR	BL	Hyb.
tillering	plant.	av.	5,27	3,03	5,10	6,73	4,07	8,83	4,84	7,07	5,37	13,33	6,22
		s.d.	2,49	1,73	2,14	3,57	2,03	3,21	1,39	4,42	1,69	3,54	1,20
	1st rat.	av.	6,80	2,80	5,00	5,03	4,93		4,91	15,00	7,37		11,18
		s.d.	4,50	1,47	3,09	2,97	3,80		1,42	5,02	3,92		5,40
internode	plant.	av.	7,10	5,89	6,46	5,73	5,10	6,19	6,06	8,56	12,37	10,53	10,46
		s.d.	1,91	1,05	1,30	1,20	1,37	1,43	0,76	1,84	2,52	1,19	2,69
	1st rat.	av.	6,75	7,82	6,44	5,14	5,64		6,36	8,97	9,06		9,02
		s.d.	0,62	0,77	1,28	0,90	1,49		1,04	1,12	1,87		0,07
diameter	plant.	av.	3,44	3,58	2,73	2,83	3,53	2,97	3,22	2,95	2,93	2,76	2,94
		s.d.	0,39	0,29	0,30	0,25	0,34	0,33	0,41	0,23	0,24	0,22	0,02
	1st rat.	av.	3,42	3,61	2,75	2,79	3,22		3,16	2,99	2,61		2,80
		s.d.	0,30	0,42	0,26	0,79	0,36		0,38	0,23	0,23		0,27
height	plant.	av.	1,58	1,88	1,36	1,34	1,13	1,20	1,46	1,94	1,88	1,32	1,91
		s.d.	0,34	0,31	0,21	0,23	0,19	0,32	0,29	0,29	0,29	0,17	0,04
	1st rat.	av.	1,49	1,89	1,21	1,02	1,04		1,33	1,90	1,98		1,94
		s.d.	0,20	0,17	0,32	0,35	0,26		0,37	0,31	0,26		0,05
Brix	plant.	av.	15,76	16,43	14,39	18,26	20,17	19,24	17,00	18,98	19,84	19,61	19,41
		s.d.	1,77	1,24	2,24	1,75	1,57	2,28	2,25	1,27	1,12	1,26	0,61
	1st rat.	av.	15,14	15,42	14,39	18,01	19,82		16,56	20,57	19,53		20,05
		s.d.	2,30	1,35	2,55	2,46	1,72		2,28	0,90	0,88		0,73

av. & s.d. : average and standard deviation relative to 30 canes sampled among each stripe
internode, diameter are in (cm) ; height is in (m) ; Brix is in degree or %age
S. off. & Hyb.: *Saccharum officinarum* and modern Hybrids averages and standard deviat- -tion between the varieties. VBP and BL wasn't counted because of their plantation in 2016

Sugarcane has been hybridized to produce more biomass and more sucrose to reach heavy yields in sucrose/ha. Crosses and selections have been made to make the sugarcane cultivars more resistant to diseases (Heinz, 1987) and easier to process (Rein, 2017). It is what we observe for the hybrids in the Table 1: (1) internodes are higher (10,46cm compare to 6,06cm after plantation); (2) diameter are lower (2,94cm vs. 3,22cm). Even if their stalk structure seems to be thicker regarding the low infestation levels by rats (Vitrac *et al.*, 2018), they correspond to sugar industry milling requirements and the fewer internodes they have the higher sucrose amount (Rein, 2017 & Moore, 2014). Finally, we can say that the group with RRV, JR and BL are considered modern varieties even found in Tahiti in 2015 without any information from the immigration or agricultural authorities. The other varieties seem to be pure *Saccharum officinarum* but nothing tells us they correspond to Otahiti canes. The use of molecular markers to compare these nine varieties should definitely allow us to separate these two groups and find or not the Otahiti canes by the introduction of Hawaiian Lahaina DNA from the HARC. Among the modern cultivars described, the JR seems to be the more productive even in cane biomass than in crushing yield and PAhl/ha. But we have to be carefully interpreting such a result because of the 3% of slope included in the plot. What it is interesting is the identification and characterization of two groups we should cultivate and experiment later. If 3C show the highest crushing yield after plantation (Figure 1) and good Brix (Table 2), we understand its attractive effect to rats (Vitrac *et al*., 2018) by the combination of thin skin, high sucrose content and precocity, which is also the case regarding PO and VE. However, in 1st ratoon, all the varieties showed a decrease not only regarding biomass and PAhL/ha and a gap regarding crushing for 3C, VE and PO. We now that no maintenance on the field occurred during 3 months during the grow stage (until June). Also we observed at this period less rains (1330 mm) than in 2015 (2093 mm). Maybe it induced functional biology tissues changes creating a curing of the skin in response of water stress (Lakshmanan & Robinson *In*: Moore, 2014) combined to weeds and rats pressure (Vitrac *et al*., 2018)? Such sensitivity indicated us some important limits for the agricultural use of these noble varieties in such a context, especially regarding 3C, VE and PO.

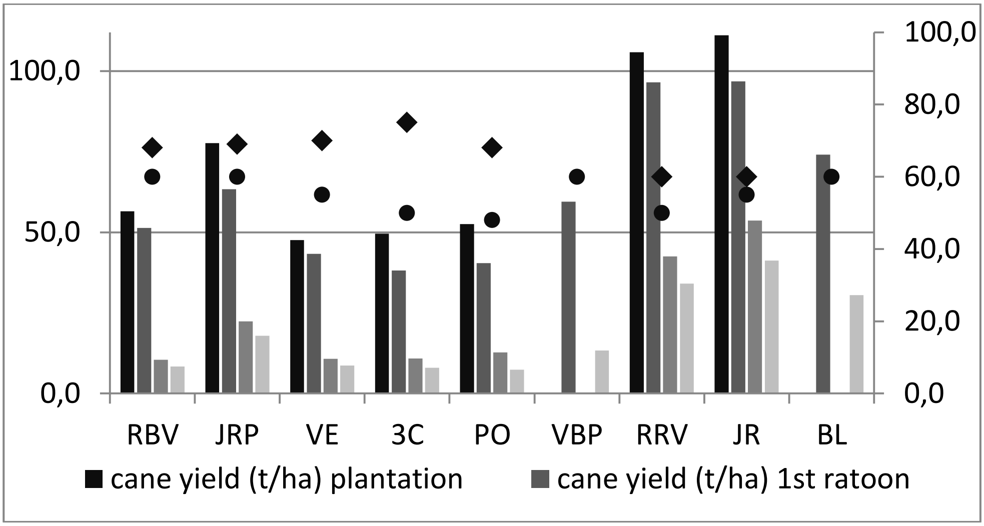

Figure 1. Yield data in cane biomass (t/ha) and Pure Alcohol hecto-Liter (PAhL/ha) (left axis) and crushing milling (%) referred to the axis on the right. All data are representative to the varieties cultivated after plantation and first ratoon.

There are few correlations between the first harvest after plantation and the different ratoons (Paulet & Glaszmann, 1994), the first being generally the most productive (Fauconnier, 1991). It was not the case in our agricultural context. As shown by Jamet (1987), the main cultivable soils are desaturated and ferralitics. But one noble variety, the JRP, showed a good potential for agronomic use with biomass yields of about 70 t/ha and 20 PAhL/ha for rum production. It also had a better crushing capacity (one press crushing) even if its diameter is higher than modern cultivars. It was the same in lesser extent for the RBV. It is thus possible that with innovative agronomic systems and some improved crushing methods, we reach better yields. After distillation (without any repetition), hedonic sensorial analysis gave us neutral results regarding the modern varieties. No acetaldehyde compounds were detected showing the quality of the fermentation process. The noble sugarcanes show a panel of different compounds, especially JRP and RBV with some notes of white truffle (DMS – Dimethyl Sulfide) which are generally observed in winery (Dagan & Schneider, 2013). Among varieties, context, climatic factors, human methods, transformation process and breeding of alcohols, there are too many points to investigate and it is difficult to draw any conclusion about aromatic advantages of noble sugarcanes. However, this study constitutes a first step to search if sugarcanes can be bred not only for sugar production but also for their aromatic qualities.

CONCLUSIONS

We found locally nine different varieties, 3 are modern hybrids cultivars and 6 are noble *Saccharum officinarum* sugarcanes. In our type of soils, the hybrids were productive even under organic standards. The noble canes seem to be very sensitive and difficult to cultivate and no Otahiti cane were found. However, two of them showed a great potential for agricultural purpose associated with aromatic particularities of the processed rum.

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GENETIC RELATIONSHIPS AMONG *PISTACIA VERA* L. F1 HYBRIDS AND THEIR PARENTS (P. *VERA*×HERMAPHRODITE GENOTYPES OF *P. ATLANTICA*) USING SSR MARKERS

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ABSTRACT

This research was conducted at the Scientific Agricultural Research center in Sweida province during (2014-2015). Breeding program was assessed in the aim to insert the bisexual phenomena of *P.atlantica* species (3 different hermaphrodite genotypes PA12, PA35, and PA37 as donators of pollen grains) to the commercial cultivars of *P.vera* (Ashouri and Batouri). Genetic relationships among the previous species and their progenies (F1, 6 genotypes of crossing program) was studied using 20 specific SSRs primer pairs, 16 of them were able to detect PCR amplification. Simple Sequence Repeat (SSR) segregation produced 44 putative alleles, out of which 40 were polymorphic (90.91%). Genetic similarity between the hybrids and their parents were closer to their female than to their male parents except for the hybrid HB3,which revealed a genetic distance 0.37 with its female parent (Batouri cultivar FB) and 0.43 with its male parent (PA35 hermaphrodite *P.atlantica* genotype). The UPGMA cluster plots based on Jaccard's coefficient grouped the genotypes into two main clusters. The number of alleles revealed by each SSR analysis ranged from 1 to 8, with a level of expected heterozygosity (He) 0.496, observed heterozygosity (Ho) 0.25, and Marker Index (MI) 19.84. These results suggested the efficiency of SSR markers for distinguishing lineage genetic studies in the *Pistacia* spp. in breeding programs to elicit new cultivars, in particularly the primer pairs Ptms-7, EPVM021, EPVM016, and EPVF019 which may form the platform to detect sex expression in the genus *Pistacia*.

Keywords: *pistachio, P.atlantica,hermaphrodite, SSR, genetic similarity.*

INTRODUCTION

Pistacia vera L. belongs to *Anacardiaceae* family, and it is one of the most economic trees in the world. All species of the genus *Pistacia* are recognized by their high genetic diversity at DNA level (Baghizadeh and Dehghan, 2018). However, Pistachio breeding programs face a lot of complicated obstacles, since it is dioecious species and therefore the potential of male parents is unknown. Pistachio juvenility cycle is somehow too long to determine the sexual genotype in early years after cultivation, because the heritage probability should be (theoretically) half for each male and female parent. In this prospect, molecular markers could facilitate breeding and allow early seedlings selection, saving time and economic resources (Vendramin *et al.*, 2009). SSR markers have been developed in some species of genus *Pistacia* (Zaloglu *et al.*, 2009; Kafkas *et al.*, 2009; Salimi *et al*., 2009).Indeed, all *Pistacia* species are dioecious and their flowers are unisexual except of some exceptional individual specimens of *P. atlantica* and *P. terebinthus* species (Kafkas, 2002; Isfendiyaroglu, 2007; Abdelkader *et al.*, 2009). Our previous study identified two new specific monoecious genotypes of *P. atlantica* (A9 and A10); in the first genotype all the flowers in the raceme are hermaphrodite with only 1-2% 0f female flowers, whereas the other genotype has four patterns of racemes (Alhajjar *et al.*, 2011). These exceptional transsexual genotypes are of importance in relation with pistachio breeding programs. Ziya Motalebipour *et al* (2016) indicated that the genome size of pistachio is about 600 Mb with a high level of heterozygosity, subsequently, this information may help in assessing stratigies for sequencing all pistachio genome. Turkeli and Kafkas (2013) assessed the first linkage map in pistachio using an inter-specific cross between *Pistacia vera* L. (siirt cultivar) and monoecious *Pistacia atlantica* Desf. (Pa-18 genotype), using ISSR, SRAP and AFLP markers. In this investigation, breeding program was assessed using three monoecious and hermaphroditic *P. atlantica* genotypes as donators and two *P. vera* female cultivars (Ashouri and Batouri) as receptors. Genetic relatedness of F1 progeny to their parents (*P. vera* × bisexual *P. atlantica*) was studied using 20 SSR markers.

MATERIALS AND METHODS

Plant Material

Three monoecious/hermaphroditic *P. atlantica* genotypes (PA12,PA35 and PA37), two commercial female *P. vrea* cultivars (Batouri FB and Ashouri FA) as receptors were used. Also, 6 F1 hybrids (HA1, HA2, HA3, HB1, HB2 and HB3) as a consequence of cross pollination between the previous species.

Methods

DNA Extraction**:** By using CTAB protocol (Porebski *et al*., 1997).

SSR markers: Extracted DNA was PCR-amplified using 20 SSR primer pairs which were previously cloned and sequenced in *P. vera* according to (Ahmad *et al.*, 2003; Vendramin *et al.*,2010)

Data Analysis

The amplified alleles were scored either as present (1) or absent (0).Genetic similarity (GS) between any two genotypes was calculated depending on Jaccard's similarity coefficient (Jaccard, 1908). A dendrogram was constructed using the unweighted pair group method using arithmetic averages UPGMA (Sneath and Sokal, 1973). Number of alleles, observed heterozygosity (Ho) according to (Wunch and Hormaza, 2007)., expected heterozygosity (He) according to (Lorenzo *et al*., 2007), and Marker Index (MI) were also determined to estimate the efficiency of SSR technique(Powell *et al*., 1996). The software used through this study was Microsoft EXCEL, SPSS17, and Past.

RESULTS AND DISCUSSION

Levels of polymorphism

Twenty SSR primer pairs were applied, 16 of them were able to detect the polymorphism, and revealed 44 alleles across all genotypes, 40 alleles were polymorphic (90.91%). These results were in agreement with Arabnezhad *et al.* (2011) as they used 18 SSR primer pairs developed from *P. khinjuk* genome. All primer pairs produced 1-8 putative alleles each with an average 2.75 alleles per locus, and the highest number of putative alleles were revealed in the progeny HB3 (FB×PA35), Table (1). Baghizadeh *et al.* (2010) reported that in SSR population analysis, four SSR primers produced 11 alleles among 31 pistachio genotypes with an average value of 2.75 alleles. The genomic EST-SSRs primer pairs (EPVM021, EPVM016, EPVF019, and EPVM056) gave sufficient polymorphism in all F1 genotypes and their parents of the two studied *Pistacia* species (*P. vera* L. and bisexual genotypes of *P. atlantica*) and were more effective in comparison with those constructed by (Ahmad *et al.*, 2003) except Ptms-7 primer pairs, Figure (1). However, some of investigated loci (EPVM021 and EPVF019) revealed specific variation between individuals within the certain locus, which reflect the importance of these loci in selection programs. However, Primers (Ptms-11, Ptms-14, Ptms-42) revealed monomorphic alleles, whereas the other primer pairs revealed polymorphic alleles. The progeny HB3 (FB×PA35) was recognized by 7 alleles using EPVM021 primer pairs, 3 of which were unique alleles (904, 748 and 633 bp, respectively) (Table 1). Using EPVM021 primer, the allele 147 bp was mutual between all *P. atlantica* bisexual genotypes and F1 genotypes (HA1, HA2, HA3, HB2 and HB3) except the hybrid HB1 (FB×PA12) which was matched with its male parent PA12. EPVM016 primer was able to detect heterozygosity in the hybrid HB3, which were 488 bp from FB3 and 550 from PA35 monoecious *P. atlantica* genotype. Heterozygosity was also detected in HA1, HA2, HA3, HB1 and HB3 hybrids using EPVF019. Also, Ptms-7 primer was able to detect heterozygosity in all studied genotypes, and it labeled the HB1 (FB×PA12) by 3 alleles. Allele size ranged between 76 bp in Ptms-11primer to 904 bp in EPVM021 primer. Vendramin *et al.*, (2010) indicated that the allele size ranged from 206 to 609 bp in pistachio, while it ranged from 213 to 766 bp in *P.integerrima*, and 219-766 bp in *P.terebinthus*.

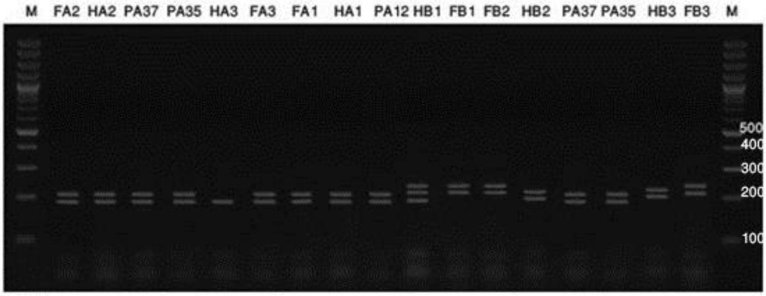

Figure (1). Putative alleles produced by using Ptms-7 SSR primer pair on studied *Pistacia* spp. genotypes (FB1, FB2 and FB3: Batouri FB; FA1, FA2 and FA3: Ashouri FA). M: 100 bp ladder

Table (1): Total number of alleles produced by 16 SSRs primer pairs, number of polymorphic alleles, polymorphism percentage and allele size (bp)

No.	**Primer**	**alleles No.**	**Polymorphic Alleles**	**Polymorphism%**	**Alleles Size bp**
1	Ptms-3	2	2	100	129-138
2	Ptms-7	4	4	100	182-202-222-242
3	Ptms-9	2	2	100	116-125
4	Ptms-11	1	0	0	76
5	Ptms-14	1	0	0	108
6	Ptms-31	2	2	100	121-129
7	Ptms-33	2	2	100	176-184
8	Ptms-40	3	3	100	192-213
9	Ptms-42	1	0	0	183
10	Ptms-45	2	2	100	154-163
11	EPVF021	7	6	88	101-147-307-439-491-633-748-904
12	EPVF013	2	2	100	633-690
13	EPVF016	2	2	100	488-550
14	EPVF056	3	3	100	300-410-520
15	EPVF019	8	8	100	667-532-377-318-202-196-161-112
16	EPVM058	2	2	100	249-273
	SUM	44	40	90.91	
	AVE	2.75	2.5		

Genetic similarity

Genetic similarity ranged from 0.24 between both *P. vrea* female cultivars; Ashouri and Batouri (FA and FB) with PA37 monoecious *P. atlantica* genotype, to 0.96 between two F1 progenies HA1 (FA×PA37) and HA2 (FA×PA12). Genetic similarity between the two studied cultivars of *P. vera* (Ashouri and Batouri) was 0.79. The genetic distances between all bisexual genotypes of *P. atlantica* and *P. vera* female cultivars (Batouri and Ashouri) were 0.282 and 0.264 respectively.

Genetic distances between the hybrids and their parents were closer to their female parents (FA and FB) than to their male parents (PA12, PA37 and PA35) except for the hybrid HB3 (FB×PA35) which revealed genetic distances 0.37 with FB and 0.43 with PA35. Pazouki *et al.* (2010) reported that genetic similarity within *Pistacia* spp. ranged from 0.03 to 0.8.

Clustering and genetic relationships among all studied genotype

The pattern of cluster analysis based on Jaccard's coefficient and UPGMA algorithm clustered the genotypes into two main clusters; the first cluster divided into three sub clusters, the first one branched into two groups, the first group contained all F1 hybrids 'between female Ashouri cultivar and all P. atlantica bisexual genotypes and the second group contained the hybrid HB1 (FB×PA12). The second sub-cluster divided into two groups; the first one contained female cultivars (FA and FB), and the second one contained only the progeny HB2 (FB×PA37). Whereas the progeny HB3 (FB × PA35) was
clustered in the third sub cluster. All P. atlantica bisexual genotypes were grouped in the second cluster which divided into two sub-clusters, the first one contained both of PA12 and PA37, while PA35 genotype was located in the second sub cluster Figure (2)

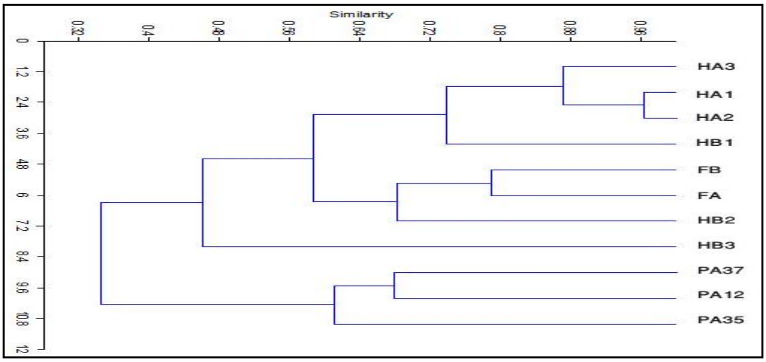

Figure (2): cluster analysis for F1 progenies and their parents using Jaccard's coefficient

To evaluate the efficiency of SSR microsatellite markers for polymorphism detection; Observed and Expected Heterozygosity (Ho and He), in addition to Marker Index (MI) were calculated. Observed heterozygosity Ho was appeared in 3 of 16 SSR loci giving an average value 0.25. Expected heterozygosity depending on allele frequency was 0.496 in all polymorphic tested loci. Marker Index (MI) was19.84. Kolahi-Zonoozi et al. (2010) indicated to a value of Observed Heterozygosity (Ho) 0.490, while the Expected Heterozygosity (He) was 0.345 in their study by using 12 primer pairs.

CONCLUSION

Depending on this investigation, it be concluded that SSR marker is an informative technique which revealed high ability to differentiate individuals and played an important role as genetic marker for lineage studies, and inheritance of sex expression as some of studied primers showed, and may form the platform to detect the sex expression in the genus Pistacia. Since hybrids (HB1, HB2 and HB3) revealed specific loci using different SSR primer pairs (particularly the hybrid HB3), they may form an essential platform for further breeding programs concerning hermaphroditism in P. vera species, in addition to introduce the male parent PA35 for this purpose. On the other hand, advances approach should be continued at different DNA levels using different informative molecular markers for sexual determination in pistachio.

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ALTERNATIVE SUBSTRATE USE IN SAGE TRANSPLANTS PRODUCTION *(SALVIA OFFICINALIS* L.)

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ABSTRACT

Mushroom production has become more popular in our environment. The most common cultivated mushroom is *Agaricus bisporus.* After mushrooms are harvested a large amount of used compost remains. This compost is a good material and producers used it as alternative substrate in plant production. The benefits of this compost are numerous like high content of organic matter and the rich mineral composition. The aim of this study was to determine the effectiveness of the use of spent mushroom compost (as alternative substrate) on growth and development of roots and above-ground parts of sage transplants (*Salvia officinalis* L.). Measurements of morphological parameters of plants (plant height, number of leaves, number of branches and plant diameter) were performed, as well as determination of fresh and dry weight of roots and above-ground parts of transplants. Application of spent mushroom substrate in the production of sage *Salvia officinalis* L. positively influenced growth and development as well as fresh and dry weight of roots and above-ground parts of treated plants compared to non-treated plants during transplanting growing stage. Plant height (+104%), number of leaves (+65%), number of branches (+143%), plant diameter (89%), were significantly increased by the spent mushroom compost application compared to the control - commercial substrate.

Keywords: *spent mushroom compost, seedlings, sage.*

INTRODUCTION

The most important problem for growing transplants in the greenhouse is provision of adequate growing media. One option to approach this subject is the use of compost that is the most economical and sustainable for organic waste management (Nair *et al*., 2005). The mushroom industry is very increasing and it has been estimated that more than 10 million metric tons of spent mushroom compost, by-product of *Agaricus bisporus* mushroom, are produced worldwide (Philippoussis *et al*., 2004; Lau *et al*., 2003). After mushroom harvesting, storage or transportation of this spent material lead to environmental contamination and costs for mushroom growers. One solution for reducing spent mushroom compost is to utilize it for plant growing media in agriculture. The spent mushroom compost is more consistent and hygienic than other composted materials, but fresh spent mushroom compost has a high proportion of potassium (K), phosphorus (P), sodium (Na), and calcium (Ca) salts. High salinity is toxic to plants and has limited its wide application in agriculture. Spent mushroom substrates are often spread onto land and allowed to weather for several years. This allows salts and nitrates (NO3) to seep out of spent materials (Gonani *et al*., 2011). However, weathering is not enough to reduce salinity to a satisfactory level. Leaching is one of the possible options to more strongly reduce the salinity of spent mushroom compost (Riahi and Arab, 2004; Riahi and Azizi, 2006). Leached spent mushroom compost has less salinity, but nitrogen (N) and carbon (C) and most essential elements as well as microbial characteristics do not change significantly compared to non-leached spent mushroom compost (Riahi *et al.*, 1998). Spent mushroom compost is rich in organic matter and constitutes an important source of macro- and micronutrients for plants and microorganisms thereby increase the soil micro flora, soil biological activity and enhance soil enzyme activity (Debosz *et al.*, 2002; Crecchio *et al.*, 2001). It contains calcium carbonate ($CaCO_3$), which provides short term buffering of the acidic waters and elevates soil pH (Rupert, 1994). Combusted farm manure is the most commonly used fertilizer in order to enrich soil and better yield. Well combusted farm manure usually yield better crop production. However, high quality farm fertilizers increase the cost. This facts forces the farmers to emphasize alternative methods for development of an efficient and quality soil improvement approach (Özgüven, 1998). This experiment was conducted to determine the effects of spent mushroom compost on growth and development of sage transplants (*Salvia officinalis* L.). The effects of spent mushroom compost on plant hight, number of leaves, number of branches and plant diameter, as well as fresh and dry weight of roots and above-ground parts of transplants were studied.

MATERIAL AND METHODS

This study was conducted in a greenhouse at Faculty of Agriculture, University of Banja Luka, Republic of Srpska/BiH. In the experiment were used already rooted sage cuttings which are growing on family farm Šušak in Prnjavor (photo 1.). For rooting cuttings were used a commercial substrate suitable for sowing seeds and cuttings Fruhstorfer Erde type: *Aussaat und Stecklingserde* from Hawita EU manufacturer. The substrate contains perlite for better aeration and friability. Other substrate characteristics are: pH 5.9; N mg/l=80; P_2O_5 mg/l=60; K_2O mg/l=90; EC 50 ms l; retention capacity 700 ml/l. Sage plantlings were transplanted into 9 cm diameter plastic pots. Experiment was set up as a split-plot design with four replications and two treatments - a commercial substrate (A1) and a mixture of commercial substrate and spent mushroom compost (button mushroom) at a ratio of 70:30 (A2). The trial consisted of a total of 80 plants, which are divided into two groups with 40 plants in each group and 10 plants per replication (photo 2.).

Commercial substrate: Klasmann-Deilmann substrate - *Potground H*, pH 6.0, very fine structure, with a mixture of white and black sphagnum peat was used as a control for sage transplants. Spent mushroom compost was previously mixed with garden soil and composted for six months and was used as a treatment . Pots were placed in greenhouse condition and slow-acting fertilizer was used only in control plants. Control plants had a slight increased and 0,3% concentration of *Yara* red cristalone 12+12+36+micro was applied. Growth promotion was recorded in every 7 days intervals during the experiment. The following morphological parameters were recorded: plant height (cm), number of leaves, number of branches, plant diameter (cm). At the end of experiment, the root was cleaned from the substrate, washed with water, and after that was measured fresh weight of above ground part and root. The plant material was placed in chamber dryer. After drying to a constant mass weighting of the dry root and above ground part was perfomed. The obtained data were statistically analyzed and the differences between specific substrate were calculated using analysis of variance with computer program VVSTAT (Vukadinović, 1994).

Photo 1. Rooted sage cuttings

Photo 2. Experiment after one month

RESULT AND DISCUSSION

Effects of spent mushroom compost on plant height (cm), number of leaves, plant diameter (cm) and number of branches are shown in Table 1. As seen in the table the effects of spent mushroom compost were statistically highly significant during transplants growth on all investigated morphological parameters. Results showed the amendment commercial substrate with 30% spent mushroom compost increased plant height highly significant (p=0.01). The largest height was observed in A2 - tretament with spent mushroom compost (11.8 cm), and the lowest in A1 - control (5.8 cm). Number of leaves were more in A2 (25.5) and less in A1 (15.5). It is the same with plant diameter - the largest in A2 with 10.6 cm, and the lowest in A1 with 5.6 cm; and with number of branches - more in A2 (4.4) and less in A1 (1.5).

Table 1. Effects of spent mushroom compost (SMC) on morphological parameters of sage transplants - *Salvia officinalis* L. (A1- control - commercial substrate - Potground H; A2- treatment - spent mushroom compost+Potground H)

Treatment (A)	Plant height (cm)	No. of leaves	Plant diameter (cm)	No. of branches
Control (A1)	5.8	15.5	5.6	1.5
Treatment (A2) SMC+Potground H	11.8	25.5	10.6	4.4
Average	8.8	20.5	8.1	3.0
Analysis of variance - F	*427.94***	*15.61***	*263.43***	*255.68***
LSD	Plant height (cm)	No.of leaves	Plant diameter (cm)	No. of branches
0,05	***0.71***	***6.22***	***0.75***	***0.45***
0,01	***1.08***	***9.43***	***1.13***	***0.69***

After analyzing the morphological parameters of growth and development of sage transplants (*Salvia officinalis* L.) measurement of fresh and dry weight of plants were carried out and the obtained results are shown in Table 2.

Table 2. Effects of spent mushroom compost (SMC) on fresh weight (FW) and dry weight (DW) of root and above ground part of sage transplants - *Salvia officinalis* L. (A1- control - commercial substrate - Potground H; A2- treatment - spent mushroom compost+Potground H)

Treatment (A)	Above-ground part FW (g)	Above-ground part DW (g)	Root FW (g)	Root DW (g)
Control (A1)	1.7	0.2	0.7	0.1
Treatment (A2) SMC+Potground H	6.9	1.3	3.0	0.5
Average	4.3	0.8	1.8	0.3
Analysis of variance - F	*480.81***	*391.42***	*52.56***	*266.37***
LSD	Above-ground part FW (g)	Above-ground part DW (g)	Root FW (g)	Root DW (g)
0,05	***0.59***	***0.13***	***0.79***	***0.06***
0,01	***0.89***	***0.20***	***1.19***	***0.09***

In the table 2. it is visible that additional of 30% of spent mushroom compost increased fresh and dry weight of root and above-ground part of sage transplants and it is also statisticaly highly significant (p=0.01). The largest fresh above-ground part and root weight were recorded in A2 - treatment with spent mushroom compost (6.9 g and 3.0 g), and the lowest in A1 - control (1.7 g and 0.7 g). As well, dry above-ground part and root weight were more in A2 (1.3 g and 0.5 g) and less in A1 (0.2 g and 0.1 g). All of the recorded parameters of growth and development were influenced by treatment, with greater plant height, plant diameter, number of branches, fresh and dry above-ground and root weight of the plants growing in alternative substrates (photo 3. and photo 4.)

Photo 3. Treatment and control sage transplants

Photo 4. Treatment and control sage transplants at the end of experiment

Numerous researchers confirm that disused mushroom compost can be used as substrate for successful cultivation of many crops (Dubsky and Sramek 2009; Vukobratović, 2008; Polat *et al.,* 2009; Gonani *et al.,* 2011). Lemaire *et al.* (1985) reported that the used mushroom compost can not be used alone due to weak water permeability, high salinity and neutral pH which is not suitable for all horticultural plants. Çelikel and Çağlar (1997) reported that higher yield and earliness for tomato and cucumber growth were found in mixtures of peat and spent mushroom compost (1:1) than for the plants that were grown in a garden soil. In the cultivation of marigold (*Tagetes patula* L.) does not recommend the use of spent mushroom compost in an amount greater than 50% relative to the amount of commercial substrates, due to the impossibility of controlling the substrate and the low salinity water capacity (Young *et al.* 2002). Spent mushroom substrate of oyster mushroom and button mushroom compost are good sources of biofertilizer as they influence the growth of *Capsicum annuum* positively. These not only affect the growth but also affect the physiochemical properties (Roy *et al.,* 2015). According to Zeljković *et al.* (2015) the application of spent mushroom compost can be used in the production of anise *Pimpinella anisum* L. as a supplement to commercial substrate caused an increase number of leaves and plant hight more than 50% compared with control. Also, spent mushroom compost can be used in the production of *Pelargonium peltatum* L. and *Petunia hybrida* Juss. transplants because of a positive impact on the growth and development of roots and above-ground parts (Zeljković *et al*., 2015a). Ahlawat *et al*. (2010) confirmed that the spent mushroom compost and its associated microflora can be used in bioremediation of fungicides. Application of spent mushroom compost in the production of geraniums *Pelargonium peltatum* L. und *Pelargonium zonale* L. positively influenced growth and development of morphological parameters and the fresh and dry weight of roots and above-ground parts of treated plants compared to non-tretated plants during two growing seasons (Parađiković *et al.*, 2017).

CONCLUSION

This study confirmed that the spent mushroom compost can be used in the production of *Sage officinalis* L. transplants as a supplement to commercial substrate in the amount of 30%. Efficient use of spent mushroom compost is reflected in an increase in the average measured value of morphological parameters of growth and development and the increase in fresh and dry weight of above ground parts or root relative to the average value of control plants. In some measured parameters values of treatments are bigger two or three times then a values obtained in control which is more then 100% increased by spent mushroom application. From the above results, it can be concluded that spent mushroom compost of button mushroom (*Agaricus bisporus*) are good sources in the preparation of alternative substrates in the production of sage transplants. Reducing the mushroom waste and re-using the spent mushroom compost as a component of growing media was the main objective of many studies. The use of spent mushroom compost as an additive to growing media not only would be economically advantageous but also, field inventories of the waste byproduct and minimize the contamination of ground-water (Gonani *et al.*, 2011).

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MICRONUTRITIENT VARIABILITY IN MAIZE INBRED LINES

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ABSTRACT

Development of micronutrienten riched staple plant foods through plant breeding holds promise for sustainable and cost-effective food-based solutions to combat micronutrient deficiencies. The first step in this process is screening aviable germplasm for micronutritient content, so the aim of this study was determination of carotenoids and tocopherols content in set of maize inbred lines. Carotenoids (lutein, zeaxanthin and *β*-carotene) and tocopherols (α, β+γ and δ) content in 101 maize inbred lines with different kernel type (37 orange, 29 yellow, 4 white,19 sweetcorn and 12 popcorn) were determined by HPLC-DAD. The mean values of L+Z, β-carotene, α-tocopherol, β+γ tocopherol, and δ-tocopherol, were 31.34, 8.72, 10.22, 49.17 and 1.81 μg/ g, respectively. Content of α -tocopherol was in the range from 2.22 to 38.14 μg/g and β+γ tocopherols from 12.10 to 105.52 μg/g, β-carotene 1.20 to 39.37 μg/g and lutein+zeaxanthin 11.28 to 69.31 μg/g. White maize lacked carotenoids in the endosperm due to the presence of recessive genes. The highest value of β-carotene had inbred line H, L+Z inbred W-4, γ-tocopherols KRW 803-3-1-2-1 and α –tocopherol P21. Orange kernel inbred lines had the highest value of L+Z and β-carotene, yellow kernel inbred lines α –tocopherol, whereas sweetcorn inbreds had the highest value of γ-tocopherols. The genetic background undoubtedly influences chemical quality and line with high content of particularly micronutritients may be used in breeding program to improve nutritional value.

Keywords: *biofortification, carotinoids, maize, tocopherols.*

INTRODUCTION

Maize as one of the most widely cultivated cereals in the world have several types of kernal, with colours such as orange, yellow, red, black, and blue. Pigmented maize has received increased attention from a nutraceutical perspective because it contains several bioactive phytochemicals such as carotenoids, tocopherols, phytic acid and phenolic compounds (Bacchetti *et al.*, 2013). Generally, maize exhibits considerable natural variation for kernel carotenoids. The carotenoids present in maize grain are classified into carotenes (*β*-carotene, *α*-carotene) and xanthophylls (lutein, zeaxanthin and *β*-criptoxanthin), with higher concentrations of lutein and zeaxanthin compared to other carotenoids.

In plants, carotenoids prevent chlorophyll from photo-oxidative damage and protect membranes from lipid peroxidation, serve as precursors to abscisic acid (ABA), which regulates plant growth, embryo development, dormancy, and stress responses (Johnson et al. 2007). Carotenoids also have significant nutritional value for humans by providing precursor molecules required for the synthesis of vitamin A. The efforts thus directed to increase provitamin A and non-provitamin A carotenoids in maize will have positive impact on the health and well-being of humans, especially women and children. Tiwari et al. (2012) and Sivaranjani et al. (2013) observed positive correlation between kernel colour and total carotenoids but kernel colour is an unpredictable indicator for β-carotene so visual selection based on kernel colour will mislead in selecting provitamin A-rich genotypes (Harjes et al. 2008). Intensive efforts are currently being undertaken to develop maize inbreds enriched with carotenoids especially provitamin A (Babu *et al.* 2013; Frano *et al*. 2014).

Tocopherols (*α*-, *β*-, *γ*-, *δ*-), the subgroup of vitamin E, are biologically active compounds. There is natural variation among different maize inbred lines for levels of tocopherols. The two predominant isomers present in maize grain are gamma-tocopherol and alpha-tocopherol. Alpha-tocopherol is considered more desirable for human and animal consumption because it has higher biological activity than gamma-tocopherol. Most maize inbred lines naturally have much more gamma-tocopherol than alpha-tocopherol. Therefore a breeding goal is to increase levels of alpha-tocopherol relative to gamma-tocopherol. However, recent research suggests that gamma-tocopherol and compounds metabolized from it have properties important to human health that are unique from properties of alpha-tocopherol. Therefore it may be desirable to not only increase levels of alpha-tocopherol in maize grain, but also levels of gamma-tocopherol.

MATERIAL AND METODS

A diverse panel of 101 inbreds having different kernal type and color was selected and grown at Zemun Polje in 2017. Of them, 70 are inbreds with standard kernal type: 37 with orange color (1-37), 29 with yelow kernal (38-66), 4 white inbred lines (67-70), 19 sweetcorn (71-89) and 12 popcorn (90-101) inbred lines. Maize seed were milled (Perten 120, Sweden) into flour (particle size < 500 μm), in order to obtain greater surface contact and stored at -20˚C prior to analysis.

Carotenoids were extracted according to the slightly modificated method proposed by Rivera *et al* .(2012). Briefly, 0.15 g of the grain maize sample was extracted twice with 5 mL of mixture methanol-ethyl actetate (6:4, v/v) for 30 min at ambient room. Collected extracts after centrifugation (3000 rpm, for 5 min) were evaporated under the stream of nitrogen to the dryness and redissolved in 1mL of the mobile phase. Afterwards, extracts were filtered through syringe filter to be analyzed by Dionex UltiMate 3000 liquid chromatography system (Thermo Scientific,Germany) fitted with photodiode array detector (DAD–3000) analytical column, Acclaim Polar Advantage II, C18 (150 × 4.6 mm, 3 µm). Mixture of methanol-acetonitrile, (90:10, v/v) was used as mobile phase. The flow rate was 1 mL/min, temperature of column was set at 25 ºC and the injection volume was 50 µL. Chromatograms were generated at 450 nm and 470 nm. Tested carotenoids were identified and quantified by comparing characteristic retention time of appropriate standards. Carotenoids content is expressed as micrograms per gram of dry matter.

Tocopherols (α-, γ+ *β*- and δ) were extracted by method proposed by Gliszczyńska-Świgło *et al* (2007) with minor modification. Approximately 0.2 g of sample was mixed with 2-propanol (4 mL) and homogenised for 30 min at room temperature. The extracts were then centrifuged at 3000 rpm for 5 min, filtered through a 0.45 µm membrane filter and a aliquot of clear supernatant was directly injected into the Dionex UltiMate 3000 liquid chromatography system (Thermo Scientific, Germany) equipped with fluorescence detector (FLD–3100). Chromatographic separation of tocopherols was accomplished on analytical column, Acclaim Polar Advantage II, C18 (150 × 4.6 mm, 3 µm) operated at 30ºC. Mixture of acetonitrile and methanol (1:1, v/v) was used as a mobile phase at flow rate of 1 mL/min. Injection volume was 5µL, while the wavelengths for excitation and emission were maintained at 290 nm and 325 nm, respectively. Tocopherols were identified and quantified comparing characteristic retention time of corresponding standards. The tocopherols content is expressed as micrograms per gram dry matter.

RESULTS AND DISCUSSION

The study revealed wide genetic variation for the kernel carotenoids lutein+ zeaxanthin and β-carotene in maize inbred lines with different kernal type and color. The mean values of L+Z and β-carotene were 31.34 and 8.72 µg/g, respectively. Content of β-carotene was in the range from 1.20 to 39.37 µg/g. The inbreds H (39.37 µg/g), W-4 (32.47 µg/g) and Fd-96 with orange kernel were found promising for β-carotene. Content of lutein+zeaxantin was in the range from 11.28 to 88.06 µg/g. The inbreds W-4 (88.06 µg/g) and TVA912 (69.31 µg/g) with orange kernal were identified with high lutein+zeaxantin. Orange kernel inbred lines had the highest mean value of L+Z and β-carotene (35.62 and 14.81 µg/g), followed by popcorn inbred lines with orange to dark yellow kernel (29.67 and 6.26 µg/ g). The carotenoid content in white kernel inbred lines had not be detected. White maize lacks carotenoids in the endosperm due to the presence of recessive *y1* or *phytoene synthase (psy1)*, the key gene that controls the first step in the carotenoid biosynthesis pathway, whereas, the yellow/orange kernel maize with dominant *Y1* contains α-carotene and β-carotene and zeaxanthin and lutein (Buckner *et al*. 1990).

Table 1. Content of carotenoides in 101 maize inbred lines

No	L+Z	β-karoten	No	L+Z	β-karoten	No	L+Z	β-karoten
1	36,08±0,03	3,38±0,00	35	22,89±0,09	15,07±0,06	69	ND	ND
2	24,84±0,38	3,20±0,03	36	36,22±0,28	8,45±0,03	70	ND	ND
3	45,40±0,27	39,37±0,24	37	69,31±0,51	10,56±0,08	71	28,67±0,32	2,66±0,03
4	46,17±0,61	16,87± 0,22	38	22,19±0,07	4,79±0,02	72	28,49±0,18	8,59±0,05
5	49,00±0,77	22,08±0,35	39	29,59±0,28	8,72±0,08	73	25,54±0,15	3,71±0,01
6	43,45±0,83	21,12±0,41	40	36,52±0,21	16,29±0,09	74	29,03±0,15	5,21±0,03
7	23,64±0,15	30,13±0,19	41	19,70±0,12	2,23±0,01	75	36,29±0,31	3,46±0,01
8	37,22±0,14	7,12±0,03	42	24,22±0,12	5,65±0,03	76	30,08±0,22	2,01±0,01
9	17,81±0,24	11,41±0,15	43	28,31±0,46	9,14±0,15	77	25,19±0,23	2,08±0,02
10	20,94±0,27	15,34±0,20	44	26,87±0,14	5,39±0,03	78	21,21±0,29	3,66±0,05
11	36,23±0,49	22,72±0,31	45	51,76±0,49	8,51±0,08	79	21,18±0,09	3,23±0,01
12	20,35±0,27	14,76±0,20	46	35,67±0,36	4,40±0,04	80	28,54±0,21	7,21±0,05
13	33,82±0,15	2,72±0,06	47	24,75±0,16	2,50±0,02	81	26,28±0,13	1,83±0,01
14	26,75±0,08	14,51±0,05	48	42,37±0,34	4,94±0,04	82	28,24±0,31	2,42±0,03
15	22,62±0,11	5,46±0,03	49	22,58±0,17	3,70±0,03	83	20,60±0,33	3,46±0,02
16	40,91±0,27	12,66±0,08	50	33,80±0,41	5,17±0,06	84	31,10±0,23	2,84±0,01
17	26,63±0,08	11,01±0,03	51	53,14±0,36	7,58±0,05	85	28,14±0,27	4,59±0,04
18	49,52±0,81	6,47±0,11	52	34,13±0,29	10,49±0,09	86	35,99±0,18	4,01±0,02
19	88,36±1,08	32,47±0,40	53	31,47±0,35	5,48±0,06	87	25,58±0,15	5,82±0,03
20	40,78±0,22	10,32±0,05	54	19,59±0,14	1,91±0,01	88	24,79±0,27	2,40±0,03
21	33,05±0,32	6,67±0,07	55	28,58±0,25	7,97±0,07	89	37,96±0,48	3,31±0,04
22	31,78±0,19	15,70±0,09	56	21,06±0,38	1,41±0,03	90	24,17±0,14	5,47±0,03
23	29,14±0,27	6,75±0,06	57	33,25±0,35	9,90±0,10	91	17,94±0,20	8,49±0,10
24	29,16±0,18	16,91±0,11	58	11,28±0,16	1,70±0,02	92	21,22±0,07	4,93±0,02
25	34,23±0,20	14,36±0,09	59	16,04±0,06	1,95±0,01	93	25,67±0,11	4,92±0,02
26	23,78±0,07	25,45±0,07	60	23,70±0,10	2,48±0,01	94	27,12±0,27	4,55±0,04
27	29,83±0,45	9,73±0,15	61	30,06±0,24	3,10±0,03	95	33,90±0,13	9,88±0,04
28	49,86±0,77	26,16±0,40	62	31,24±0,18	3,34±0,02	96	24,03±0,14	4,81±0,03
29	39,05±0,11	8,65±0,02	63	28,90±0,43	3,28±0,05	97	30,78±0,41	4,12±0,05
30	17,56±0,18	10,13±0,10	64	17,13±0,16	1,20±0,01	98	38,12±0,39	10,14±0,10
31	27,56±0,25	16,69±0,15	65	29,29±0,12	5,56±0,02	99	39,69±0,49	11,67±0,14
32	33,30±0,08	8,79±0,02	66	25,69±0,21	1,30±0,01	100	31,07 ±0,18	2,33±0,01
33	31,60±0,06	25,05±0,04	67	ND	ND	101	42,36±0,27	4,04±0,03
34	39,21 ±0,14	14,67±0,05	68	ND	ND			

The genetic variation for various carotenoids among maize inbreds showed zeaxanthin and lutein were the most predominant carotenoids. Vignesh *et al.* (2012) reported the same low range of kernel β-carotene as well as Chander *et al.* (2008) observed a similar trend of variation while evaluating a set of Chinese germplasm, and found less provitamin A concentration and more of lutein and zeaxanthin. The carotenes (α- and β-carotene) are the intermediates in the carotenoid biosynthesis pathway, leading to the more synthesis of non-provitamin A (lutein and zeaxanthin), which is why maize kernels generally have limited provitamin A despite being rich in other carotenoids (Vallabhaneni *et al.* 2009).

Table 2. Content of tocopherols in 101 maize inbred lines

No	δ	β+γ	α	No	δ	β+γ	α
1	2,04±0,01	28,55±0,13	10,59±0,05	52	0,95±0,01	53,30±0,39	2,68±0,02
2	1,98±0,02	35,73±0,28	14,15±0,11	53	1,85±0,01	30,90±0,23	17,91±0,13
3	1,08±0,01	25,18±0,30	24,04±0,29	54	1,04±0,03	69,69±0,32	6,33±0,03
4	1,68±0,05	79,01±0,56	4,26±0,03	55	1,21±0,01	35,81±0,11	9,76±0,03
5	2,95±0,03	51,61±0,60	8,16±0,09	56	0,81±0,00	23,33±0,10	19,12±0,08
6	2,53±0,06	105,52±1,37	5,94±0,08	57	2,02±0,01	41,88±0,27	19,78±0,13
7	1,80±0,01	44,54±0,20	7,23±0,03	58	1,62±0,01	28,89±0,14	13,62±0,07
8	0,74±0,01	18,25±0,21	9,73±0,11	59	1,46±0,01	46,60±0,20	14,24±0,06
9	1,29±0,03	37,51±0,33	4,67±0,04	60	1,60±0,01	7 2,18±0,41	6,80±0,09
10	1,38±0,01	16,31±0,08	2,90±0,01	61	0,66±0,01	30,31±0,34	16,15±0,18
11	4,39±0,03	53,09±0,35	10,84±0,07	62	0,92±0,01	44,11±0,34	15,56±0,12
12	1,18±0,01	29,08±0,16	8,66±0,05	63	1,26±0,01	45,00±0,47	13,82±0,15
13	2,08±0,02	58,89±0,68	4,72±0,05	64	0,49±0,00	27,16±0,13	4,41±0,07
14	2,99±0,02	81,69±0,66	7,60±0,06	65	1,36±0,00	23,11±0,05	9,81±0,02
15	1,15±0,01	33,35±0,36	12,16±0,13	66	0,49±0,00	16,19±0,14	11,16±0,09
16	1,40±0,01	39,05±0,23	3,38±0,01	67	1,45±0,04	72,13±0,66	3,72±0,03
17	1,73±0,00	63,77±0,18	18,00±0,05	68	1,22±0,01	33,89±0,16	13,35±0,06
18	2,77±0,03	62,40±0,64	4,41±0,04	69	0,61±0,00	35,74±0,13	18,70±0,07
19	2,29±0,02	77,46±0,54	9,85±0,07	70	1,20±0,00	43,97±0,11	12,37±0,03
20	0,77±0,01	41,42±0,31	9,44±0,07	71	1,65±0,03	50,09±0,81	15,60±0,25
21	5,48±0,05	105,02±0,92	9,82±0,09	72	1,80±0,01	53,88±0,21	13,73±0,05
22	1,39±0,01	28,16±0,28	3,74±0,02	73	0,94±0,02	69,26±0,39	3,22±0,02
23	2,29±0,02	41,24±0,38	38,14±0,35	74	2,14±0,03	57,05±0,78	6,25±0,09
24	2,74±0,03	71,30±0,80	10,62±0,12	75	2,62±0,05	77,11±1,43	7,66±0,14
25	0,50±0,01	14,17±0,17	7,40±0,09	76	2,17±0,01	52,95±0,21	14,99±0,06
26	0,79±0,00	19,20±0,11	12,93±0,08	77	3,66±0,02	75,26±034	10,68±0,05
27	0,85±0,01	27,49±0,17	2,35±00,01	78	3,51±0,02	86,12±0,38	16,58±0,11
28	2,01±0,01	62,17±0,24	10,05±0,04	79	2,38±0,02	83,87±0,30	8,83±0,03
29	4,27±0,03	71,70±0,53	21,73±0,16	80	2,52±0,02	63,32±0,38	8,41±0,05
30	1,23±0,03	57,28±0,50	3,87±0,03	81	4,66±0,11	99,26±0,24	12,28±0,28
31	2,77±0,02	63,77±0,40	11,31±0,07	82	2,70±0,28	59,99±0,28	12,96±0,36
32	0,46±0,01	42,63±0,34	2,67±0,02	83	4,01±0,02	101,32±0,54	17,00±0,09
33	6,56±0,06	56,60±0,50	12,37±0,11	84	1,16±0,09	88,38±0,15	3,97±0,05
34	1,74±0,01	20,11±0,17	7,86±0,07	85	1,69±0,08	82,99±0,48	9,20±0,16
35	0,96±0,01	12,10±0,10	7,84±0,06	86	2,14±0,01	59,79±0,15	13,57±0,03
36	3,83±0,02	64,59±0,34	7,81±0,04	87	3,01±0,02	78,67±0,61	17,79±0,14
37	1,90±0,01	45,64±0,18	17,26±0,07	88	2,09±0,02	53,61±0,47	9,40±0,08
38	1,82±0,01	47,73±0,30	3,33±0,02	89	1,29±0,05	64,47±0,95	10,70±0,16
39	1,21±0,01	21,76±0,24	13,24±0,15	90	2,31±0,02	36,33±0,37	6,36±0,06
40	1,25±0,06	73,15±0,72	6,73±0,02	91	1,45±0,06	40,27±0,30	5,20±0,01
41	0,77±0,03	55,25±0,47	3,72±0,01	92	1,73±0,07	39,19±0,56	4,14±0,02
42	1,69±0,01	47,63±0,40	8,77±0,07	93	2,03±0,02	38,08±0,16	5,60±0,02
43	0,83±0,05	61,51±0,82	2,22±0,03	94	0,98±0,01	35,15±0,12	3,50±0,01
44	4,53±0,04	40,40±0,37	10,80±0,10	95	1,59±0,02	48,23±0,32	3,39±0,01
45	0,70±0,00	24,27±0,05	12,99±0,03	96	0,70±0,04	39,73±0,39	3,12±0,01
46	0,59±0,00	21,89±0,18	20,52±0,17	97	1,27±0,02	29,19±0,21	3,65±0,02
47	1,20±0,01	31,72±0,20	20,24±0,13	98	1,57±0,05	48,22±0,44	5,76±0,03
48	0,79±0,00	20,20±0,08	12,12±0,05	99	1,19±0,01	45,11±0,17	4,53±0,01
49	1,43±0,01	36,01±0,24	3,10±0,01	100	0,71±0,03	41,09±0,39	3,75±0,02
50	1,74±0,01	33,82±0,14	13,67±0,06	101	1,13±0,02	60,45±0,19	4,49±0,01
51	1,18±0,01	32,34±0,20	16,66±0,11				

In breeding program for selection of high carotenes maze lines, Safawo *et a*l. (2010) have also determined high variation in β-carotene in maize grains. Vignesh *et al* (2015) found wide genetic variation for lutein (0.36–15.75 μg/g), zeaxanthin (0.25–22.76 μg/g), and β-carotene (0.07–17.41 μg/g) in 48 diverse maize inbreds. According to Chander *et al.* (2008) allelic variations and dosage effects may be responsible for the wide range of variability for carotenoids in yellow maize. Inbreds with high lutein and zeaxanthin identified in the study can be used in developing hybrids specifically for poultry industry and inbreds with high β-carotene (~15 μg/g) would be useful in developing provitamin A enriched hybrids to alleviate vitamin A deficiency.

In the reverse phase system, which was employed in this study, there is no possibility to separate *β*- and *γ*- tocopherol, so their content is expressed as their sum (Abidi, 2009, Gliszczyńska-Świgło *et al.*, 2004). The mean values of α-tocopherol, β+γ tocopherol, and δ-tocopherol, were 10.22, 49.17 and 1.81 μg/g, respectively. Sweetcorn inbred lines have the highest average value of *β+γ* tocopherols (59.61 μg/g) followed by orange kernal inbred lines (48.26 μg/g), popcorn (41.75 μg/g), white kernal inbred lines (40.38 μg/g) and yellow kernal inbred lines (39.18 μg/g). That are in accordinace with results of our previously study of tocopherols content in different set of inbred lines (Drinic *et al*., 2017) as sweetcorn inbred lines had higher *β+γ* tocopherols content than standard kernel type inbred lines. Average values of *α* tocopherol in orange, yelow, white inbred lines, sweetcorn and popcorn was 9.96, 12.04, 12.03, 8.65 and 4.48μg/g, respectively. The highest value of γ-tocopherols have inbred line KRW 803-3-1-2-1 (105.52 μg/g) and α –tocopherol TVA973 (38.14 μg/g). The inbred line W-4 with orange kernels had a high level of *β*-carotene and lutein+zeaxantin, as well as moderate content of tocopherols. Orange kernel inbred lines had the highest value of L+Z and β-carotene, yelow kernel inbred lines α –tocopherol, whereas sweetcorn inbreds had the highest value of γ-tocopherols. The genetic background undoubtedly influences chemical quality and line with high content of particularly micronutritients may be used in breeding program to improve nutritional value.

CONCLUSIONS

Carotenoides and tocopherols are compounds present in maize kernal that provide health and economic benefits, which potentially could be captured by both producers and consumers to add value to the grain. So, increased levels of caritenoids and tocopherols in maize grain, because of their antioxidant activity, should increase the nutritive value of maize. The great natural variation for carotenoids and tocopherolos are presented in maize inbred lines with different kernal type (normal, sweetcorn, popcorn) and kernal color (white, yellow, orange). The inbred line W-4 with orange kernels had a highest content of lutein+zeaxantin and high β-carotene, as well as moderate content of tocopherols so could be use in breeding program for parallel improvement of both carotenoids and tocopherols.

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THE EFFECTS OF PARAFFIN AND PARAFILM APPLICATIONS AND DIFFERENT ROOTSTOCKS ON YIELD OF GRAFTED VINE IN 'BLACK MAGIC' GRAPE CULTIVAR

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ABSTRACT

The scions belonging to 'Black Magic' grape cultivar were grafted on '41B', '1103 P', 'Fercal' and 'SO4' American rootstock cuttings using omega grafting method in the experiment. Paraffin was applied to one half of the grafted cuttings. The other half was wrapped with parafilm and then paraffin was applied. Grafted cuttings were planted in perlite medium at 3.0 x 3.5 cm intervals inside plastic buckets and kept in the stratification room for 4 weeks. Grafted cuttings, which had been maintained under room conditions for one week, were then grown for two months under unheated greenhouse conditions. In order to determine rootstock and application effects, bud burst ratio (%), callus formation rate (%), callus formation degree (0-4), rooting ratio (%), rooting degree (0-4), shoot length (cm), shoot diameter (mm) and grafted vine ratio (%) were examined. According to the results of the study, it was determined that paraffin+parafilm application yielded better results than paraffin application in terms of bud burst ratio (respectively 70.83%, 53.33%), callus formation ratio (respectively 83.75%, 69.58%) and yield of grafted-vines (respectively 68.33%, 51.67%). In terms of the rootstocks, bud burst ratio was determined to be the highest in SO4 rootstock (71.67%) and the lowest in Fercal (54.17%). 1103 P rootstock yielded a highest value than other rootstocks in terms of shoot length (26.17 cm), callus rate at grafting surface (88.33%), and rooting ratio (97.50%). The effect of rootstocks on shoot diameter, rooting degree, and grafted vine ratio was found to be similar.

Key words: *Grafted vine, Rootstocks, Paraffin, Parafilm.*

INTRODUCTION

The production of grapes for early table consumption is becoming more popular in the Mediterranean region of Turkey (Kamiloğlu et al., 2011). In Turkey, the Mediterranean region has an important role in grape production, producing 715.781 t in 79.468 ha of vineyard (TUİK, 2017). The most appropriate ecology for early grapes cultivars that can be marketed easily and for higher prices in domestic and foreign markets is the coastline of the Mediterranean region (Söylemezoğlu et al., 2005). In the ecology of Mediterranean region, it is observed that producers in the area are quite willing to try early varieties aimed at early-grown grape cultivation. Black magic, which is a new grape variety for the region, is an early variety, with good cluster and berry shape. The clusters are medium sized, conical occasionally winged shaped and loose with an average bunch weight of 450-500 g. The berry is medium sized to large with an average weight of 5.5-6.0 g. The berry is ovoid in shape with dark blue coloration of the skin, neutral taste and 1-2 grape seeds per berry (Dimovska et al., 2013).

The grape phylloxera is one of the worst threats to modern viticulture world-wild. Most of the vineyard areas in Turkey are contaminated by phylloxera. In region where phylloxera occurs, grafting is an indispensable technic of Turkish viticulture. The grafting of Vitis vinifera varieties on American rootstocks is still considered to be the most effective means of controlling phylloxera (Vrsic et al., 2015).

The bench grafting is the main technique used for grapevine propagation (Regina et al., 2012). The most common graft section technique in this aspect is Omega. This grafting technique mainly involves the use of cuttings with a diameter of 7 to 10 mm. Tight grip of rootstock and scion tissues throughout callus formation process influences the success of grafting (Çelik et al., 1998). It is considered that, besides its effects on changes on the internal structure of cutting, cutting diameter physically affects tighter grip of scion and rootstock in omega grafting, and increases the success of grafting. As a matter of fact, Çelik et al. (1992) reported that cuttings with a diameter of 12 to 15 mm increase the grafted cutting ratio to be planted in the nursery by 27.92% to 43.14% more than cuttings with a diameter of 8 mm. Accordingly, in this grafting technique, it is important to ensure tight contact between scion and rootstock until callus formation is ensured particularly on cuttings with a certain diameter range.

Vine sapling production in Turkey is 3.362.663 in 2017 (FÜAB, 2017). This value is well below our country’s annual vine sapling requirement. The production of grapevine sapling in Mediterranean region (163.400) is also quite low in agricultural regions of Turkey.

The main goal of this research was to study the influences of paraffin and parafilm applications and some grape rootstocks; 41B’, ‘1103 P’, ‘Fercal’ and ‘SO4’ on yield of grafted vine of ‘Black Magic’ grapevines in Eastern Mediterranean Region.

MATERIAL AND METHODS

This research was conducted in 2016 at the Department of Horticulture of Hatay Mustafa Kemal University (Turkey). The scions belonging to 'Black Magic' grape cultivar were grafted on ‘41B’, ‘1103 P’, ‘Fercal’ and ‘SO4’ American rootstock cuttings using omega grafting method in the experiment (on March 21, 2016). Paraffin was applied to one half of the grafted cuttings, the other half was rolled with special plastic parafilm (Zenginbal, 2015) and then paraffin was applied (Hamdan and Basheer-Salimia, 2010), (Figure 1a,b,c). Grafted cuttings were

planted in perlite medium at 3.0 x 3.5 cm intervals and 15 cm depth inside plastic buckets and kept in the stratification room for 4 weeks (Figure 1d,e). Grafted cuttings, which had been maintained under room conditions for one week, were then grown for about 2 months under unheated greenhouse conditions (Figure 1f). In order to determine rootstock and application effects, rootstock cutting diameter (mm), callus formation rate (%), callus formation degree (0-4), rooting ratio (%), rooting degree (0-4), bud burst ratio (%), shoot length (cm), shoot diameter (mm) and grafted vine ratio (%) were examined (Figure 1g). In experiment, temperature and humidity values within the stratification room and the greenhouse were recorded with 30-minute intervals using a datalogger.
The research was planned so that there would be three replications and 20 grafted cuttings would be involved in each replication in 'Two Factor Completely Randomised Design' pattern. Variance analyses of obtained data were made by using Mstat-C computer software. Differences between the averages were evaluated by Tukey test at 0.05 or 0.01. Angular transformation was applied to % values.

Figure 1. General appearances in some stages of the experiment

RESULT AND DISCUSSION

Temperature and humidity values recorded in the experiment are provided in Figure 2. as weekly averages. In order to accelerate the formation of callus at the graft surface, temperature should be kept at 26-28°C, while relative humidity should be kept at 85-90% in the stratification room (Celik, 2007). During the study, temperature varied between 24.37-26.18 °C and relative humidity varied between 89.55-96.26% in the stratification room. During the growing period in the greenhouse (8 weeks), average humidity was recorded as 67.43±6.88% and average temperature was recorded as 25.36±2.51 °C

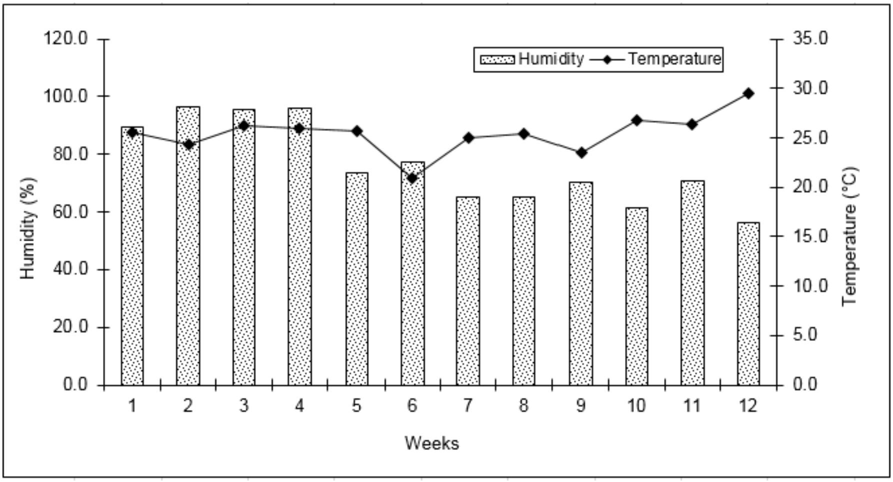

Figure 2. Changes in temperature and relative humidity during the experiment

The results obtained in terms of examined parameters according to rootstocks and applications in the study are given in Table 1 and Table 2.

During the trial, rootstock, application, and rootstock x application interaction were found to be statistically significant at the level of 1 % in terms of the ratio of callus formation at grafting surface. While callus formation ratio was 95.00 % on cuttings grafted on 1103 P rootstock, on which parafilm + paraffin was applied, cuttings grafted on 41 B and Fercal rootstocks, on which paraffin was applied, yielded the lowest value (respectively 56.67 %, 58.33 %). While application and rootstock x application interaction was found to be insignificant in terms of callus formation degree at grafting surface, the difference between rootstocks was found to be statistically significant (1%). The highest callus formation degree was identified on SO4 rootstock with 2.92. Although no difference was observed between rootstocks and applications in terms of rooting degree of cuttings, it was determined that 1103 P yielded the highest value (97.50 %) and Fercal yielded the lowest value (81.67 %) in terms of rooting ratio (Table 1).

During their study conducted to find out the effect of different IBA doses, Celik and Gargın (2009) reported that rooting degrees varied between 2.31-2.97 according to rootstock averages. Kamiloglu and Güler (2014) reported in their study that 1103 P rootstock yielded a higher value than SO4 and 41 B in terms of rooting ratio. It is seen that the findings obtained from this study support the results of the researchers.

There are endogenous and exogenous factors that affect the termination of dormancy in Vitis vinifera. Exogenous factors are temperature, light intensity, day length, oxygen, water, minerals, and cultural operations. On the other hand, internal factors are plant growth regulators, enzymes, amino acids, proteins, lipids and carbohydrates (Eris and Celik, 1981). It is seen in this study that rootstocks are effective on the bursting of buds in grafted cuttings. As a matter of fact, in terms of bud burst ratio, the differences between rootstocks were found to be significant at the level of 5 %, and the differences between applications were found to be significant at the level of 1%, while rootstock x application interaction was not found to be statistically significant. Bud burst ratio was determined to be the highest in SO4 rootstock (71.67 %) and the lowest in Fercal (54.17%).

Table 1. The effects of paraffin and parafilm+paraffin on rooting and callus formation for Black Magic grafted onto 4 rootstock

Rootstock	Treatment	Rootstock cutting diameter (mm)	Callus formation rate (%)	Callus formation degree (0-4)	Rooting ratio (%)	Rooting degree (0-4)
41 B	Paraffin	7.49 abc[1]	56.67(48.87) d	2.43	88.33(70.50)	2.82
	Parafilm+paraffin	7.78 a	86.67(68.67) b	3.03	90.00(71.97)	2.53
	Mean	7.64 A	71.67(58.77) C	2.73 AB	89.17(71.23) AB	2.68
1103 P	Paraffin	7.19 bcd	81.67(64.67) bc	2.33	98.33(85.70)	2.90
	Parafilm+Paraffin	7.19 bcd	95.00(77.10) a	2.20	96.67(83.87)	2.92
	Mean	7.19 B	88.33(70.88) A	2.27 B	97.50(84.78) A	2.91
Fercal	Paraffin	6.65 d	58.33(49.83) d	2.80	75.00(60.27)	2.80
	Parafilm+Paraffin	6.96 cd	70.00(56.83) cd	2.70	83.33(73.53)	2.86
	Mean	6.81 C	64.17(53.33) D	2.75 AB	81.67(66.90) B	2.83
SO4	Paraffin	7.70 ab	81.67(64.67) bc	2.60	83.33(66.83)	2.80
	Parafilm+Paraffin	6.90 d	83.33(65.93) b	3.23	93.33(75.27)	3.18
	Mean	7.30 AB	82.50(65.30) B	2.92 A	88.33(71.05) AB	2.98
Treatment Mean	Paraffin	7.26	69.58(57.01) Y	2.54	86.25(70.83)	2.83
	Parafilm+paraffin	7.21	83.75(67.13) X	2.79	92.08(76.16)	2.87
$D_{Rootstock}$		**	**	**	**	N.S.
$D_{Treatment}$		N.S.	**	N.S.	N.S.	N.S.
$D_{RootstockxTreatment.}$		**	**	N.S.	N.S.	N.S.

[1]:Values not associated with the same letter are significantly different (P<0.05 or P<0.01)
*: Significant at 0.05 level ; **: Significant at 0.01 level; N.S.: Not Significant

However, it can be suggested that, in addition to the rootstocks, the differences in internal structure of scions used for grafting might also have been effective on the bursting ratio of buds. Parafilm + paraffin application (70.83 %) yielded the highest values in terms of this characteristic. The longer shoots were obtained from plants grafted on 1103 P than other rootstocks. The effects of rootstocks on shoot diameter and grafted vine ratio was not found significant. In terms of treatments, shoot diameter and grafted grapevines were found to be statistically significant at the levels of respectively 5 % and 1 %. Parafilm+paraffin application yielded better results than paraffin application (respectively 68.33 %, 51.67 %) in terms of grafted vine ratio (Table 2).

Table 2. The effects of paraffin and parafilm+paraffin on bud burst and shoot growth and grafted vine ratio for Black Magic grafted onto 4 rootstock

Rootstock	Treatment	Bud burst ratio (%)	Shoot length (cm)	Shoot diameter (mm)	Grafted vine ratio (%)
41 B	Paraffin	53.33(46.93)	20.83	2.92	53.33(46.93)
	Parafilm+paraffin	65.00(53.87)	19.47	2.86	61.67(51.93)
	Mean	**59.17(50.40) AB**[1]	**20.15 B**	**2.89**	**57.50(49.43)**
1103 P	Paraffin	55.00(48.07)	24.43	2.59	55.00(48.07)
	Parafilm+Paraffin	71.67(58.07)	27.90	2.78	71.67(58.07)

		Mean	**63.33(53.07) AB**	**26.17 A**	**2.69**	**63.33(53.07)**
Fercal	Paraffin		38.33(38.17)	17.03	3.22	35.00(36.23)
	Parafilm+Paraffin		70.00(57.00)	15.63	2.24	68.33(55.87)
		Mean	**54.17(47.58) B**	**16.33 B**	**2.73**	**51.67(46.05)**
SO4	Paraffin		66.67(54.83)	20.20	2.92	63.33(52.80)
	Parafilm+Paraffin		76.67(61.13)	20.20	2.36	71.67(57.90)
		Mean	**71.67(57.98) A**	**20.20 B**	**2.64**	**67.50(55.35)**
Treatment Mean	Paraffin		**50.33(47.00) Y**	**20.63**	**2.92 X**	**51.67(46.01) Y**
	Parafilm+paraffin		**70.83(57.52) X**	**20.80**	**2.56 Y**	**68.33(55.94) X**
$D_{Rootstock}$			*	**	N.S.	N.S.
$D_{Treatment}$			**	N.S.	*	**
$D_{RootstockxTreatment.}$			N.S.	N.S.	N.S.	N.S.

[1]:Values not associated with the same letter are significantly different (P<0.05 or P<0.01)
*: Significant at 0.05 level ; **: Significant at 0.01 level; N.S.: Not Significant

Similar studies have not been found in the literature in terms of parafilm+paraffin application. Therefore, the findings obtained from the study cannot be compared with other studies. But; this application generally gave higher values than the paraffin application in terms of the properties examined. American grapevine rootstocks can give similar or different results in terms of features examined in grafting studies. In our study, 1103 P yielded higher values than other rootstocks in terms of shoot length, callus formation rate and rooting ratio. When the results of some researches are examined; 1103 P rootstock shoot length (Dardeniz and Şahin, 2005), callus formation (Kamiloglu and Güler, 2014; Köse et al., 2015) and root development (Sağlam et al. 2005; Kamiloglu and Güler, 2014; Köse et al., 2015) gave better results than the other rootstocks.

CONCLUSION

Wrapping at the graft union is not a common practice in the omega grafting technique. However, this practice, performed on thin diameter rootstock cuttings, have a positive effect on the success of grafting. This is caused by the increase in the ratio of grafted vines due to parafilm+paraffin application. According to the results of the study, parafilm+paraffin application provided 16.00 % improvement on the ratio of grafted vines in comparison with application of only paraffin. In terms of the rootstocks, 1103 P yielded better performance than other rootstocks in terms of shoot length (26.17 cm), callus formation rate at grafting surface (88.33 %), and rooting ratio (97.50%). The effects of rootstocks on shoot diameter, rooting degree, and grafted vine ratio were found to be similar.

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PRION PROTEIN GENE SEQUENCES ANALYSIS IN TWELVE SHEEP BREEDS OF PAKISTAN

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ABSTRACT

Prions are considered the only agents of transmissible spongiform encephalopathies (TSEs) and are harmful pathogens of mammals. These infectious agents of host are made up through aggregation of conformational isomers (PrPSc) and encode glycoprotein (PrPC) of 33-35 kDa. TSEs are the fatal group of diseases which are neurodegenerative and include chronic wasting disease in deer and elk, Creutzfeldt-Jakob disease (CJD) and transmissible mink encephalopathy (TME) in humans and scrapie in goats and sheep. The accumulation of abnormal form of the normal protein (PrP) is common in all diseases related TSE. This abnormal form of PrP called PrPSc is resistant to proteolysis as well as infectious. Present study was conducted in order to do sequence analysis of prion protein gene in twelve breeds of the sheep. We studied this gene to elucidate 12 of Pakistani sheep breeds and to compare gene order with other mammalian species. PCR amplification of 771 bp fragment was done on selected samples from all twelve breeds followed by sequencing. Sequence analysis was done and some sites were found to be heterozygous. These findings on prion protein gene in sheep will provide assistance for further studies on pathogenesis, cross-species transmission, breeding programs, resistance and susceptibility to scrapie.

Key words: Prions, TSEs, scrapie, neurodegenerative, sheep

INTRODUCTION

Prion diseases are a group of rapidly progressive, and fatal neurologic diseases which are commonly known as transmissible spongiform encephalopathies (TSEs). Infectious agent of these diseases, a protein as suggested by (Griffith, 1967; Bolton et al., 1982), was isolated as a protease-resistant sialoglycoprotein through the use of progressive enrichment of brain homogenates for infectivity. This protein is major component in the infective brain fractions and was shown to accumulate in the affected brains (Griffith, 1967; Bolton et al, 1982; Prusiner et al, 1982). The term prion was coined by Prusiner in 1982 in order to differentiate these proteinaceous infectious particles from viroids or viruses. The protein component of these particles was then recognized as Prion protein (PrP) and for this work

Prusiner was awarded Nobel Prize (et al., 2003). Prions, the smallest and simplest infectious pathogens, have no nucleic acid. While all other infectious agents like fungi, bacteria and viruses have genomes composed of RNA or DNA that direct the synthesis of their progeny (Pramood et al., 2003). The unique feature of prion diseases is the abnormal metabolism of the prion protein (PrP), as this protein is in two conformational states with various physicochemical properties. PrPC is the normal form of protein and is cell surface protein attached through glycophosphatidyl inositol anchor. This protein is highly conserved and expressed in different types of cells, specifically in neuronal cells. The molecular weight of PrPC is 33 to 35 kDa with high content of β-helical secondary structure, and it is soluble in detergents and sensitive to protease treatment. In the early embryogenesis, the PrPC is expressed while in adults it occurs in spinal cord and in neurons of the brain at the highest level (McKintosh et al. 2003). PrPC is also found in number of peripheral cell types and in glial cells of the CNS at lower levels (Moser et al, 1995; Ford et al, 2002). PrPC molecules normally present on the surface of cell attached to lipid bilayer through C-terminal, glycosyl-phosphatidylinositol (GPI) anchor (Stahl et al., 1987). The biosynthetic pathway of PrPC is exactly the same as of other membrane and secreted proteins which includes synthesis on rough ER, transfer to the Golgi, and then delivery to surface of the cell (Harris et al., 2004). PrPC is considered to be important protein in the context of biological functions due to its highly conservation in evolution. Therefore, the investigation of the biological activity of PrPC is very important for understanding the pathogenesis of prion diseases as change in its function could play a crucial role in the process of disease (Westergard et al., 2007). PrPSc is the disease associated isoform and is only present in the brains as an aggregated form. It has high content of β-sheet secondary structure and partially resistant to protease treatment and is not soluble in detergents (McKintosh et al., 2003). Diseases related prion occur due to the conversion of normal, cell surface glycoprotein (PrPC) into conformationally altered isoform (PrPSc) (Westergard et al., 2007). This conversion of PrPC to PrPSc is the basic reason of prion diseases (David et al., 2011). It is very important to develop novel diagnostic tools, due to the lackability of prion-directed immune response, for early prion disease diagnosis (Hussein and Al-Mufarrej, 2004).

Prion infections have been able to cross-species transmission to have infected human beings. Therefore, there is an urgent need to develop safe and effective vaccines against these fatal and currently incurable diseases (Mabbott, 2014). Although the molecular mechanisms of prion disease pathogenesis remains unclear, single-nucleotide polymorphisms (SNPs) of PrP were found to be associated with the incubation period, susceptibility, and species barrier to the scrapie disease in sheep (wang 2009). Sheep is an important meat animal of Pakistan and prion disease if present could be a public health threat. There were no reports on prion gene sequence and polymorphism analysis in Pakistani sheep breeds. In the present study, blood samples were collected from different sheep breads all over Pakistan, and PrP gene sequencing analysis was carried out. The findings on sequence analysis could assist in breeding programs and TSE pathogenesis and cross species transmission studies.

MATERIALS AND METHODS

Sample collection:

Five mL blood was collected in falcon tubes containing 200 µL anticoagulant i.e. Ethylenediamine tetra-acetic acid (0.5 M EDTA) from all four provinces of Pakistan, Azad Jammu and Kashmir region (AJK) from different sheep breeds (Table 1). Proper record was maintained containing the information regarding the breed, animal ID, age, sex and location of animal. Field blood samples were placed on ice immediately after their collection and brought to the Molecular Biology and Genomic Laboratory and stored temporarily in freezer at -20 °C before DNA extraction.

DNA isolation:

Genomic DNA was extracted from blood samples using standard protocol, which involved RBCs lysis, protein digestion, and precipitation followed by DNA isolation and purification. The DNA samples were dissolved in Tris-EDTA(TE) buffer (pH 8.0) and stored at -20 °C for further use. Quantification of the DNA of the collected samples was done with the help of agarose gel electrophoresis (0.8 %). Standard DNA/ DNA ladder was used for simultaneous and accurate determination of amplified DNA fragment size. All samples were brought to same concentration level i.e. Approx. 50 ng/ µL.

Primer designing:

For the amplification of Prion protein gene (PrP); specific primers were designed from already reported sequences (Acession No. DQ346682) available on GenBank, National Centre for Biotechnology Information (NCBI) using Primer 3 software and in-silico PCR web facility (Rozen and Skaletsky, 2000) using the reference sequences of this gene.

PCR amplification and sequencing:

The PCR amplification of 876 bp containing the entire coding region in exon 3 of the PrP gene was carried out using forward (5’-1CTTTAAGTGATTTTTACGTGG21-3’) and reverse (5’-854TGGCAAAGATTAAGAA GATAATG876-3’) primers. Sequencing was done using ABI Genetic Analyzer 3130 XL (Applied Biosystems, USA).

RESULTS AND DISCUSSION

Sequence alignment and analysis:

Sequence alignment was done by using CodonCode Aligner software. 771 bp portion of PrP gene was aligned in all sheep breed and SNPs (single nucleotide polymorphisms) identification were done. 670 bp portion was selected for phylogenetic tree construction with other mammalian species (listed in table 2) by MEGA6 using UPGMA method with 1000 bootstrap value.

Table 1. SNPs Identified in the PrP gene in Sheep breeds.

Breed _sample no	SNPs Position	SNP Nucleotide	Change with	Transition/ Transversion
Blochi_06	572	C	Y	Transition
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Blochi_11	511	C	R	Transversion
Blochi_12	437	A	R	Transition
Balochi_21	512	A	G	Transition
Blochi_46	512	A	G	Transition
Blochi_61	691	A	M	Heterozygous
	711	C	S	Heterozygous
Blochi_83	379	G	A	Transition
Blochi_84	513	G	K	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Blochi_94	414	C	T	Transition
	428	A	G	Transition
	718	T	C	Transition
Balochi_98	404	G	R	Transition
	414	C	T	Transition
	718	T	C	Transition
Bulkhi_09	380	G	K	Heterozygous
Bulkhi_18	380	G	T	Transversion
Bulkhi_19	380	G	K	Heterozygous
Dumri_01	513	G	K	Heterozygous
	691	A	C	Transversion
	711	C	G	Transversion
	512	A	R	Transition
Dumri_02	512	A	G	Transition
Dumri_03	379	G	A	Transition
Dumri_07	691	A	M	Heterozygous
	711	C	S	Heterozygous
Dumri_11	513	G	K	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Dumri_10	513	G	K	Heterozygous
	711	C	S	Heterozygous
Dumri_12	379	G	R	Transition
	512	A	R	Transition
Dumri_16	512	A	G	Transition
Kaghani_01	379	G	R	Transition
	566	A	W	Heterozygous
Kaghani_04	566	A	W	Heterozygous
Kaghani_06	566	A	W	Heterozygous
Kaghani_10	414	C	T	Transition
	718	T	C	Transition
Kaghani_11	512	A	R	Transition
	566	A	W	Heterozygous
Kaghani_13	379	G	A	Transition
Kaghani_15	566	A	W	Heterozygous
Kaghani_16	379	G	R	Transition

	566	A	W	Heterozygous
Kaghani_17	437	A	G	Transition
Kaghani_19	437	A	R	Transition
Khail_106	512	A	R	Transition
	513	G	A	Transition
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Khail_114	566	A	W	Heterozygous
Khail_123	691	A	C	Transversion
	711	C	G	Transversion
Khail_128	379	G	R	Transition
Koka_01	455	A	T	Transversion
	566	A	W	Heterozygous
Koka_02	566	A	W	Heterozygous
Koka_03	566	A	T	Transversion
Koka_04	566	A	W	Heterozygous
Koka_05	379	G	R	Transition
Koka_06	566	A	W	Heterozygous
Koka_08	513	G	K	Heterozygous
	566	A	W	Heterozygous
	691	A	M	Heterozygous
	711	C	G	Transversion
Koka_09	566	A	W	Heterozygous
Koka_10	566	A	W	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Koka_11	379	G	R	Transition
	566	A	W	Heterozygous
Koka_12	455	A	T	Transversion
Koka_14	566	A	W	-
Koka_15	566	A	T	Transversion
Mangli_05	512	A	R	Transition
Mangli_11	-	-	-	
Mangli_16	-	-	-	
Mangli_21	414	C	Y	Transition
	428	A	G	Transition
	718	T	Y	Transition
Mangli_38	379	G	R	Transition
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Mangli_54	379	G	R	Transition
	511	C	M	Heterozygous
Mangli_55	437	A	R	Transition
Mangli_57	379	G	R	Transition
	572	C	Y	Transition
Mangli_58	-	-	-	-
Mangli_64	512	A	R	Transition
	566	A	W	Heterozygous
Mangli_65	566	A	W	Heterozygous
Poonchi_05	566	A	T	Transversion
Poonchi_06	513	G	K	Heterozygous
	691	A	C	Transversion
	711	C	G	Transversion
Poonchi_08	512	A	R	Transition

	566	A	W	Heterozygous
Poonchi_09	379	G	R	Transition
Poonchi_12	407	C	Y	Heterozygous
	461	G	R	Transition
Poonchi_15	512	A	R	Transition
	691	A	M	Heterozygous
	711	C	G	Transversion
Poonchi_16	512	A	G	Transition
Rakhshani_05	711	C	S	Heterozygous
Rakhshani_07	511	C	R	Transversion
	512	A	R	Transition
Rakhshani_10	513	G	K	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Rakhshani_13	513	G	K	Heterozygous
	691	A	M	Heterozygous
	711	C	G	Transversion
Rakhshani_17	-	-	-	-
Rakhshani_18	512	A	R	Transition
	566	A	W	Heterozygous
Ramboullet_01	512	A	G	Transition
Ramboullet_23	511	C	M	Heterozygous
Ramboullet_33	512	A	R	Heterozygous
Ramboullet_37	512	A	R	Transition
	566	A	W	Heterozygous
Ramboullet_44	461	G	R	Transition
	691	A	M	Heterozygous
	711	C	G	Transversion
Ramboullet_54	566	A	W	Heterozygous
	691	A	M	Heterozygous
Salt Range_01	379	G	R	Transition
Salt Range_02	512	A	R	Transition
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Salt Range_03	437	A	R	Transition
Salt Range_04	513	G	T	Transversion
Salt Range_05	566	A	W	Heterozygous
Salt Range_06	-	-	-	-
Salt Range_07	513	G	K	
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Salt Range_09	437	A	R	Transition
Salt Range_10	379	G	R	Transition
	566	A	W	Heterozygous
Salt Range_13	379	G	R	Transition
	513	G	K	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Salt Range_14	566	A	W	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Salt Range_15	513	G	T	Transversion
	691	A	M	Heterozygous
	711	C	G	Transversion

Shenwari_04	-	-	-	-
Shenwari_07	566	A	W	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Shenwari_09	566	A	W	Heterozygous
Shenwari_11	-	-	-	-
Shenwari_21	379	G	R	Heterozygous
Shenwari_22	-	-	-	-
Shenwari_24	512	A	R	Heterozygous
Shenwari_33	566	A	W	Heterozygous
	572	C	Y	Heterozygous
	691	A	M	Heterozygous
	711	C	S	Heterozygous
Shenwari_32	512	A	R	Transition
	566	A	W	Heterozygous

Phylogenetic analysis:

A Phylogenetic tree using MEGA6 software (Tamura et al., 2011) was constructed from prion protein gene in twelve sheep breeds of Pakistan and some species were taken as an outgroup. The tree showed different clades with branching and re branching pattern.

Table 2. Samples collected from different sheep breeds at different locations

Breeds	**Province**	**Purpose**	**Samples(n)**
Salt Range	Punjab	Meat and wool	12
Bulkhi	Khyber Pakhtunkhwa	Meat and wool	11
Kaghani	Khyber Pakhtunkhwa	Meat and wool	10
Koka	Sindh	Meat and wool	13
Balochi	Balochistan	Meat and wool	11
Dumri	Balochistan	Meat and wool	10
Mangli	Balochistan	Meat and wool	11
Rakhshani	Balochistan	Meat and wool	6
Shenwari	Balochistan	Meat and wool	9
Kail	AJK	Meat and wool	12
Poonchi	AJK	Meat and wool	11
Ramboullet	AJK	Meat and wool	13
			129

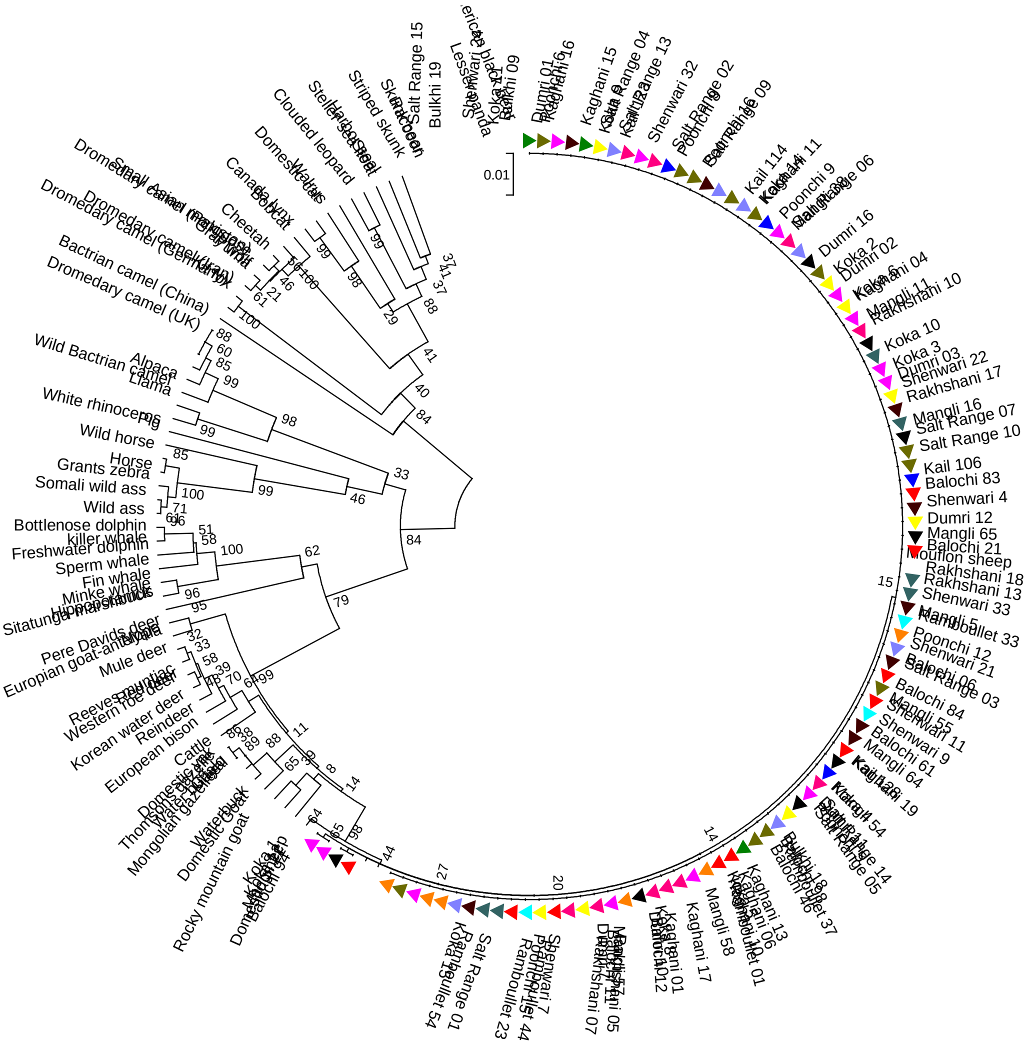

Figure 1: Neighbor Joining (circular) Phylogenetic Tree for Prion protein gene (MEGA6) of twelve Pakistani sheep breeds with other mammalian PrP sequences available on GenBank NCBI.

Our study leaded to some of great important findings and exemplifies the first screening made on prion gene sequence and polymorphism analysis in Pakistani sheep breeds. The most striking result to emerge from the data is that 6 single nucleotide polymorphism was observed at position 379, 380, 404, 407, 414, 428, 437, 455, 461, 511, 512, 513, 566, 572, 691, 711, 718 using software MEGA 6 for conducting sequence alignment. Further, some of these mutations were of transitional nature and some were transversional one. Furthermore, pylogenetic tree was constructed to infer the evolutionary relationships among prion protein gene sequence in different species. Interestingly, the more distant species doesn't share the same clade. By comparing PrP coding sequences of goats with those of sheep, high genetic conservation was revealed. This result is in line with that of Goldmann et al.(1996).

The common name for transmissible spongiform encephalopathy (TSE) is scrapie that affects goats, mouflfons and sheeps of the world. As all prion diseases, scrapie is also fatal and neurodegenerative disease. This disease is associated with different signs which may be of neurological or behavioral type abnormalities. Immunodetection of PrPSc depositions in central nervous system using biochemical and immunohistochemical methods is primarily required in order to diagnose disease. The findings of this study have a number of important implications for developing diagnostic markers for prion diseases, and providing assistance for further studies on pathogenesis, cross-species transmission, breeding programs, resistance and susceptibility to scrapie.

Prion ailments are infrequent neurodegenerative conditions bringing about exceptionally variable clinical disorders, which regularly incorporate unmistakable neuropsychiatric manifestations. (Thompson et al) did a clinical investigation of behavioral and psychiatric indications in a substantial planned companion of patients with prion disease in the United Kingdom, permitting us to operationalise particular behavioral/psychiatric phenotypes as qualities in human prion infection. They particularly analyzed a determination of competitor SNPs that have indicated expansive relationship with psychiatric conditions in beforehand distributed studies, and the codon 129 polymorphism of the prion protein quality, which is known not different parts of the phenotype of prion sickness. No SNPs achieved all inclusive centrality and there was no confirmation of modified weight of known psychiatric danger alleles in applicable prion cases. SNPs demonstrating suggestive confirmation of affiliation incorporated a few lying close qualities already involved in affiliation investigations of other psychiatric and neurodegenerative maladies. These incorporate ANK3, SORL1 and a district of chromosome 6p containing a few qualities ensnared in schizophrenia and bipolar disorder.

Prion sicknesses are a differing gathering of neurodegenerative conditions, brought about by the templated misfolding of prion protein. Beside the strong genetic hazard presented by different variations of the prion protein quality (PrPn), a few different variations have been proposed to give hazard in the most well-known sort, sporadic Creutzfeldt-Jakob sickness (sCJD) or in the obtained prion maladies. Substantial and uncommon duplicate number variations (CNVs) are known not hazard in a few related issue including Alzheimer's malady (at APP), schizophrenia, epilepsy, mental hindrance, and a mental imbalance. Lukic et al. (2016), reported the main all inclusive investigation for CNV-related danger utilizing information got from a late universal synergistic affiliation study in sCJD (n = 1147 after quality control) and openly accessible controls (n = 5427). A cell-based prion contamination test did not give steady confirmation to a part for PARK2 in prion ailment defenselessness. This information are steady with an unobtrusive effect of CNVs on danger recently onset neurologic conditions and propose that, not at all like APP, PRNP duplication is not a causal high-hazard change.

A similar type of study was conducted by Hussain et al. [4] for finding novel polymorphisms in prp gene of two Pakistani sheep breeds (Damani and Hashtnagri) and two Pakistani goats breeds (kamori and local hairy). They broke down the PrP quality succession to decide the recurrence of polymorphisms in 56 sheep (28 each from Damani and Hashtnagri breeds) and 56 goats (28 each from Kamori and Local Hairy breeds). An aggregate of 7 amino corrosive polymorphisms were recognized in the PrP quality for sheep and 4 for goats. These amino corrosive polymorphisms were joined in 13 alleles and 15 genotypes in sheep and 5 alleles and 6 genotypes in goats. The general recurrence of the most sheep scrapie-safe polymorphism (Q171R) was ascertained to be 0.107. The most scrapie- susceptible polymorphism (A136V) was not identified in any of the concentrated on sheep. The general recurrence of scrapie-related polymorphism (H143R) in goats was observed to be 0.152. Alongside already known amino polymorphisms, two novel polymorphisms were additionally recognized for each of sheep (Q171N and T191I) and goats (G22C and P63L).

There is a settled relationship between sheep prion protein (PrP) genotype and the danger of death from scrapie. Certain genotypes are plainly connected with vulnerability to the infection and others to resistance. In the established structure, scrapie susceptibility is very identified with changes in particular amino acids that prompt a modified type of the prion protein (PrPsc). Polymorphisms in the host-encoded prion quality (PRNP) are real determinants of susceptibility to exemplary scrapie, with varieties at codons 136, 154, and 171 passing on variable degrees of resistance. There are more than 15 polymorphisms reported in PrPn Of these, just three codons (codon 136, 154 and 171) have been accounted for to influence susceptibility to the illness. Susceptibility to ovine scrapie is likewise controlled by the infective scrapie strain. Two strains of scrapie have been characterized. Sort A produces the ailment in sheep that are either homozygous or heterozygous for a valine at codon 136 while sort C causes illness in sheep that are homozygous for a glutamine at codon 171.

The codon 136, valine (V) is connected with high scrapie vulnerability while alanine (An) is connected with low susceptibility, despite the fact that this may rely on upon the strain of scrapie operators (Goldmann 1994). At codon 154, arginine (R) is connected with susceptibility while histidine (H) is connected with halfway resistance. At codon 171, glutamine (Q) and histidine (H) are connected with vulnerability while arginine (R) is connected with resistance (Baylis et al. 2004). Codon variations at positions other than 136, 154, and 171 are additionally connected with scrapie resistance. A M112T variation on the ARQ haplotype has been connected with scrapie resistance in orally-immunized Suffolk sheep in the U.S. (Laegreid et al. 2008). M137T and N176K variations on the ARQ haplotype have been connected with scrapie resistance in intercranially-inoculated, orally-inoculated, and naturally-infected Italian Sarda breed sheep. Varieties at codon 141 can be identified with the atypical scrapie structure Nor98.

Zečević et al. conducted a similar study on the Pramenka sheep breed, Vlasic (Dubski) strain, of Bosnia and Herzegovina. Their research showed that locus (codon) 171, which is responsible for the synthesis of four amino acids (arginine, glutamine, histidine, and lysine), was present in their study with only two variants: glutamine and arginine. Arginine synthesis at codon 171 forms non-risk haplotype ARR, while the glutamine variant increases the risk of TSE. In the studied Bosnian population, arginine is represented with a frequency of 83.33%, while glutamine was with a frequency of 16.67%. A combination of the alanine variant from codon 136 with the arginine variant of codon 171 makes the scrapie risk-free ARR haplotype. Besides investigations on polymorphisms of those three well-known scrapie susceptible codons (loci), our examination was extended to investigations on polymorphisms of four other loci (codons 145, 185, 231, and 237) with unknown effects on the development of scrapie in sheep (unknown and undefined levels of risk). The absence of any polymorphisms was found at codons 145 and 185 in the Bosnian population under investigation, while silent mutations were recorded on codons 231 (AGG → CGG) and 237 (CTC→ CTG). In both cases, there was no amino acid changes found. The silent mutation that was found at locus (codon) 231, which is responsible for the synthesis of amino acid arginine, had a frequency of variant AGG 85.71%, while a variant CGG was present with a frequency of 14.29%. A silent mutation that was found at codon 237 (responsible for the synthesis of amino acid leucine) was present with 88.10% frequency of variant CTC and 11.90% frequency of variant CT.

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ORGANIC CARBON STOCKS IN ARABLE LAND OF REPUBLIC OF SRPSKA - BOSNIA AND HERZEGOVINA

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ABSTRACT

On the territory of Republic of Srpska (RS – Entity of Bosnia and Herzegovina), in the period 2014 - 2017, the fertility control of arable land was performed in 4125 average samples *(taken from top soil, 0 - 30 cm)* representing the surface area of 5776 ha. All samples were geo-positioned and linked to the SOTER database *(soil and terrain databases)*. RS is divided into 262 SOTER units. In each soil sample humus was analysed (colorimetric method, wet burning with K2Cr2O7 and conc H_2SO_4). Soil organic carbon (SOC) was calculated from humus (% humus x factor 0.58). SOC stock *(t ha^{-1})* for each plot were calculated on the basis of the volume mass *(mg m^{-3})* of the soil type on which the plot was located, the soil weights up to 30 cm *(kg ha^{-1})* and the area of the plot *(ha)*. SOC stock on 5776 ha of agricultural land was 225168 t ha^{-1}. The analyzed area was represented by 24 types of soil *(FAO class)*. The highest average SOC stocks of 130 t ha^{-1} *(based on 31 samples)* was found in Calacaric Cambisol and the lowest in Stagnic Luvisol 38 t ha^{-1} *(based on 464 samples)*. In 84% of the tested samples, representing 89% of researched area, the SOC stocks were less than 57 t ha^{-1}. Estimation of the SOC stocks on the total arable land was prepared by GIS analysis interpolation of the SOC results for 4125 samples on the agricultural land area *(arable land, gardens, orchards, vineyards and meadows)*. Estimated SOC stocks on 578894 ha of arable land were 32833549 t. The result of this research is the first step towards the establishment of SOC monitoring system in RS.

Key words: *soil organic carbon, arable land, GIS, Republic of Srpska.*

INTRODUCTION

The term Soil Organic Matter (SOM) is used to describe the organic constituents in soil in various stages of decomposition such as tissues from dead plants and animals, materials less than 2 mm in size, and soil organisms (FAO, 2017). SOM turnover plays a crucial role in soil ecosystem functioning and global warming. SOM is critical for the stabilization of soil structure, retention and release of plant nutrients and maintenance of water-holding capacity, thus making it a key indicator not only for agricultural productivity, but also environmental resilience. SOM contains roughly 55–60 percent C by mass. In many soils, this C comprises most or all of the C stock – referred to as SOC – except where inorganic forms of soil C occur (FAO and ITPS, 2015). Soil plays an important role in the carbon cycle on Earth. The magnitude of the SOC storage is spatially and temporally variable and determined by different abiotic and biotic factors (Weissert *et al.*, 2016). Global SOC stocks have been estimated to be about 1500 PgC for the topmost 1 m (FAO and ITPS, 2015). In most soil types (except for calcareous ones), carbon is typically contained in organic compounds, *i.e.*, in the form of organic carbon (Batjes & Sombroek, 1997). This suggests that changes in organic carbon stocks in the soil (increases or decreases) may be of global significance and they may mitigate or exacerbate climate changes. In addition to soil organic carbon having a positive impact on climate changes, proper land management aimed at raising the level of organic carbon, can increase the productivity and sustainability of agricultural ecosystems (Cole *et al.*, 1997). Increase in SOC concentration to above the threshold level has numerous co-benefits such as increase in food and nutritional security through improvements in soil health and the attendant increase in use efficiency of inputs (*e.g.*, fertilizer, water, energy) (Lal, 2017). To evaluate the role of soil in carbon cycling, it is necessary to estimate organic carbon stocks (Yang *et al.*, 2007). Such assessment is necessary from the points of both, environmental protection and agricultural production. Considering the vital importance of organic carbon for the functioning of ecosystems, its effect on soil structure and soil water capacity, and its role in numerous chemical and physical soil properties, it is important to establish its baseline status in order to be able to monitor its variations over time. It is a source of nutrients and is crucial for agricultural production. (FAO, 2017). Soil organic carbon stocks were investigated in different regions and countries. Spatial distribution of soil organic carbon and SOC sequestration potentials were investigated in the soils of Republic of Serbia. Organic carbon stocks were estimated for soil layers 0-30 cm and 0-100 cm based on the results from a database and using soil and land use maps (Vidojevic *et all*, 2015) . Organic carbon stocks were also estimated for agricultural soils. The mean value of organic carbon up to the depth of 30 cm was found to be 68.99 t ha^{-1}, or 1.58%, which is considered as low (1-2%) (Vidojević *et al.*, 2014). Statistically significant differences in the variations of organic matter content over time can be obtained only when an adequate database is available (Sleutel *et al.*, 2003; Van Meirvenne *et al.*, 1996). This paper presents an assessment of organic carbon stocks in the soils in the Republic of Srpska. The assessment of organic carbon stocks in the soils in the Republic of Srpska was carried out in the period 2014-2017. The result of this research is the first step towards the establishment of SOC monitoring system in the Republic of Srpska.

MATERIAL AND METHODS

On the territory of the Republic of Srpska (RS), in the period 2014 - 2017, the fertility control of arable land was performed in 4125 average samples representing the surface area of 5776 ha. The analyzed area is represented by 23 types of soil (FAO class). An average sample consists of 15 to 25 individual samples taken from top soil (0 - 30 cm). The average tested plot area was 1.4 hectares. All samples are geo-positioned and linked to the SOTER database (land and terain databases). The Republic of Srpska is divided into 262 SOTER units (surfaces which are similar by the geological surface, soil type and geomorfology).

In each soil sample humus was analysed (colorimetric method, wet burning with $K_2Cr_2O_7$ and conc H_2SO_4). Soil organic carbon (SOC) was calculated from humus (% humus x factor 0.58). SOC stock (t ha^{-1}) for each plot were calculated on the basis of the volume mass (mg m^{-3}) of the soil type on which the plot is located, the soil weights up to 30 cm (kg ha^{-1}) and the area of the plot (ha). The assessment of SOC stocks on the total arable land of the Republic of Srpska (RS) was performed using ARCMAP 10.0. Agriculture lands are separated from the LCLU maps of RS 1:100 000 and represented by the following polygons: arable land, meadows, orchards, vineyards and fragmented areas (polygons) on which they dominate arable lands, meadows, orchards and vineyards. Abandoned areas (such as mined areas) and pastures (there are not fertility control on these areas) are not included in the research. The estimation of SOC stocks on agricultural areas (polygons) was obtained by IDW interpolation method of SOC values in 4125 samples. For the estimated SOC stocks content by the soil types, SOTER map of dominant FAO soil types was used. Mean values of the content of organic carbon in the soil and standard deviation were calculated with Statistica Version 8.0 (2007)

RESULTS AND DISCUSSION

The results of SOC content analysis in RS are presented in two levels: measured values and value estimates. The measured values of SOC were obtained on the basis of the results of the SOC analysis in 4125 average soil samples (0-30 cm) collected from 5777 ha of cultivable surfaces (1% of total cultivable area of RS). The estimated values of SOC were obtained by GIS interpolation of measured values on the total cultivable area of Republic of Srpska (578894 ha). The results of the measured SOC values are shown in Table 1. The analyzed area (5777 ha) is represented by 23 types of soil (FAO class).

Table 1. The measured values of SOC and SOC stocks in analyzed cultivable areas shown by the dominant FAO soil types.

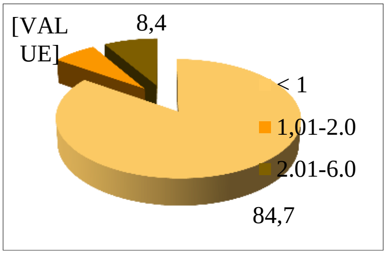
1	2	3	4	5	6	7	8	9	10	11	12
No.	FAO SOIL CLASS	Sample No.	Area ha	Bulk density g cm^{-3}		SOC %	SOC stocks t	SOC content t ha^{-1}			
				min	max			Mean	Min	Max	SD
1.	Stagnic Luvisols	464	826	1.07	1.32	0.6	17521	37.8	0.4	153.9	21.6
2.	Stagnic Podzoluvisols	618	1167	1.11	1.43	0.7	32421	52.5	1.9	157.6	26.0
3.	Chromic Luvisols	102	136	1.08	1.33	0.9	4503	44.0	0.8	110.0	25.7
4.	Eutric Fluvisols	318	435	1.16	1.50	0.9	16352	51.4	8.1	115.1	21.8
5.	Vertic Luvisols	425	544	1.10	1.40	0.9	18501	43.5	0.1	241.2	23.0
6.	Calcaric Fluvisols	272	388	1.11	1.54	1.0	15517	57.0	2.2	163.5	28.2
7.	Eutric Gleysols	631	858	1.21	1.52	1.0	33649	53.3	0.5	303.1	28.1
8.	Eutric Vertisols	222	321	1.03	1.29	1.0	10749	48.4	11.6	158.4	21.3
9.	Ferric Luvisols	189	220	1.19	1.19	1.0	8214	43.5	8.3	121.1	20.1
10.	Calcic Vertisols	57	62	1.05	1.36	1.2	2747	48.2	12.1	98.9	18.2
11.	Ferric Acrisols	34	39	1.34	1.65	1.2	2178	64.1	28.0	123.5	23.4
12.	Haplic Luvisols	104	125	1.21	1.34	1.2	5509	53.0	0.3	170.5	27.1
13.	Mollic Gleysols	51	65	0.54	1.24	1.3	2263	44.4	11.2	120.3	24.1
14.	Eutric Cambisols	97	104	1.02	1.36	1.9	7074	72.9	11.3	254.1	39.2
15.	Dystric Cambisols	48	44	0.99	1.47	2.2	3551	74.0	14.0	146.1	30.6
16.	Ferralic Cambisols	117	105	1.06	1.52	2.4	9848	84.2	18.2	317.1	52.2
17.	Eutric Leptosols	3	4	1.00	1.01	2.5	300	84.8	39.0	143.0	54.0
18.	Vertic Cambisols	65	56	0.98	1.29	2.8	5337	82.1	23.2	177.3	35.6
19.	Humic Cambisols	117	111	0.67	1.15	3.2	9584	81.9	16.0	261.5	39.5
20.	Mollic Leptosols	91	89	0.9	1.23	3.2	9090	99.9	18.8	354.9	54.3
21.	Rendzic Leptosols	54	48	0.71	1.26	3.6	5111	94.6	15.0	226.3	49.5
22.	Umbric Leptosols	15	8	1.16	1.16	4.1	1120	74.7	24.2	114.4	30.4
23.	Calcaric Cambisols	31	22	1.08	1.49	4.7	4030	130.0	36.3	351.4	69.5
Total:		4125	5777	-	-	-	225168	38-130	0,1-39	98,9-354,9	

The SOC stocks (column 8) in the analyzed soil types increases with the increase in the number of analyzed average samples (column 3), as this increases the area of the cultivated land (column 4, an average sample represents an area of 1.4 ha). However, a more realistic situation of SOC is obtained by analyzing % SOC (column 7). Table 2 shows content of SOC by classes of coverage according to Van Ranst *et al.* (1995). Data from Table 2 and Graph 1 show that agricultural production in RS on 91.5% of analyzed areas occurs with very low (84.7%) and low SOC content (6.8%). However, in Table 1, columns 10 and 11, a large variation range between the minimum and maximum SOC content (t ha^{-1}) for all types of soil is observed. For example, the lowest average value of SOC content is established in Stagnic Luvisol and is 37.8 t ha^{-1} or 1.05% (low content) SOC (Table1, number 1, column 9), and variation between minimum and maximum value of SOC is 153.4 t ha^{-1} and ranges from very low content of 0.5 t ha^{-1} to 153.9 t ha^{-1} and 4.3 %, which presents the class of medium SOC content. On this type of land, control of 464 soil samples (about 11% of all samples) was performed.

Table 2. SOC classes according to Van Ranst *et al. (1995)*

SOC Classes		No. of samples	% of samples	Area ha	% Area
Very low	< 1%	3241	78.6	4895	84.7
Low	1.01-2.0	343	8.3	395	6.8
Medium	2.01-6.0	541	13.1	487	8.4
High	> 6.01%	0	-	0	-

Graph 1. Content of SOC (%) in analyzed area



The highest average SOC value of 130 t ha^{-1} and 3.3% (medium content) was found in Calcaric Cambisols (Table 1, number 23, column 9). SOC variations in Calacaric Cambisolu ranged from very low content of 36.3 t ha^{-1} (0.9%) to high content of 651.4 t ha^{-1} or 9.1% OC Such a large variations of SOC is associated with the use of cultivable land and the application of good agricultural practice, i.e. with the structure of plant production. Table 2 shows stocks of SOC depending on the structure of plant production on examined cultivable land.

Table 3. SOC stocks on the analyzed cultivable land depending on the structure of plant production.

1	2	3	4	5	6
No.	Crops and plantings	Area ha	Area %	Average SOC t ha^{-1}	SOC %
1	Grain crops	4098	70.9	32.7	0.9
2	Vegetable	191	3.3	67.9	1.9
3	Orchards and vineyards	423	7.3	46.5	1.3
4	Meadows	1065	18.4	54.8	1.5
	Total:	5777	100.0	-	-

The results in Table 3 show that the analyzed cultivable land is mostly used for grain crops cultivation (71%), but also that the lowest average value of SOC 32.7 t ha^{-1} or 0.9% is found in these soils, which is very low SOC content. The lowest area of cultivable land is used for vegetable growing (3.3%), but in these soils twice the average SOC content of 1.9% (low level) is found.

The reason for this state of OC in the soil, on which vegetables are grown, is because of the intensive production on smaller areas where regular organic fertilizers are applied. When growing cereals, organic fertilizers are used to a lesser extent, mainly mineral fertilizers are used, and therefore the content of OC in these soils decreases. For these reasons, the conclusion is that the content of OC in the agricultural soil, besides the type of land, depends to a large extent on the way of use i.e. the structure of plant production, the regular application of measures for increasing the organic matter in soil and the type of agricultural production (organic, integral ...). Predic *et al. 2016* came to similar conclusions it was found that the results of the basic parameters of soil fertility indicate that it is the soil of different levels of fertility, which is related to the land use and soil type. The average content of OC in meadow areas, permanent crops, i.e. in soils which are occasionally processed, depend to a great extent on the type of land.

For evaluation of SOC stock in soils in RS, GIS charts LC/LU were used, according to which RS agricultural area occupies 1047724 ha (42.5%) (Predić *et al.* 2011). The arable land of 578894 ha, marked out of the agricultural land, are dominated by the following: meadows, orchards, vineyards and fragmented areas (polygons). Currently abandoned arable areas (mine risk ...) are not analyzed in this paper because fertility control on these surfaces was not carried out. Figure 2 shows the spatial distribution of estimated OC content on arable land, and Table 4 shows the distribution percentage of the estimated OC content in agricultural land of RS.

Table 4. Class percentage of the estimated CO content in RS arable land

Class OC		ha	%
Very low	< 1%	469967	81.2
Low	1.01-2.0	100199	17.3
Medium	2.01-6.0	8363	1.4
High	> 6.01%	365	0.1
	Total:	578894	100

On the basis of the data obtained (Table 4), it can be concluded that agricultural production in RS on 81.5% of the arable areas is performed on very low OC soils, and on 17.3% of the low OC content soils which is in total 98.5% or 570166 ha of arable land with insufficient OC content. The estimated SOC stock is shown in Table 5.

Table 5. The estimated SOC stock by FAO soil classes (the total arable land area of RS)

1	2	3	4	5	6	7	8	9	10
No.	FAO SOIL CLASS	Area ha	%	SOC stocks t	SOC %	SOC content t ha^{-1}			
						Mean	Min	Max	SD
1.	Eutric Leptosols	151	0.03	10100	0.03	66.9	13.5	74.7	9.8
2.	Umbric Leptosols	5238	0.9	453610	1.4	86.6	34.6	120.8	16.7
3.	Calcaric Cambisols	5596	1.0	492467	1.5	88.0	33.3	286.3	40.8
4.	Vertic Cambisols	7290	1.3	568609	1.7	78.0	23.2	177.3	26.7
5.	Mollic Gleysols	10899	1.9	573266	1.8	52.6	11.2	145.0	25.7
6.	Dystric Cambisols	12897	2.2	753162	2.3	58.4	14.0	131.9	20.9
7.	Calcic Vertisols	17576	3.0	868257	2.7	49.4	10.5	98.8	10.6
8.	Eutric Fluvisols	18645	3.2	939703	2.9	50.4	8.1	114.6	15.3
9.	Ferric Luvisols	23238	4.0	1036410	3.2	44.6	8.3	121.1	13.0
10.	Ferric Acrisols	23009	4.0	1069916	3.3	46.5	18.3	93.2	11.1
11.	Ferralic Cambisols	14675	2.5	1071304	3.3	73.0	18.2	185.8	35.5
12.	Chromic Luvisols	23886	4.1	1084444	3.3	45.4	0.9	107.1	16.9
13.	Rendzic Leptosols	15552	2.7	1286171	3.9	82.7	19.8	207.0	29.8
14.	Mollic Leptosols	18724	3.2	1421123	4.4	75.9	18.8	278.8	27.0
15.	Haplic Luvisols	27597	4.8	1437804	4.4	52.1	0.3	168.2	16.2
16.	Eutric Vertisols	26155	4.5	1558829	4.8	59.6	12.6	148.8	22.4
17.	Eutric Cambisols	36385	6.3	2252220	6.9	61.9	12.3	254.0	23.5
18.	Humic Cambisols	29228	5.0	2326586	7.1	79.6	16.0	186.5	32.3
19.	Stagnic Podzoluvisols	46012	7.9	2383418	7.3	51.8	2.2	157.4	17.4
20.	Vertic Luvisols	47723	8.2	2510256	7.7	52.6	0.1	241.0	17.7
21.	Eutric Gleysols	46783	8.1	2666629	8.2	57.0	0.5	302.8	17.3
22.	Stagnic Luvisols	70341	12.2	2855860	8.8	40.6	0.4	150.5	13.5
23.	Calcaric Fluvisols	51293	8.9	2964757	9.1	57.8	2.2	163.4	21.3
Total		578894	100.0	32584900	100.0	44.6 - 88.0	0.1 -34.6	74.7-302,8	-

Table 4 shows soil types according to SOC content. The results show that Stagnic Luvisols is the most common type of soil (12.2%) on the arable areas of RS, while the largest SOC stocks are in Calcaric Fluvisol (9.1%). The estimated SOC stock in arable agricultural land of RS is 32584900 t, which is 56.3 t ha^{-1} in average or 1.56% of RS, respectively. Therefore, the arable land in RS is classified as low OC content land by Van Ranst *et al.* (1995). Similar results were obtained in Serbia, where the mean value of SOC was established at 68.99 t ha^{-1} or 1.58% to 30 cm of depth (Vidojević *et al., 2014*).

CONCLUSIONS

The SOC stock in arable agricultural land on 578894 ha in RS is estimated to 32584900 t. Average OC content is 56.3 t ha^{-1} or 1.56% which classifies RS arable land in low OC content land, according to Van Ranst *et al.* (1995). 98.5% or 570166 ha of the arable land in RS is with the insufficient OC content for agricultural production, while in 81.5% of the arable land a very low OC content was determined. OC in the agricultural soil, besides the type of land, depends to a large extent on the way of use i.e. the structure of plant production, the regular application of measures for increasing the organic matter in soil and the type of agricultural production (organic, integral ...). The result of this research is the first step towards the establishment of SOC monitoring system in Republic of Srpska.

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EFFECTS OF WEANING SYSTEM ON MILK AND EXTERNAL MAMMARY CONFORMATION TRAITS OF SICILO-SARDE TUNISIAN DAIRY EWE

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ABSTRACT

A better development of the Sicilo-Sarde dairy sheep in Tunisia needs a review of its behavior by adopting early weaning and generalizing mechanical milking that involves an adaptation of the external mammary morphology (MM). Thirty ewes were divided into two groups (early and late weaning; EW and LW respectively) to study the effects of weaning system (WS) and milking time (MT) on milk and external MM traits in early milking period. MM was evaluated by six measurements and three scores of udder and teat. EW group had higher milk production (MP) and lower fat and protein amounts that increased with MT advancement ($P<0.001$). WS did not affect MM traits ($P>0.05$), only teat length was higher for LW ewes ($P<0.05$). Udder depth, teat diameter, distance between teats and teat angle score decreased with MT ($P<0.01$). Significant interactions were noted between WS and MT for most traits studied. MP was negatively correlated with fat and protein amounts (-0.38 and -0.50 respectively) and moderately correlated with udder depth, cistern height, teat diameter (from 0.31 to 0.42). Fat and protein had negative correlation with udder depth, teat diameter and distance between teats. Higher correlation was determined between udder depth and distance between teats ($r=0.60$; $P<0.001$). In conclusion, EW system allows a better start of the milk production in early milking period. Cistern height, teat length and teat angle score are the prominent traits which affect milk ability and adaptation of ewe to machine milking and consequently they must be included in selection program.

Keywords: Dairy sheep, milk composition, mammary conformation, Correlation, milkability.

INTRODUCTION

The level of milk production (MP) in early lactation and especially during the first two months is crucial for both total milk production ewes and lamb growth. Thus, MP increases rapidly until the peak of lactation reached at 3-4 weeks after lambing (Cappio-Borlino et *al*., 1997). In addition, about 25% of total MP is obtained during the first month of lactation (McKusick et *al*., 2001). In Tunisia, in Sicilo-Sarde farms, the only dairy sheep in northern Africa, ewes are milked after a long suckling period that exceeds two and a half months and sometimes 100 days (Meraï et *al*., 2014; Aloulou et *al*. 2018) and can probably explains, among others, the low level of MP of this breed. Thus, this farming mode is not economic for dairy ewes. Efforts should be conducted to increasing both the commercial milk yield and growth performance of lambs. More appropriate behavior by an earlier start of the milking period is required.

In other hand, mechanical milking of Sicilo-Sarde ewes is a recent technological advance that is developing in dairy sheep farms. Furthermore, the mechanical milking ability of the ewe is under control of several effects including mammary conformation (Rovai et *al*., 1999; Marnet and Negrão, 2000; Milerski et *al*., 2006; Gelasakis et *al*., 2012). In fact, under perfect conditions of milking, characteristics of mammary morphology are very important for the milkability of dairy ewes and also for their udder health, the quantity and the quality of milk (Dzidic et *al*., 2004). The udder of Sicilo-Sarde ewe is quite well developed, with a strong attachment with straight teats (Khaldi and Farid, 1981). The external mammary morphology of the Sicilo-Sarde in Tunisia has been studied only once in the region of Bizerte (Ayadi et *al*., 2011). Thus, the present work aims to evaluate the effects of weaning system (early *vs.* late) and milking time on milk and external mammary morphology traits in early milking period.

MATERIALS AND METHODS

Thirty multiparous Sicilo-Sarde ewes were used at the farm of the Cooperative Unit of Agricultural Production of Methline (Bizerte, Tunisia) to study the effects of weaning system (WS) and milking time (MT) on milk and external mammary morphology traits in early milking period. Ewes were divided into two groups according to WS (early and late weaning; EW and LW respectively). During the experimental trial, ewes were reared indoor and received daily 1.8 and 0.7 kg/head of oat hay and commercial concentrate respectively.

Ewes were weaned from their lambs at 30-35 and 60-70 days *postpartum* respectively for EW and LW groups. Ewes were machine milked twice daily at 09:00 and 21:00 h. The amount and chemical composition of milk were recorded weekly for the two first measuring thereafter the dairy control was done every two weeks during the 45 days of starting exclusive milking. Chemical composition of milk was determined from samples of the morning milking using a MilkoScan FT 4000 (Foss Electric, integrated Milk Testing).

External mammary morphology (MM) was evaluated by six measurements and three linear scores of udder and teat according to Milerski et *al*. (2006) and the modified method described by Marie-Etancelin et *al*. (2005), respectively. Udder and teat morphology included: udder depth (UD), udder height (UH), cistern height (CH), teat length (TL), teat diameter at the base (TD) and distance between teats (DBT). Subjective linear scores evaluation of udder cleft (UCS), udder-hock distance (UHDS) and teat angle (TAS) were carried out using a scale from 1 to 9 : UCS (1= udder cleft absent, 9= glands were clearly divided), UHDS (1=udder-floor well below hocks; 9= udder-floor above hocks) and TAS with regard to the vertical position (1= horizontal; 9= vertical). The measurements of udder and teat morphology traits were measured one to three hours after the morning milking.

The data of milk traits and mammary morphology were analyzed using the GLM procedure (SAS, version 9.1), with weaning system (WS) and milking time (MT) as variation factors. Differences between Least Square Means were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Weaning system (WS) had significant effects on all milk traits ($P \leq 0.05$) in early milking period (Table 1). Ewes of EW group produced significantly higher daily milk production (DMP) by +123 ml than those of LW group. These findings agree with previous studies (McKusick et *al*., 2001; Dikmen et *al*., 2007; Mohamed et *al*., 2008) that have found an increase in the production of milk marketed for early weaning system. It could be associated with the fact that dairy ewes produced about 38 and 30% of total milk in the first and second months of lactation (McDonald et *al*. 1995). McKusick et *al*. (2001) also reported that about 25% of total milk production is obtained in the first four weeks of lactation.

As for milk chemical composition, milk of LW group contain higher amount of milk fat (+13.3 g/kg), protein (+3.4 g/kg) and total solids (+15.6 g/kg). Also, its parts had increased with the advancement of milking period by +9.7, 6.5 and 11.4 g/kg respectively for milk fat, protein and total solids as reported by Bencini and Pulina (1997) and Komprej et *al*. (2012). Thus, these authors found that production and composition of milk vary with lactation stage and mainly milk fat content and milk production change inversely.

Significant interactions also were noted between WS and MT for production and composition of milk. For EW ewes, the DMP had decreased by 41% during the first month of milking. However, MT did not affect ($P > 0.05$) the DMP for LW ewes which were more persistent in their milk production in early lactation period. Also, Marnet and Negrão (2000) reported that the transition from suckling to exclusive milking is usually accompanied by a drop in milk production of about 30-40%. The rate of milk drop was higher in early lactation than after (Bencini and Pulina, 1997; Marnet and Negrão, 2000) may be related to the important maternal effect during this period. For this reason, the DMP of EW ewes had declined more rapidly. In this context, previous work recommended in the pre-weaning period to combined suckling and milking whose purpose is to reduce the negative effects of weaning shock for both ewe and lamb (McKusick et *al*., 2001; Dikmen et *al*., 2007).

Table 1. Least squares mean for the effects of weaning mode and milking time on milk yield and chemical composition (g/kg) in early milking period of Sicilo-Sarde ewes

	Mean	**Weaning system**		**Milking time**		**Weaning system * milking time**			
		EW	**LW**	**T1**	**T2**	**EW-1**	**EW-2**	**LW-1**	**LW-2**
DMP(ml)	589	658^{a}	525^{b}	706^{a}	477^{b}	829^{a}	486^{b}	583^{b}	468^{b}
Fat	69.2	62.3^{b}	75.6^{a}	64.1^{b}	73.8^{a}	47.97^{c}	76.71^{ab}	80.25^{a}	70.88^{b}
Protein	52.0	50.2^{b}	53.6^{a}	48.7^{b}	55.2^{a}	44.55^{b}	55.87^{a}	52.76^{a}	54.47^{a}
Total Solids	172.3	164.2^{b}	179.8^{a}	166.3^{b}	177.7^{a}	147.59^{c}	180.74^{ab}	185.06^{a}	174.58^{b}

[a,b,c,] means, in the same line and for the same effect, with different superscript are significantly different (P< 0.05); DMP: Daily Milk Production; EW, Early Weaning; LW, Late Weaning; ml, millilitre;

Results of external mammary morphology (MM) are shown in Table 2. No significant differences between the two WS were observed for all udder and teat traits except teat length (TL). LW ewes had longer teat than these of EW groups (28.4 *vs.* 25.0 mm). Also, Rovai et *al*. (1999) found an increase in the TL when lactation progress. However, udder depth (UD), teat diameter at the base (TD) and distance between teats (DBT) had decreased and udder-hock distance score (UHDS) increased with the advancement of MT. These results agree with those of Rovai et *al*. (1999). The other measurements of MM were not affected (P > 0.05) by the MT. Significant interactions were noted between WS and MT for most MM traits. The values of UD, TD, and DBT were higher and udder-hock distance score (UHDS) was lower for EW ewes at the beginning of milking as DMP was higher. However, TL was higher for LW group at the beginning of MT. For both groups, the means of teat angle score (TAS) indicated that teats became positioned more horizontally with the advancement of lactation as reported by de la Fuente et *al*. (1996).

Overall, the mean obtained for UH (202.6 mm) was in the range given by Gelasakis et *al*. (2012) for Chios ewes. The average of CH (37.9 mm) was lower than 46-54 mm reported by Gelasakis et *al*., (2012). The average of TL (26.7 mm) for the Sicilo-Sarde ewe were shorter than 33.6-36.5 and 43-47 mm reported respectively by Milerski et *al*. (2006) and Gelasakis et *al*. (2012). The difference between studies may be related to breed, parity and stage of lactation (Milerski et *al*., 2006; Gelasakis et *al*., 2012). As for the subjective linear scores, UCS, UHDS and TAS averaged 6.52, 6.00 and 3.30 respectively. These means indicated that ewes had glands visibly divided, their udder-floor was slightly above the hock and the teat had an angle of about 64° to the vertical.

Table 2. Least squares mean for the effects of weaning system and milking time on mammary morphology characteristics in early milking period of Sicilo-Sarde ewes

	Mean	Weaning system		Milking time		Weaning system * Milking stage			
		EW	LW	T1	T2	EW-T1	EW-T2	LW-T1	LW-T2
Udder and teat measurements (mm)									
UD	80.0	81.9	78.2	88.3[a]	71.8[b]	93.5[a]	70.36[c]	83.00[b]	73.33[c]
UH	202.6	203.4	202.0	202.6	202.7	202.14	204.64	203.17	200.83
CH	37.9	39.3	36.6	38.0	37.9	40.32	38.23	35.62	37.52
TL	26.7	25.0[b]	28.4[a]	27.1	26.2	23.75[b]	26.20[b]	30.47[a]	26.28[b]
TD	15.2	15.8	14.7	17.0[a]	13.5[b]	18.42[a]	13.18[c]	15.59[b]	13.88[bc]
DBT	106.9	108.2	105.7	112.2[a]	101.7[b]	114.64[a]	101.79[b]	109.67[ab]	101.67[b]
Subjective linear scores									
UCS	6.52	6.54	6.50	6.20	6.83	6.14	6.93	6.27	6.73
UHDS	6.00	5.75	6.23	5.60[b]	6.38[a]	5.00[b]	6.50[a]	6.20[a]	6.27[a]
TAS	3.30	3.14	3.45	3.89[a]	2.70[b]	3.75[a]	2.54[b]	4.03[a]	2.87[ab]

[a,b,c,] means, in the same line and for the same effect, with different superscript are significantly different (P< 0.05); EW, Early Weaning; LW, Late Weaning; T, Time; mm, millimetre, MT, milking Time ; UD, Udder Depth ; UH, Udder Height; CH, Cistern Height; TL, Teat Length; TD, Teat Diameter; DBT, Distance Between Teats; UCS, Udder Cleft Score; UHDS, Udder-Hock Distance Score; TAS, Teat Angle Score

Relationships between traits of milk and MM measurements were studied by determine the Pearson correlation coefficients (Tableau 3). Fat and protein amounts were negatively correlated with DMP (r= -0.38 and -0.50, respectively) as reported by Bencini and Pulina (1997). DMP was moderately correlated with UD, CH, TD (r=0.31-0.42). Also, suggesting the use of these traits in selection program to improve the productivity of this ewe. Fat and protein amounts were correlated negatively with UD, TD and DBT and had positive correlation with UHDS. MM traits were little correlated between them. The highest correlation coefficient was determined between UD and DBT (r=0.60; P<0.001). CH, TL and TAS are the prominent traits which affect milkability and adaptation of ewe to machine milking and consequently they must be included in selection program.

Table 3. Pearson correlation coefficients between milk yield traits and mammary morphology characteristics in early milking period of Sicilo-Sarde ewes

	DMP	Fat	Protein	UD	UH	CH	TL	TD	DBT	UCS	UHDS
Fat	-0.38 **	-									
Protein	-0.50 ***	0.54 ***	-								
UD	0.31 *	-0.41 **	-0.31 *	-							
UH	0.25 NS	-0.03 NS	0.04 NS	0.32 *	-						
CH	0.42 **	-0.09 NS	-0.14 NS	0.08 NS	0.45 ***	-					
TL	-0.01 NS	0.23 NS	0.04 NS	-0.18 NS	-0.02 NS	-0.08 NS	-				
TD	0.38 **	-0.49 ***	-0.47 ***	0.33 *	-0.01 NS	0.03 NS	0.24 NS	-			
DBT	0.31 *	-0.29 *	-0.33 *	0.60 ***	0.26 *	0.14 NS	0.02 NS	0.39 **	-		
UCS	0.06 NS	0.21 NS	0.05 NS	-0.36 *	-0.12 NS	0.09 NS	-0.03 NS	-0.10 NS	0.00 NS	-	
UHDS	-0.20 NS	0.34 **	0.29 *	-0.27 *	-0.23 NS	-0.22 NS	0.09 NS	-0.34 **	-0.57 ***	-0.07 NS	-
TAS	0.00 NS	-0.19 NS	-0.17 NS	0.23 NS	-0.18 NS	-0.53 ***	0.35 **	0.45 ***	0.26 *	-0.22 NS	-0.21 NS

UD, Udder Depth; UH, Udder Height; CH, Cistern Height; TL, Teat Length; TD, Teat Diameter; DBT, Distance Between Teats; UCS, Udder Cleft Score; UHDS, Udder-Hock Distance; TAS, Teat Angle Score; NS, Not Significant; *, P≤0.05; **, P≤0.01; ***, P≤0.001

CONCLUSION

During the early milking period, early weaning system allows a better start of the milk production. However, mammary morphology traits did not change with weaning system. Cistern height, teat length and teat angle score are the prominent traits which affect milkability and adaptation of ewe to machine milking and consequently they must be included in selection program of this breed. More detailed study with a high number of animals should be carried out to better characterize of the mammary morphology of Sicilo-Sarde ewe.

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PLANT HEIGHT CONTROL OF *HYACINTHUS ORIENTALIS* BY GIBBERELLIN INHIBITORS

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ABSTRACT

In this study, effect of gibberellin inhibtors as preplant bulb soaks on plant height of *Hyacinthus orientalis* cv. 'Jan Bos' grown in pots were investigated. Bulbs of hyacints were soaked in flurprimidol at 0, 10, 20 ppm and paclobutrazol at 0, 100, 200 ppm before planting. Effect of gibberellin inhibitors on the flowering time, flower diameter and length, leaf length, plant height, flower life, chlorophyll content of leaves were determined. In addition, after hyacints grown in pots in the greenhouse arrived at the sales stage to determine the changes that occur in the plant height, plants were taken to the laboratory where temperature was held constant at 20 °C. The shortest plant height was obtained from the 200 ppm paclobutrazol and 20 ppm flurprimidol treatment as given bulb soaks. In this treatments, plant heihgt was 7.33 and 8.61 cm and were 49% , 41% shorter than untreated control. The lower dose of 10 ppm flurprimidol and 100 ppm paclobutrazol were also effective on height control with 9.11 and 9.71 cm plant height, respectively. Gibberellin inhibitors also shortened leaf lenght. Flurprimidol and paclobutrazol treatments resulted in higher chlorophyll content per unit area in the leaves than untreated controls. The highest chlorophyll contetnt was obtained from the plants treated 200 ppm paclobutrazol with 83.36 CCI (Chlorophyll content index), while the control was 50.56 CCI. The effects of treatments on plant height were maintained in lab conditions (home-office). The shortest plant height was obtained from 200 paclobutrazol treatment with 9.75 cm, while the control was 21.5 cm during post production period.

Keywords: *Flurprimidol, Paclobutrazol, Bulb Soak, Hyacinthus, Plant Height.*

INTRODUCTION

Hyacinthus orientalis from the Hyacinthaceae family is perennial plant (Samuitiene et al., 2007; Addai, 2010). It is a horticulturally important plant and native of the West and Central Asia (Addai, 2010). *H. orientalis* is a spring flowering bulb, they have a strong sweet fragrance (Gender, 1994). They are also commonly used in parks, gardens and refuges as ornamental plants. In addition, *Hyancinthus* is also used as potted plant in the indoor. But the fact that their excessive elongation after production at consumer conditions with low light makes it difficult to use as indoor plants (Çelikel et al., 2016). Elongation causes also downward curvature of the flower stem with heavy inflorescence. Therefore, plant height control is important for maintaining compactness and aesthetically pleasing appearance, as well as preventing damage during transportation and marketing due to stem elongation (Çelikel et al., 2016). We may control plant height either by physical methods with environmental factors (light, temperature, etc.), or by chemical methods with growth regulators mostly gibberellin inhibitors (Demir and Çelikel, 2013; Çelikel et al., 2016). These inhibitors are paclobutrazol, flurprimidol, ancymidol, uniconazole, chlormequat chloride and daminozide (Currey and Lopez, 2017).

Flurprimidol as bulb soaks at 20 mg L^{-1} controlled the plant height of ‘Pink Pearl’ hyacinth cultivar cultivars at anthesis and postharvest evaluation (Krug et al., 2006a). Flurprimidol ≥ 10 mg L^{-1} controlled plant height of ‘Delft Blue’ hyacinth cultivar during postharvest evaluation (Krug et al., 2006a). Uniconazole at 30 mg L^{-1} controlled height of ‘Delft Blue’ during the postharvest evaluation and plants treated with uniconazole were 8% shorter than untreated control (Krug et al., 2006a). ‘Jan Bos’ hyacinth cultivar treated with ≥ 10 mg L^{-1} flurprimidol were 4% shorter than untreated control during greenhouse forcing (Krug et al., 2006a). Miller (2010a), reported that growth regulators such as ancymidol, paclobutrazol, flurprimidol act to reduce gibberellin levels in the plant thereby causing shorter plants. Pre-plant flurprimidol dips show excellent control for hyacinth growth regulation (Miller, 2010a). Paclobutrazol or uniconazole pre-plant dips were effective in controlling height of prepared ‘Anna Marie’ hyacinths (Miller, 2002). Plant height of ‘Carlton’ narcissus cultivar was controlled by flurprimidol and paclobutrazol as soil drench at 3 mg/pot concentration (Miller, 2010b). Flurprimidol bulb soaks controlled plant height of narcissus during both greenhouse and postproduction evaluation (Krug et al., 2006b).

Effects of flurprimidol and paclobutrazol were investigated on plant height of *Hyacinthus orientalis cv.* ‘Jan Bos’ and other *Hyacinthus* cultivars before but other parameters (leaf length, flower diameter and length, chlorophyll content of leaves) except plant height were not studied on *H. orientalis cv.* ‘Jan Bos’. In addition there is no previous study on plant height control of *H. orientalis* cultivars in Turkey. Therefore, we investigated the effects of these chemical treatments as preplant bulb soaks on plant height and other properties of *Hyacinthus orientalis* cv. ‘Jan Bos’ grown in pots.

MATERIAL AND METHOD

Hyacinthus orientalis cv. ‘Jan Bos’ bulbs with circumference of 12 cm from Asya Lale (Konya, Turkey) were used in this study. Bulbs were soaked into flurprimidol (FP,Sigma-Aldrich) solutions of 0, 10, 20 ppm or paclobutrazol (PBZ, 25% Cultar; Syngenta) of 0, 100, 200 ppm for 30 min before planting. Ethanol (2%) was used as a solvent of flurprimidol. Therefore a control for the solvent was included in these experiments. Bulbs were allowed to air dry and were planted into a 15 cm diameter plastic pots (1.6 volume) containing soil, peat and perlite (1:1:1) as one bulb per pot on the day of treatment (11th October 2013). Plants grown in a polyethylene covered greenhouse were irrigated as needed with tap water.

Postproduction evaluation: When hyacinths reached to the sale stage (50% open flower in a stem with buds), four replicate plants randomly selected from each treatment were taken to the laboratory. Postproduction life and quality of pot plants were evaluated in this laboratory at 20 °C illuminated with Cool White Fluorescent light of 1000 lux at bench level, under a diurnal cycle of 12 h day, 12 h night as standard conditions (Çelikel and Karaçalı, 1991; Çelikel, 1993).

Flowering time and flower life: Flowering time was determined as number of days from planting time to opening of the 50% flower in stem. Flower life was calculated as the number of days from the opening of the 50 % flower to the wilting of the more than 50% flower in stem.

Chlorophyll content: Chlorophyll content of leaves was measure by chlorophyll meter (Apogee). It was determined as Chlorophyll content index (CCI).

Plant height and Leaf length: The plant height (from the pot rim to the uppermost of the inflorescence) and leaf length (the longest leaf) were started to measure respectively 103 days (22nd January 2014) and 117 (5th February 2014) after planting. Measurements were made weekly.

Flower diameter and length: the flower diameter and length was measured by a caliper at anthesis time.

Data Analysis: Data were tested by one way analysis of variance (ANOVA) using a completely randomized design. The study was conducted with 10 replications except 4 replications for postproduction evaluation. The obtained data were analyzed statistically by using the SPSS package program. The mean and standard error ($\overline{X} \pm S\overline{x}$) values were determined. Differences between means were separated by Duncan's multiple range test ($P \leq 0.01$).

RESULTS AND DISCUSSION

Flowering time, flower life and chlorophyll content; Plant growth regulators delayed the flowering time of hyacinths. The delay of flowering time was found significant ($P \leq 0.01$). The latest flowering was obtained from 200 ppm PBZ with 128 days, while control and ethanol were 118 and 119 days, respectively (Table 1). The gibberellin inhibitors delayed flowering time about 3-9 days. A delay was observed in some *Iris* cultivars in the visible appearance buds in plants treated with paclobutrazol (Francescangeli, 2009). The application of paclobutrazol delayed the appearance of the flower color in *Petunia* (Francescangeli and Zagabria, 2009). Flurprimidol application caused flowering delay of *Ornithogalum saundersiae* (Salachana and Zawadzińska, 2013). Flowering time of 'Mona Lisa' lily cultivar was slightly delayed with flurprimidol treatment (Pobudkiewicz and Treder, 2006). Flurprimidol has been reported as a highly effective retardant on a number of flowering ornamentals (Barrett 1983; Criley, 1997).

In our study gibberellin inhibitors did not affect the flower life of hyacinths except 200 ppm PBZ treatment (Table 1). The flower life of 'Jan Bos' changed in 15-22 days. There was no difference among the applications except higher dose (200

ppm) of PBZ in flower life of hyacinths (Table 2). The higher doses of PBZ shortened the flower life 5 days, while the lower dose of PBZ and FP treatments didn't affect the flower life. In addition the higher doses of these plant growth regulators also reduced significantly the plant height. Blázquez et al., (1998) reported that the gibberellin class of plant hormones has been implicated in the control of flowering in several species. It was reported that exogenous GA_2 promote the switch from vegetative growth to flowering in a variety of plants by Wilson et al., 1992. Therefore the gibberellin inhibitors used in this study, effected flowering. Treatments affected the duration of the cycle.
There was significant difference ($P \leq 0.01$) in chlorophyll content of leaves in hyacinths (Table 1). PBZ and FP treatments caused an increase in chlorophyll content of leaves (Table 1). The highest chlorophyll content was obtained from 200 ppm PBZ treatments with 83.36 CCI, while control plants were 50.56 CCI (Table 1). In addition the lower doses (100 ppm) of paclobutrazol and flurprimidol aplications also increased the chlorophyll content of hyacinths (Table 1). Chlorophyll content of hyacinths treated with 100 ppm paclobutrazol, 10 and 20 ppm flurprimidol were 64.74, 60.19 and 66.00 CCI, respectively. Paclobutrazol and uniconazole increased foliar chlorophyll content and leaf thickness in soybean (Barnes et al., 1989). The use of flurprimidol resulted plants with an increased relative chlorophyll content of *Ornithogalum saundersiae* (Salachna and Zawadzińska, 2013). It was reported that total chlorophyll content of leaves increased by 15 and 16% in tomato treated with paclobutrazol (Berova and Zlatev, 2000). Triazoles (Paclobutrazol, uniconazole) have generally increased chlorophyll content of treated plants, although exceptions have been reported (Wieland and Wample 1985; Barnes et al., 1989). In our study also gibberellin inhibitors of paclobutrazol and flurprimidol increased the chlororphyll content of leaves in hyacinths. Therefore leaves were dark green than control plants.

Table 1. The effects of flurprimidol (FP) and paclobutrazol (PBZ) on flowering time flower life and chlorophyll content of *Hyacinthus orientalis* cv. 'Jan Bos' Mean ± Standard Error ($\bar{X} \pm S\bar{x}$)

Treatments	Flowering time	Flower life (days)	Chlorophyll content (CCI)
Control	117.86 ± 0.94 c	19.00 ± 0.87 a	50.56 ± 4.80 c
Ethanol (%2)	118.88 ± 1.30 c	20.37 ± 1.03 a	50.67 ± 2.41 c
10 ppm FP	121.33 ± 1.01 bc	20.11 ± 0.96 a	60.19 ± 2.87 bc
20 ppm FP	121.22 ± 1.33 bc	20.56 ± 1.18 a	66.00 ± 4.26 b
100 ppm PBZ	123.83 ± 0.91 b	19.00 ± 0.97 a	64.74 ± 6.32 b
200 ppm PBZ	127.67 ± 1.31 a	14.56 ± 0.53 b	83.36 ± 4.98 a
Significance	0.000	0.000	0.000

* Different letters in the same columns indicate differences among treatments according to Duncan multiple range test (1%).

Plant height, leaf length, flower diameter and length; In our study the gibberellin inhibitors applications decreased the plant height, leaf length, flower diameter and length (Table 2). The shortest plant height (7.33, 8.61, 9.11, 9.71 cm) was obtained from 200 ppm PBZ, 20 ppm FP, 10 ppm FP and 100 ppm PBZ treatments, respectively, whereas the control plants (14.5 cm) were the longest ones (Table 2, Figure 1). There was no difference between ethanol and control plants (Table 2). Plants applied gibberellin inhibitors were 49% (200 ppm PBZ), 41% (20 ppm FP), 37% (10 ppm FP) and 33% (100 ppm PBZ) shorter than control (Figure 3, 4). It was reported that flurprimidol was effective growth retardant in reducing stem extension of ‘Mona Lisa’ lily cultivar without adverse side-effects (Pobudkiewicz and Treder, 2006). Paclobutrazol substrate drenches control plant height of ‘Tete a Tete’ narcissus cultivar during greenhouse forcing (Krug et al., 2006b). Soil drenches of uniconazole retarded shoot and petiole elongation of *Brassaia actinophylla* (Wang et al., 1990). In another study flurprimidol, paclobutrazol and uniconazole suppressed height of *Impatiens hawker* cultivars (Currey et al., 2016). *Hyacinths* treated with plant growth regulators in this study were shorter than control plants during post production evaluation (Figure 2). The height differences between control and treated plants were maintained in the post-production period (Figure 2). ‘Tete a Tete’ narcissus treated with 0.69 mg / pot flurprimidol were 15% shorter than control at the end of the post production evaluation (Krug et al., 2006b). We found that FP and PBZ effectively controlled the plant height not only during production in greenhouse but also after production and there was no significant difference between low and high doses of chemicals (Figure 2). Our results clearly indicated that the effect of plant growth regulators on plant height (Figure 2), leaf length, flower diameter and length continued in the laboratory (home-office) conditions during the post-production period. The leaf area and plant size of kalanchoe was decreased by gibberellin inhibitors of paclobutrazol and uniconazole treatments (Hwang et al., 2008). Similarly we found that FP was effective for height control of hyacinths in our study.

The shortest leaf length was obtained from 200 ppm PBZ, 20 ppm FP and 100 ppm PBZ application with 5.33, 5.94 and 6.07 cm, respectively. The longest leaf length is 7.8 and 7.7 cm in ethanol application and untreated control (Table 2). There was a significant ($P \leq 0.01$) difference among application for leaf length (Table 2). It was reported that Topflor (flurprimidol) treatment shortened leaf length of hyacinths cultivars (Miller 2010a). Uniconazole foliar spray caused to reduction in leaf length and width of *Fuchsia x hybrida* (Kim, 1995). In our study the gibberellin inhibitors decreased leaf length, flower diameter and length (Table 2). There was significant difference ($P \leq 0.01$) in flower diameter and length of ‘Jan Bos’ hyacinths (Table 2). The smallest flower diameters were 54.99, 55.04, 55.93 and 55.96 mm from 200 ppm PBZ, 20 ppm FP, 10 ppm FP and 100 ppm PBZ, respectively. Flower diameter of control and ethanol were 58.51 and 58.81 mm. The shortest flower length was obtained from 200 PBZ and 20 ppm FP with 5.81 and 6.63 cm, respectively, while control and ethanol was 9.65 and 9.12 cm, respectively. The tepal size, leaf size and pedicel length of plants applied flurprimidol were smaller than the control plants in 'Mona Lisa' lily cultivar (Pobudkiewicz and Treder, 2006). Flurprimidol application caused a reduction the inflorescence and flower diameter of *Ornithogalum saundersiae* (Salachana and Zawadzińska, 2013). The use of flurprimidol resulted with the shorter leaves in *Ornithogalum saundersiae* (Salachana and Zawadzińska, 2013) and *Zantedeschia aethiopica* (Gonzalez et al., 1999). Similarly in our study gibberellin inhibitors of FP and PBZ caused a reduction in flower diameter and length, were effective to shorten leaf length of hyacinth both during greenhouse and post-production period.

Table 2. The effects of flurprimidol (FP) and paclobutrazol (PBZ) on plant height, leaf length flower diameter and length of *Hyacinthus orientalis* cv. 'Jan Bos' Mean ± Standard Error ($\bar{X} \pm S\bar{x}$)

Treatments	Plant height (cm)	Leaf length (cm)	Flower diameter (mm)	Flower length (cm)
Control	14.50 ± 2.05 a	7.69 ± 0.67 a	58.51 ± 1.76 a	9.65 ± 1.31 a
Ethanol (%2)	13.25 ± 0.80 a	7.83 ± 0.46 a	58.81 ± 2.31 a	9.12 ± 0.59 a
10 ppm FP	9.11 ± 0.35 c	6.33 ± 0.28 bc	55.93 ± 0.48 b	7.38 ± 0.31 b
20 ppm FP	8.61 ± 0.40 c	5.94 ± 0.21 c	55.04 ± 2.55 b	6.63 ± 0.21 bc
100 ppm PBZ	9.71 ± 1.15 c	6.07 ± 0.95 c	55.96 ± 0.26 b	7.50 ± 0.45 b
200 ppm PBZ	7.33 ± 0.69 c	5.33 ± 0.26 c	54.99 ± 1.31 b	5.81 ± 0.46 c
Significance	0.000	0.003	0.000	0.000

* Different letters in the same columns indicate differences among treatments according to Duncan multiple range test (1%).

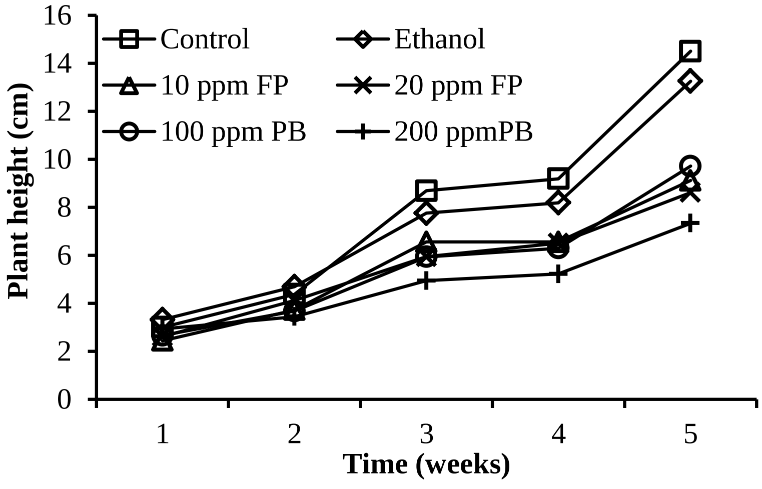

Figure 1. Effect of flurprimidol (FP) and paclobutrazol (PB) treatments on plant height of *Hyacinthus orientalis* cv. 'Jan Bos' during greenhouse production period The plant height was started to measure 103 days after planting (1. week)

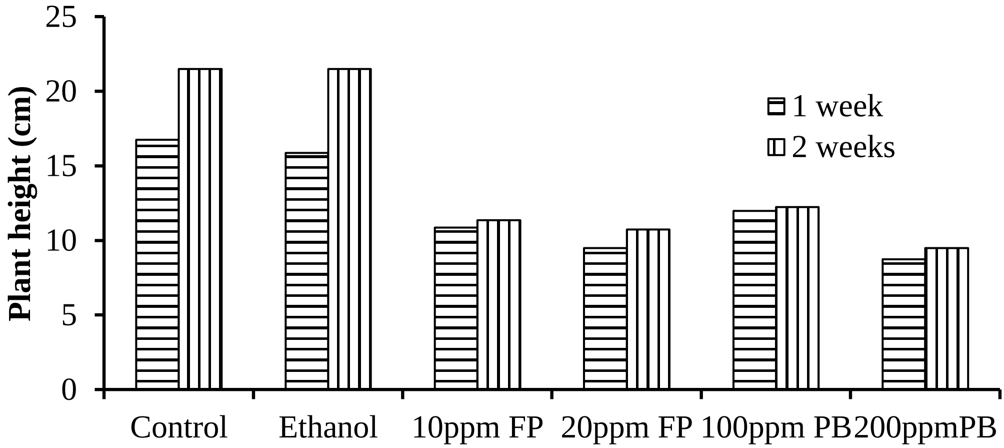

Figure 2. Effect of flurprimidol (FP) and paclobutrazol (PB) treatments on plant height of *Hyacinthus orientalis* cv. 'Jan Bos' during post production period in lab conditions. Plant height was started to measure 138 days after planting (1 week; 26 February 2014) in lab.

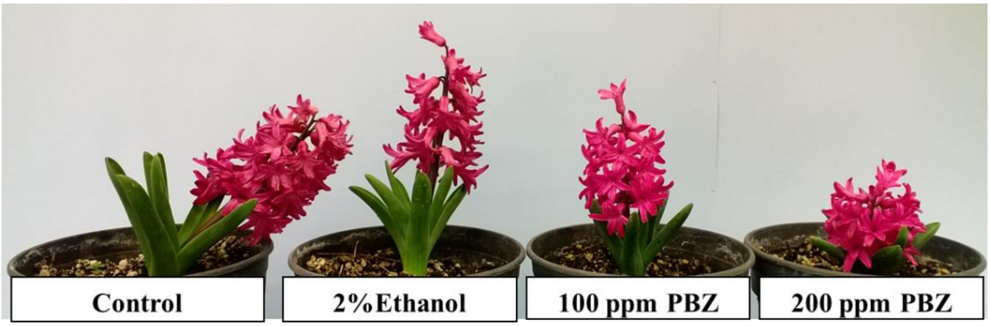

Figure 3. The effects of paclobutrazol (PBZ) bulb soak on *H. orientalis* cv. 'Jan Bos'

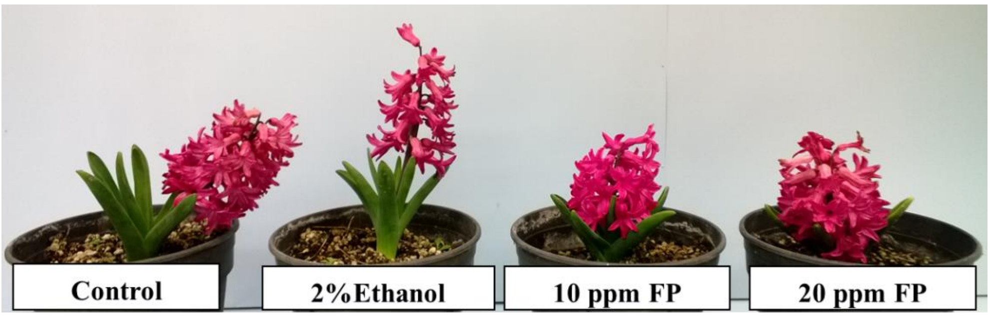

Figure 4. The effects of flurprimidoll (FP) bulb soak on *H. orientalis* cv. 'Jan Bos'

CONCLUSIONS

In conclusion gibberellin inhibitors controlled the plant height in hyacinths both during greenhouse and postproduction period. Paclobutrazol and flurprimidol decreased the leaf length, flower diameter and length and increased chlorophyll content of leaves. We found that there was no difference in flower life among the applications except higher dose (200 ppm) of PBZ. As a conclusion, we suggest 10 ppm flurprimiol or 100 ppm paclobutrazol, treatment as preplant bulb soak in order to provide plant height control and maintain post production quality of *H. orientalis* cv. 'Jan Bos' grown in pots.

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INFLUENCE OF CLIMATIC FACTORS ON THE QUALITY OF MERLOT GRAPEVINE VARIETY IN TREBINJE REGION VINEYARDS (BOSNIA AND HERZEGOVINA)

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ABSTRACT

The aim of this paper is to analyzed climatic influence on quality of yield of Merlot grapevine variety, in Trebinje region (Entity of Republic of Srpska, Bosnia and Herzegovina) vineyards, during the vegetation 2016 and 2017. We investigated the mechanical properties of bunch and berry (bunch weight, number of berries on the bunch, weight of 100 berries, weight of 100 berries flesh, weight of the berries skin, weight of seeds in 100 berries) and quality properties of grapes (sugar content and total acid content in the must). The highest bunch weight, as well as the number of berries on the bunch was achieved in 2017 (276.84 g, i.e. 183.03), while the lowest bunch weight (193.6 g) and number of berries on the bunch (158.53) were measured in 2016. Weight of 100 berries, weight of 100 berries flesh, weight of the berries skin, weight of seeds in 100 berries were larger in 2017. The highest sugar content was measured during 2017 and the highest level of acid in must was measured during 2016. During the years 2016 and 2017 a significant influence of climatic factors on the quality characteristics of the studied variety was observed. Although both considered years had above average temperature and less precipitation, further analysis of differences in monthly values of climatologically parameters could provide an explanation for differences in mechanical and quality properties of grapes.

Keywords: *climate, influence, Merlot, Trebinje vineyards*.

INTRODUCTION

The temperature characteristics of an area are primary to assess its suitability for growing vines. The area of Herzegovina is suitable for growing vines due to the high temperature sum of vegetation and mild winters whose absolute minimum rarely exceeds -10°C (*Kojić*, 2000). Trebinje is located in a zone of altered Mediterranean climate. It is characterized by a large number of sunny days (260), low relative humidity and cloudiness, rains in winters and warm summers. Autumn is significantly warmer than spring, the snow falls very rarely and when it falls, it does not stay for too long. The relief of the municipality is dominated by the mountain Leotar, the river Trebišnjica, Trebinje field and Popovo field. It is located at an altitude of 273 m. The reconstruction of the vineyards in the area of Herzegovina started in the last decade and new varieties have been introduced since then, Merlot variety is one of them.

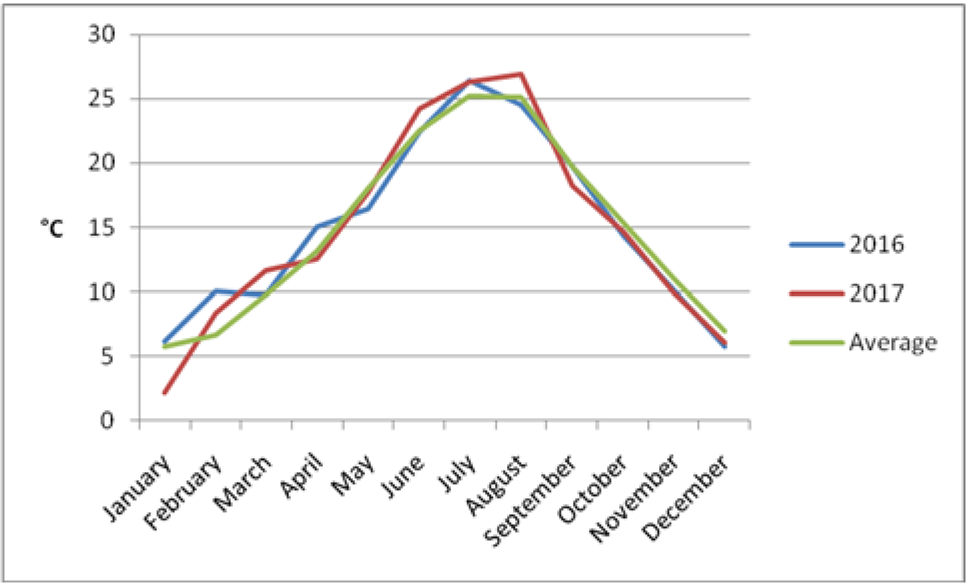
The aim of this paper is to analyzed climatic influence to quality of yield of Merlot grapevine variety and the possibility of its cultivation in climatic conditions of the Trebinje region and other wine regions of similar agro ecological conditions.

MATERIAL AND METHODS

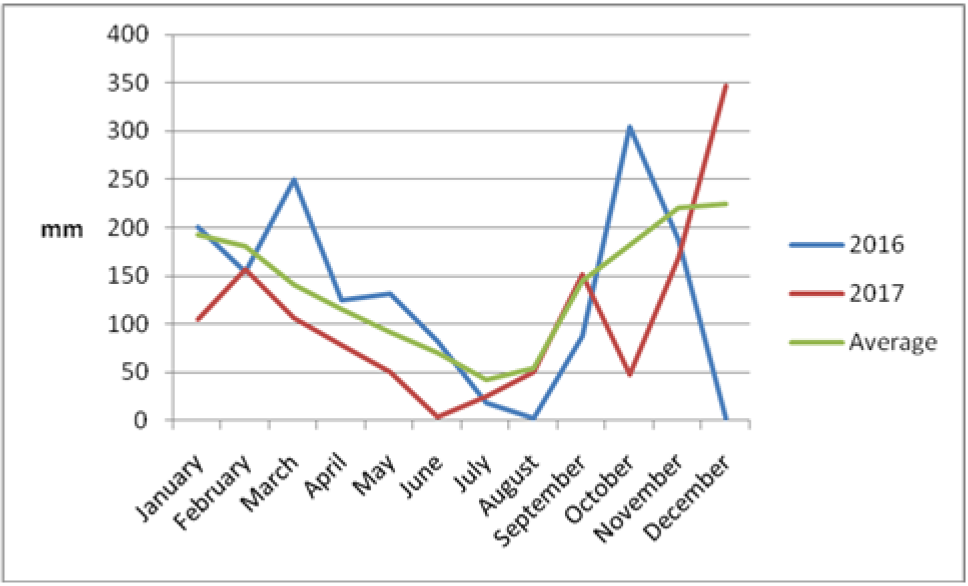
The research of yield quality of Merlot variety was carried out during the growing season in 2016 and 2017, in Trebinje area (Entity of Republic of Srpska, Bosnia and Herzegovina). The experiment was set up at the location of Petrovo field. This vineyard was founded in 2004. The distance between plantings was 2.8 x 0.9 m. 10 vines were selected for the research and each vine served as a separate experimental unit in the research. During the pruning, 14 buds were left on the vine. Basic measures of pruning were applied during the two years of research in experimental plantation, as well as basic measures for the protection of the most important causes of disease and pests. During the research, the "drop by drop" method of irrigation was used on the plantations. The investigations include the mechanical properties of bunch and berry (bunch weight, number of berries on the bunch, weight of 100 berries, weight of 100 berries flesh, weight of the berries skin, weight of seeds in 100 berries) and quality properties of grapes (sugar content and total acid content in the must). The data from Hydro meteorological Institute of the Republic of Srpska was used for the analysis of meteorological conditions observed during the two years.

RESULTS AND DISCUSSION

The amount and quality of grape yield depends largely on the climate and meteorological conditions that prevail in the regions of production. Therefore, in this paper the most important climatic elements were analyzed by comparing their values during studied years with the average values from the period of twenty years. Compared to long-term averages (1997-2017) both observed years were warmer and had less rainfalls (Graph 1. and Graph 2.). In January, March, April, May, June, October and November 2016, rainfall was considerably higher than in 2017, while in July, August and September rainfall was considerably higher in 2017.

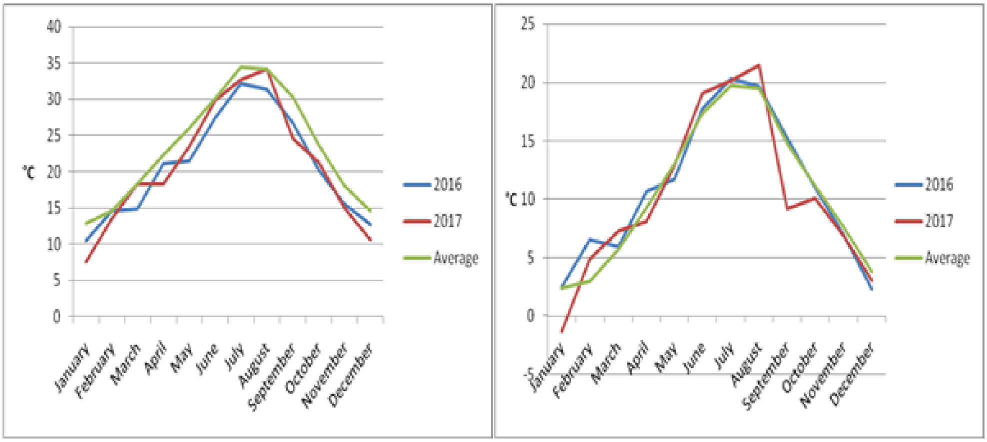


Graph 1. Average monthly temperature for the period of 1997-2017
as well as for 2016 and 2017 years.



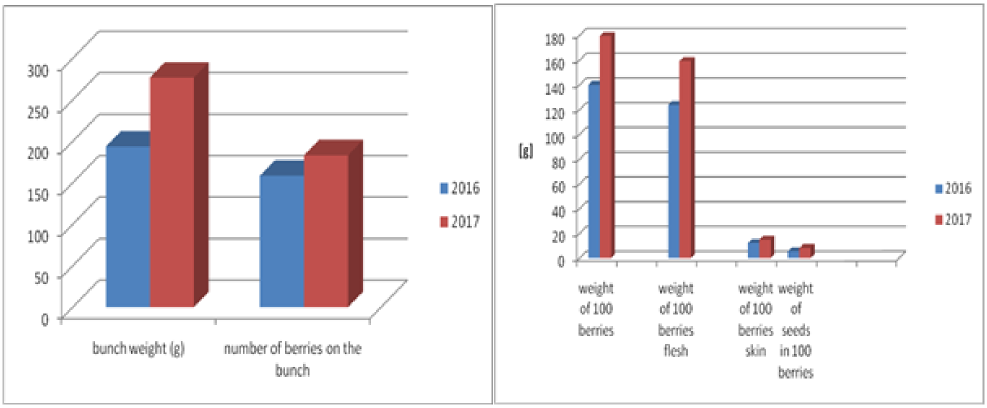
Graph 2. Average monthly rainfall for the period of 1997-2017
as well as for 2016 and 2017 years.

In Trebinje vineyards the warmest summer months are July and August, which is shown in Graph 3. The high temperatures during July and August were not adversely affecting the growth of the wine grape. The average maximum monthly temperature in May, June, July and August 2017 was higher than in 2016, but the average maximum monthly temperature in both studied years were lower compared to the perennial average (1997-2017).



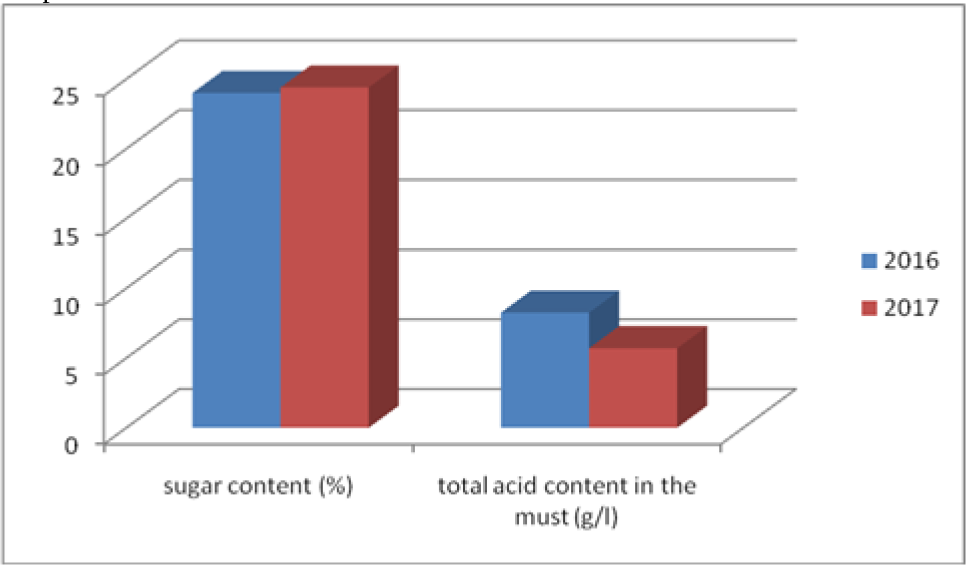
Graph 3. Average monthly maximum and minimum temperatures for the period of 1997-2012 as well as for 2013 and 2014 years.

The absolute and relative values of the mechanical composition are different for different varieties of grapevine and for different conditions of cultivation. Knowing the mechanical composition of clusters and berries has a special practical significance in assessing grapes as a raw material for processing and consumption in a fresh state. The mechanical composition of the bunches and berries is crucial for the grape must in wine ratio (*Blesić*, 2006). The values of certain parameters of the mechanical composition of the bunch of the Merlot variety are presented in Graph 4.



Graph 4. Mechanical composition of bunch of Merlot variety

In 2016, the average bunch weight (193.6 g) was less than the year 2017, when the average weight of 276.84 g was measured. A larger weight of 100 berries (179.42 g) and weight of 100 berries flesh (156.4 g) was in 2017, while in 2016 were measured weight of 100 berries (139.92 g) and weight of 100 berries flesh (122.24 g). Average weight of 100 berries skin were 14.88 g and weight of seeds in 100 berries were 8.14 g in 2017, while in 2016 weight of 100 berries skin were 12.23 g and weight of seeds in 100 berries were 5.45 g. The obtained values are higher compared to the results of *Pajović et al* (2009). Higher average monthly temperatures and precipitation in July, August and September 2017 contributed to higher values of parameters of the mechanical composition of bunch in 2017 compared to 2016.



Graph 5. Chemical composition of the must of Merlot variety

Influencing the physiological processes, the ecological potential of the production region significantly affect the quality of the grapes which is primarily reflected in the change of the sugar content, acid content, coloured and aromatic compounds, etc. The quantity of sugar in the grapes, depending on variety, degree of ripeness and health significantly depends on the climatic conditions in the ripening stage of grapes (*Ranković-Vasić et al.,* 2011). Based on the results given in Graph 5 it can be noticed that higher sugar content in grapes was measured in 2017 (24.4 %) and that particular year had higher average temperature during the May, June, July and August. Lower sugar content was obtained in 2016 (24 %). Total acid content in the must is an important indicator of the quality of grapes and it is linked to the taste and a harmony of grape and later wine (*Popović et al*.,2013). The total acid content in 2017 (5.7 g/l) was lower compared to 2016 (8.25 g/l) as a result of higher precipitation in July, August and September. These results are in accordance with the results of *Vukosavljević et al* (2011), *Garić et al* (2010) and *Avramov et al* (2003).

CONCLUSIONS

Based on the presented results the following conclusions on the influence of climatic factors on Merlot variety cultivated in agro ecological conditions of Trebinje can be formed:

- In Trebinje vineyards there are favorable agroecological conditions for growing Merlot variety.
- The results showed a significant influence of climatic factors on the quality of the yield of Merlot variety.
- Bunch weight and number of berries were lower in 2016, which was assigned to the lack of precipitation during the July, August and September. Weight of 100 berries, weight of 100 berries flesh, weight of the berries skin, weight of seeds in 100 berries also were lower in 2016.
- Higher sugar content had been measured in 2017 which was the result of higher average monthly temperature in May, June, July and August. The total acid content in 2017 was lower compared to 2016 as a result of higher precipitation in July, August and September.

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SPATIO-TEMPORAL DYNAMIC OF LAND DEGRADATION USING REMOTE SENSING-BASED INDEX

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ABSTRACT

Land degradation is the major issue which affect watershed sustainability and following social, economic and environmental of livelihood people. So, early detection of land degradation is necessary for policy-makers to make appropriate decision. In this way, remote sensing method is a candidate choice for assessments and monitoring. In this study, land degradation was assessed using Rain-Use Efficiency (RUE) in the Shazand Watershed, Iran in 1986, 1998, 2008 and 2016. Thus, annual rainfall was calculated using inverse distance weight (IDW), net primary productivity (NPP) were calculated using Landsat images. The results indicated that RUE had increasing and then decreasing trends which were 10.66, 33.77, 20.03 and 9.47 kg C ha^{-1} yr^{-1}. The results also illustrate that the mean value of RUE in different land uses varied between the irrigated land and orchard that had the highest value and outcrop dominant areas and bareland had the lowest value of RUE among land use categories. It is also established that spatio-temporal analysis of RUE can provide valuable information about the trend of watershed's sustainability over years.

Key Words: *Land use/cover, Watershed Sustainability, Watershed Health, Watershed Management.*

INTRODUCTION

Land degradation (LD) is one of the most serious environmental and socio-economic problems (Wang et al., 2014) due to which more than 250 million people are directly influenced. The UNCCD, the Convention on Biodiversity, the Kyoto Protocol on global climate change, and the Millennium Development Goals have highlighted land degradation as a development challenge (Yengoh et al., 2015). So, identification, rehabilitation and applying preventative measurements are necessary for LD assessment to efficiently mitigate LD-related issues. Vegetation cover is a good indicator for LD. Different researchers have used various vegetation-based indices to analyze LD in global, national, local and field levels (Wang et al., 2014). Among different vegetation-based indices, the

Normalized Difference Vegetation Index (NDVI) is one of the most effective indicator for monitoring watershed health, watershed sustainability and LD (Li et al., 2015). By considering that climatic conditions affect NDVI, so exploring the relationship between NDVI and precipitation is a helpful manner to mirror LD. Accordingly, Rain Use Efficiency (RUE) which is defined as the ratio between Net Primary Production (NPP) and rainfall (Vermeire et al., 2009), is a useful indicator for land degradation and vegetation cover (Fensholt et al., 2013) reflecting the ecosystem functioning and structure.

RUE in unhealthy watersheds are more than that of degraded one with the similar rainfall. It is due to more runoff generation and less biomass production in degraded watersheds (Wessels et al., 2007). There is a strong relation between rainfall and RUE in that increasing and decreasing of RUE may be in the result of rainfall increasing and decreasing. However, this positive relation doesn't exist in all region because RUE is controlled by several factors such as vegetation composition, soil condition and biogeochemical constraints (Bhandari et al., 2015). Hence, spatial pattern of RUE and effective factors are different from site to site (Jia et al., 2015). RUE is an appealing concept which is calculated by both remote sensing and ecological interpretation (Dardel et al., 2014; Huang and Xu, 2016; Liu et al., 2018). Many researchers consider Satellite images as an ideal technology for LD (Vermeire et al., 2009; Dan et al., 2018; Kundu et al., 2018). It provides conditions to analyze timely and high temporal frequencies in large areas (Wang et al., 2014). In this way, many researches have calculated NPP using vegetation indices particularly NDVI.

The present study has tried to investigate LD by analyzing the spatial and temporal dynamic of RUE in different year of 1986, 1998, 2008 and 2016. Furthermore, its relation with rainfall and in various land uses/covers were also assessed.

MATERIAL AND METHOD

Study area

The Shanzand watershed is located in Markazi Province, Iran (49° 4' to 49° 52' E and 33° 44' to 34° 12' N) covering an area of 1,740 km^2. The climate of the watershed is moderate semi-arid to cold semi-arid. The elevation varies between 1800 to 3300 m which resulted in spatial and temporal distribution of rainfall. The mean annual rainfall is 420 mm the mean annual temperature is 12 °C (Hazbavi and Sadeghi, 2017). Farming was dominant in past decades. Whilst, land use change especially urban and industries development and orchard extension occurred after 1998 (Davudirad et al., 2016).

Land use classification

The land use maps of the Shazand watershed for 1986, 1998 and 2008 were extracted from existing researches (Davudirad et al., 2016) and for 2016 was developed using maximum likelihood method. Some eight land uses/ covers viz. bareland, dry farming, forest, irrigation farming, orchard, rangeland, residential and outcrops dominant areas were recognized.

RUE calculation

NPP and NDVI of MODIS products were downloaded for study area and their relationship was obtained. The monthly images of Landsat TM, ETM+ and OLI (1986, 1998, 2008 and 2016) were also collected. The necessary geo-referenced, radiometric and atmospheric corrections were performed and NDVI map was calculated to use for analyzing the relation between NDVI of Landsat and MODIS. Then NPP was calculated in 30 m using extracted equation. RUE was eventually calculated using following equations:

$$\mathrm{RUE} = \frac{\mathrm{NPP}}{\mathrm{P}} \quad (1)$$

where P is annual rainfall and NPP is net primary production.

The rainfall datasets of eight stations in and around the watershed were received from the Markazi Meteorological Bureau and averaged with the help of Inverse Distance Weighted (IDW) technique in ArcGIS 10.4.1 environment.

RESULT AND DISCUSSION

Land use/cover maps

Based on methodology explained above, land uses/covers maps were developed for the study years as depicted in Fig. 1.

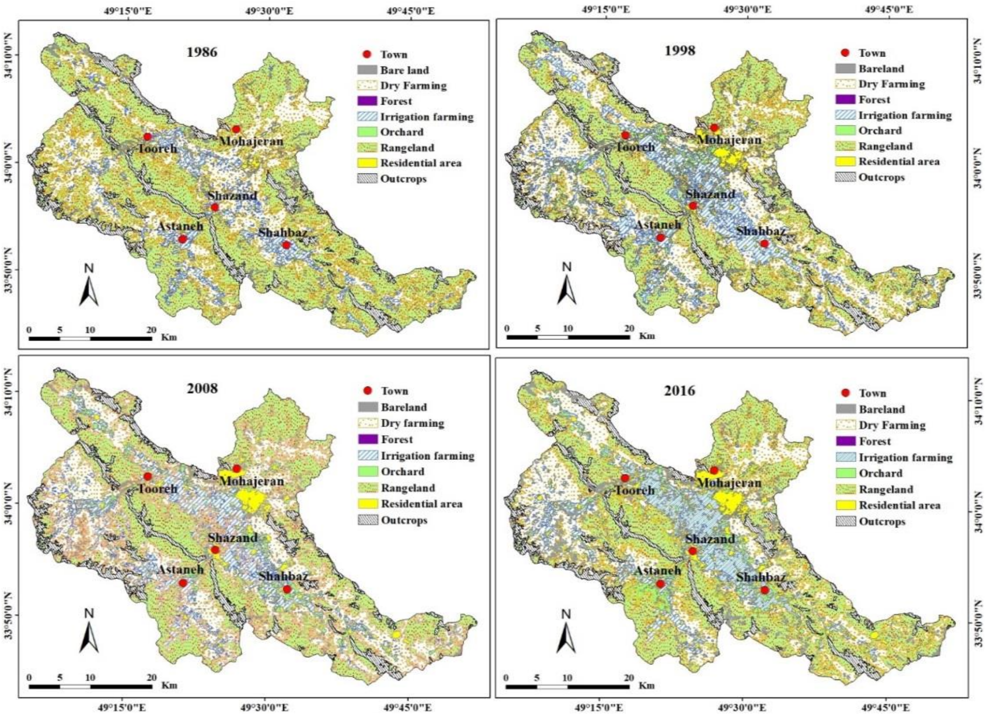

Figure 1. Land uses/covers maps for study node years in the Shazand Watershed, Iran

Spatial and temporal variation of RUE

The spatial distributions of RUE for study node years of 1986, 1998, 2008 and 2016 with mean values of 10.66, 33.77, 20.03 and 9.47 kg C ha^{-1} mm^{-1} have been mapped in Fig.2. Fig. 3 shows that RUE value had an increasing trend until 2008 and then it decreased to the lowest value in 2016. It means the watershed function tended to be degraded and the vegetation ability to use water decreased in 2016. High values of RUE in 1998 and 2008 indicate a better condition of RUE and more efficiency of rainfall to produce vegetative biomass.

Annual rainfall for 1986, 1998, 2008 and 2016 as respective amount of 455.93, 458.41, 311.31 and 479.71 mm verified a negative relationship with RUE. So that, dry years and years followed by dry years had the high value of RUE. Inversely, wet years had the low value of RUE. This implied that others biotic and abiotic factors affect RUE to get access to maximum level of efficiency. This results is consistent with Huang and Xu (2016), Liu and Huang (2016), and Sun and Du (2017) that indicated a negative correlation between RUE and rainfall. Furthermore, this result agreed with Zhang et al. (2014) who concluded that rainfall variations in current- and previous-year affected RUE. However, they found a dry year preceded by a wet year resulted in the highest RUE. The results also verified fragile condition of the study watershed in viewpoint of LD as already reported by Davudirad et al. (2016) and Hazbavi and Sadeghi (2017), and Sadeghi and Hazbavi (2017).

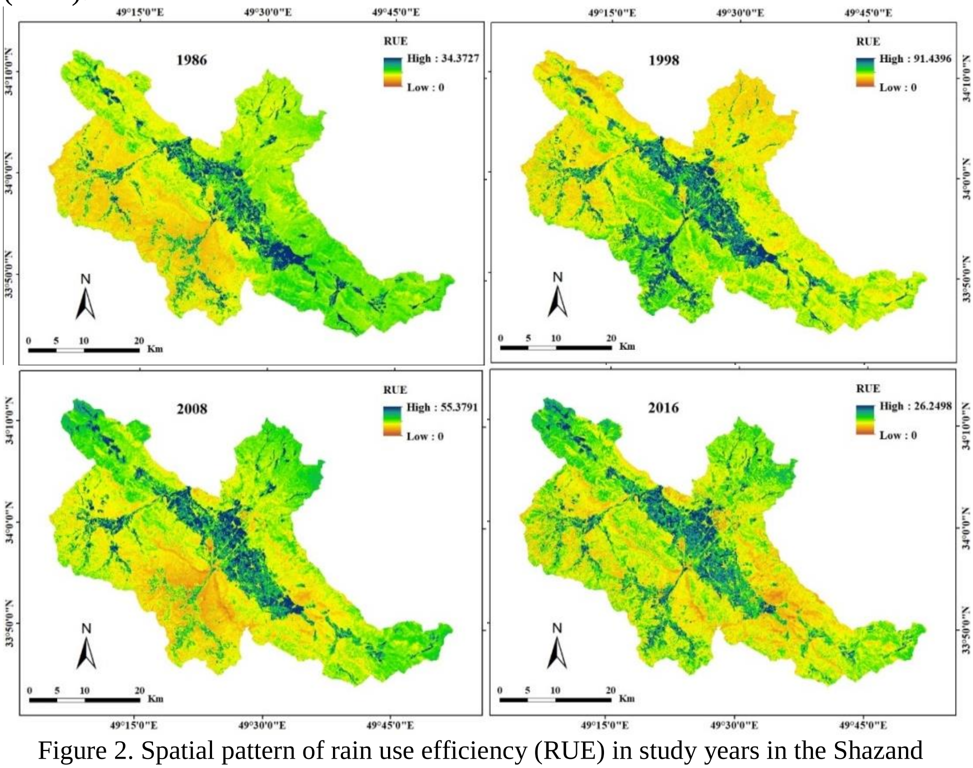

Figure 2. Spatial pattern of rain use efficiency (RUE) in study years in the Shazand Watershed, Iran

Relationship between RUE and land use/cover

The relationship between RUE and land use/cover was investigated in study years to examine land degradation condition. Table 1 illustrates the mean value of RUE in different land uses. It can be seen that the irrigation lands and orchards had the highest value and outcrops dominant areas and sparse forest had the lowest value of RUE among land use categories in study years.

Table 1. Rain use efficiencies in different and uses/covers from 1986 to 2016 in the Shazand Watershed, Iran

Node year	Rangeland	Dry farming	Irrigation farming	Orchard	Residential	Outcrops dominant areas	Bareland	Sparse Forest
1986	9.84	11.08	18.95	20.02	11.50	9.00	9.71	7.44
1998	30.95	31.13	51.72	48.23	31.93	28.21	29.15	32.42
2008	19.15	18.78	26.80	24.00	19.35	17.19	18.10	14.46
2016	8.96	9.02	12.54	10.85	9.11	8.12	8.40	8.69

CONCLUSION

Monitoring and evaluating the watershed health is vital for proper watershed management and implementation of revitalization measures leading to the functional sustainability of the system. Identification of land degradation in low intensity or early stages help policy makers take restorative or preventative actions before its damages became worse. In this study, the dynamics of RUE was examined in the Shazand Watershed in relation to rainfall and land use/cover. The results showed that RUE variations are not solely controlled by the rainfall of the year under consideration. Furthermore, mean value of RUE in different land uses/covers were different so the irrigation land and orchard had the highest value and outcrops dominant areas and bareland had the lowest value of RUE. The result provided an important platform in watershed monitoring and decision-making. However, the further studies with comprehensive factors are needed to minutely investigating RUE evolution.

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MODELLING ON-FARM DIVERSIFICATION THROUGH PORTFOLIO OPTIMIZATION AND GOAL PROGRAMMING: A CASE STUDY FROM BOLIVIA

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ABSTRACT

Modern Portfolio Theory provides a theoretical framework for agricultural risk reduction. Powerful yet accessible tools have been developed to optimize scarce capital/labor allocation to increase returns and reduce correlated risks via diversification. Such tools are used to assess rural livelihood diversification induced by an incentive-based program for watershed conservation piloted between 2003 and 2011 in a context of rural poverty in Bolivia. The tools assembled and tested in this study may provide low-cost diagnostics to improve implementers' understanding of risks and returns in a specific rural context. Comparing alternative portfolio frontiers may represent a useful and transformative tool to understand socio-ecological systems such as watersheds, facilitating regime shifts that benefit ecosystem services and livelihoods.

Key-words: Socio-Ecological Resilience, Modern Portfolio Theory, Goal Programming.

INTRODUCTION

Agricultural risk reduction

Agricultural risk reduction is an elusive goal and an urgent one for the global rural poor. Agricultural risk is site specific and multilayered, but similarly to risk in stock market investments, it can be reduced via adequate diversification. Modern Portfolio Theory and the set of tools available to compute alternative investment allocations represent a largely untapped resource to alleviate poverty by reducing agricultural risk. If agricultural risk reduction in itself does not dramatically increase incomes, at least it can stabilize them and potentially create room for savings and borrowing capability.

The case study for the application of portfolio theory to rural household economies was provided by a regional incentive-based watershed conservation program called *WaterShared* (Asquith 2016), piloted by Natura Bolivia Foundation, and adopted by a growing number of sub-national and national governments in Bolivia, Ecuador, Peru and Colombia. The *WaterShared* program has been ranked as a good practice for adaptive management (IIED 2010). Incentivizing alternative livelihoods is a recommended practice to neutralize leakage in conservation projects where farmers enroll marginal land but continue deforesting on more suitable land (Angelsen, A. (ed.) 2008). Natura provides in-kind incentives and training to diversify household economy, productive assets and skills aimed at neutralizing leackage and alleviate poverty for households that enroll forest land in a conservation program. Beekeeping, fruticulture and intensified livestock management, in this order, have been promoted by Natura contributing to diversifying livelihoods away from the dominant productive matrix in the region: itinerant annual agriculture and extensive cattle rearing. Was introduced diversification adequate to reduce risks and increase returns?

Poshiwa et al. (2013) used asset portfolio metrics to assess how conservation initiatives may help reducing rural households´ annual income fluctuations due to rainfall variation through diversification of wildlife use in Zimbabwe, relating agricultural risk reduction to alternative livelihoods in favor of conservation. Similar studies tend to estimate agricultural returns based on farm-gate price historical data and a "one size fits all" costs estimates based on secondary information (Seitz and Torre 2014). Though a reasonable approximation with potential to generate sound investment recommendations for farmers, the outcome of such studies may oversimplify the real-world complexity given by heterogeneity across farmers. There is a wild variability of returns in a single agricultural season and across a relatively homogeneous region: timing in all important to i.e. get the right amount of rain at the right time, or a good price at harvest. Skills and assets vary widely across a sample of farmers as well as random fluctuations in local markets and climates.

The rural context across the developing world is one of informal economy, poverty and accounting illiteracy. The cost of collecting field data at the household level is high and few institutions ever generate proper time series. Recent trends in program evaluation are considering "right-fit" evidence strategies for program monitoring and improvement (). Thus, the objectives of this paper are to (i) demonstrate the use of cross-sectional field data to determine expected returns from, and variability of, various land-based activities; and (ii) to calculate the optimal capital and labor allocation following the principles of modern portfolio theory (Markowitz, 1952; 1959) adopting rather inexpensive cross-sectional data. Presented here is the result of a quest for a robust yet "right-fit" method to qualify introduced on-farm diversification.

The resilience framework and modern portfolio theory.

A unifying paradigm emerged in the past three decades to frame adaptive co-management of social systems and the ecological systems they depend upon. That unifying and still unfolding paradigm is called Socio-Ecological Resilience (Holling 1986, 2001; Folke et al. 2002, 2016). Social and ecological resilience are related and increasingly treated as features of unified socio-ecological systems (SES), which are nested systems with complex feedbacks and interactions, subject to environmental changes due to natural and human induced processes. Resilience is defined as the ability to maintain functions under stress and bounce back from shocks (Walker and Salt 2006). Resilience can be described as the capacity to respond to "unforeseen surprises" without losing the system's integrity and functionality and is chiefly maintained by diversity (Walker and Salt 2012; Nemec et al. 2013).

How can external experts recommend or assess if diversification is the "right" kind? For instance: 1) diversifying crops that have the same agricultural calendar (planting and harvest time) may actually worsen risk rather than reduce it. 2) If expected returns on investments (ROI) are high on average, but extremely variable , the asset may not be appropriate for a stable portfolio. Assets such as capital, labor, productive inputs and skills will be understood as synonymous to the productive activities they enable. To cross the language bridge with finance we may also say that rural livelihoods allow just long-only positions. I intend to qualify the asset and households' diversified portfolios in terms of risk reduction. Markowitz (1952; 1959) devised enduring principles for capital allocation meant to diversify away investments' risk while maximizing returns: modern portfolio theory (MPT). The model for portfolios of risky assets is conceptually simple (minimize the variance (x_i ; … x_h) and co-variance (σ_{ih}) of expected ROI while maximizing ROI (ri ; … xi), formally expressed as:

$$Min \frac{1}{2} \sum_{i,h=1}^{n} \text{xi xh } \sigma\text{ih}; \qquad Max \sum_{i,h=1}^{n} \text{ri xi}$$

Subject to:

$$\sum_{i=1}^{n} \text{xi} = 1; \qquad \text{xi} \geq 0; \qquad \text{i} = 1 \ldots \text{n}; \qquad x \in D$$

Diversification *per se* is no panacea in a setting of limited capital it may prevent specialization with negative impacts on farmers income (Townsend 1994; Morduch 1995). As Markovitz (1952) originally put it: "Since the future is not known with certainty, it must be "expected" returns which we discount […] If we ignore market imperfections the foregoing rule *never* implies that there is a diversified portfolio which is preferable to all non-diversified portfolios." Markovitz's procedure for portfolio selection is a mathematically sound best guess to reduce exposure to non-systematic risk based on the admittedly unverified assumption that the future will look just like the past.

The maximum entropy principle (MEP) has been successfully used in diverse fields ranging from population ecology to quantitative finance (Harte, 2011; Usta and Kantar, 2011) in order to extend prior knowledge by finding the least biased distribution consistent with that knowledge. Entropy is a measure of diversity and is at its maximum when portfolio weights are equally distributed. Informational entropy represents the number of bits required to encapsulate the information relative to a probability distribution. An equally distributed four assets portfolio is an example of maximum entropy, portfolio's entropy is at its maximum and equals 0.60 bit. Entropy may be expressed as a percentage of this maximum value (which varies according to the assets included in a portfolio). One empirical study on financial time series found that the solution to the optimization problem that maximizes entropy (H) of the assets' weights, while keeping risk low, performed better in out of sample (OOS) tests than the classical solution that maximizes returns keeping risk low (Usta and Kantar, 2011). Less efficient, more diversified and sub-optimal asset selection, performed better than optimal solutions OOS (Rongxi et al. 2013; Geman et al. 2014). Applying the MEP reduced the risk of over-fitting the model to the data and allows for more performing portfolio selection facing the fundamental unpredictability of the real world. Through goal programming is it possible to test assumptions in a flexible framework of optimal farming investment allocations including an H target function:

$$\text{Information Entropy } (H) = -\sum_{i=l}^{k} pi \ln pi$$

Where *pi* represents the share of capital allocated to an asset included in the portfolio. Applying the MEP in this context equals to setting a higher diversity target in addition to reducing risk and increasing expected returns, potentially generating better performing portfolios out of sample. If diversity is one of the main ingredients in the recipe for adaptive performance in both theories 1) socio-ecological resilience and 2) modern portfolio, and it is measurable as entropy on available cross-sectional data, then we can qualify rural households' portfolios consistently for the time period under exam (12 months).

MATERIAL AND METHOD

The dataset

The original dataset is a cross-section with valid surveys from 97 households, based on 2015-2016 returns on investment in the main on-farm assets. Assets were aggregated in four categories: 1) annual agriculture, 2) fruticulture, 3) livestock and 4) beekeeping. Such dataset represents a detailed reconstruction of on-farm cash flow by activity for a sample that can be classified almost homogeneously as poor and very poor, territorially spread on five Municipalities. The impacts on income of local micro-climatic conditions, markets, skills, capital, etc. and the *WaterShared* program are all discounted by a single metric: return on investment in each relevant asset during the 12 months prior to the survey. Households' production costs are represented mainly by own-labor, monetized at an average rate of USD 14.60 for agricultural daily wages in the study area. Return on investments were computed as follows:

- Cost: monetized own labor based on local daily wages + inputs and services acquired.
- Net income by crop/asset: ((own-consumption + production sold) * average price) - Cost.
- Return on investment (ROI): Net income / Cost.

Theory and Calculations

Data augmentation and portfolio optimization

Portfolio optimization algorithms begin with the calculation of the variance-covariance matrix. Most households surveyed rely on two or three main on-farm assets, so the dataset built with ROI per asset presents empty cells (sheet 1 in attached spreadsheet). Quantitative is a field plagued with partial and incomplete time series. The solution developed and adopted in the field consists of a commonly used data augmentation technique that allows generating synthetic data (correlated normals) with the same statistical properties as the original dataset. Portfolio level sensitivity and value-at-risk (VAR) analyses are rarely done other than via historical simulation (i.e. Andersen, G. et al., 2007). Parametric methods are used extensively to develop historical simulations and reduce to a minimum the likelihood of “unforeseen surprises”, exploring the correlated risks of low-probability/high-impact events in the furthest section of the PDF´s tail.

The calculation of the augmented matrix starts with a symmetrical covariance matrix calculated on the original dataset (a *real* co-variance matrix). Missing values determine pairwise exclusion. Eigenvalues and eigenvectors are calculated on this covariance matrix, computed on the original data. The transformation matrix is the result of matrix multiplication of transposed eigenvectors times the square root of the diagonal matrix of eigenvalues. Correlated normal are obtained multiplying the uncorrelated random normals array by the transformation matrix.

The standard procedure for portfolio optimization is then applied to computed standard normals whose averages, standard deviations (STD), correlation and covariance matrixes are identical to those calculated on the field data (sheet 2 in attached spreadsheet). (Abbot, K. 2013). The algorithm used to nonlinearly optimize portfolios according to alternate goals is the generalized reduced gradient (GRG) developed by Lasdon, L. et al. (1973), calculations were implemented in excel 2016 (Wright, C. 2012 a, b).

RESULTS AND DISCUSSION

Rural poverty and agricultural risk

Classifying surveyed beneficiaries with the Unsatisfied Basic Needs Index 89% of 109 can be defined as poor and very poor in terms of assets and economic capability by national standards (INE, 2004). The best performing asset provided by the program for this relatively homogeneous group was beekeeping, averaging a ROI above 400% which obviously doesn’t include beehives acquired or received as an incentive before the current agricultural year. Such excellent performance is due to a very low investment required to maintain the beehives, most surveyed farmers barely invest in renewing pre-printed wax or provide bees with additional calories in winter (amounting to USD 3-4/year). The bulk of the cost in 2015-2016 was represented by own labor invested in harvesting honey twice a year, averaging 13 work-days. Noticeably most surveyed farmers (60%) depend primarily on annual agriculture and secondarily on livestock, worst performing assets on both ROI and risk.

Table 1. Returns and risks associated to each asset in 2015-2016.

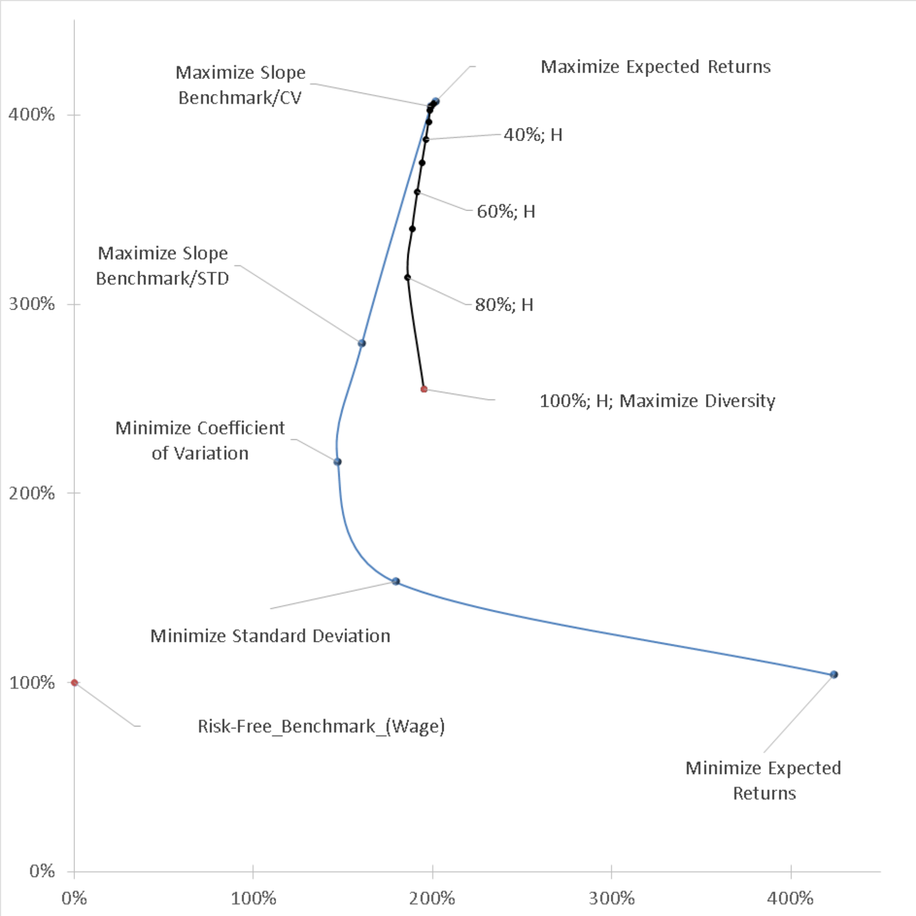
	Annual Agric. ANAG	**Livestock LIVE**	**Fruticulture FRUT**	**Beekeeping BEE**
Expected Returns (ROI)	104%	135%	374%	407%
Standard Deviation (STD)	434%	350%	1263%	727%
Coefficient of Variation (CV)	417%	258%	338%	178%

Efficient portfolios

The sector's benchmark for the risk-free investment is the agricultural daily wage, averaging USD 14.60 in the study area. The most efficient diversified portfolio (2) did not select livestock but only beekeeping and fruticulture, while the second-best portfolio (3: Maximize Slope Benchmark / CV constraint: Entropy target 40%) selected 2% of total capital/labor available within the household to be invested in livestock. Higher H targets (4, 5) increase the share of capital allocated to livestock and fruticulture, turning such selections into better candidates for OOS performance. A qualitative leap appears when risk is conceptualized in absolute as opposed to relative terms (6) with a drop in ROI, sliding abruptly on the inefficient side of the portfolio frontier. Risk conceptualization in relative terms generates higher returns but smallholder farmers will rarely take paper-risk. The most risk averse allocation choice (min STD) corresponds to local preferences as the majority of surveyed households primarily relies on annual agriculture and covets more livestock. In this Bolivian case-study a mix of expert opinion and local preferences helped selecting adequate on-farm diversification (as per 2015-2016 field data) through a participatory process. The incentivized productive assets (beekeeping, fruit trees and intensified livestock) and diversified portfolios including combinations of them generate some of the most efficient portfolios whit higher H targets (slightly sub-optimal). Despite persisting asset poverty and relatively low agricultural incomes it is clear that Watershared supported alternative livelihoods and contributed to transition household economies up the efficient side of the portfolio frontier by increasing capital/labor allocation to Beekeeping, fruticulture and intensified livestock management.

Table 2. Portfolio selection, rank ordered by decreasing ROI.

N.	**Target Function**	**Assets**					**Performance / statistics**			
		ANAG	FRUT	LIVE	BEE	TOT	ROI	STD	CV	H
1	Maximize Expected Returns (ROI)	0%	0%	0%	100%	100%	407%	823%	202%	0%
2	Maximize Slope: Benchmark / CV	0%	8%	0%	92%	100%	405%	806%	199%	19%
3	Entropy target 40%	0%	17%	2%	81%	100%	396%	783%	198%	40%
4	Entropy target 60%	2%	20%	8%	71%	100%	375%	727%	194%	60%
5	Entropy target 80%	6%	22%	16%	57%	100%	340%	642%	189%	80%
6	Maximize Slope: Benchmark / STD	0%	14%	45%	41%	100%	279%	449%	161%	72%
7	Maximize Diversity	25%	25%	25%	25%	100%	255%	498%	195%	100%
8	Minimize Coefficient of Variation	0%	15%	68%	17%	100%	217%	319%	147%	61%
9	Minimize Standard Deviation	25%	6%	65%	4%	100%	153%	275%	180%	67%
10	Minimize Expected Returns	100%	0%	0%	0%	100%	104%	442%	424%	0%

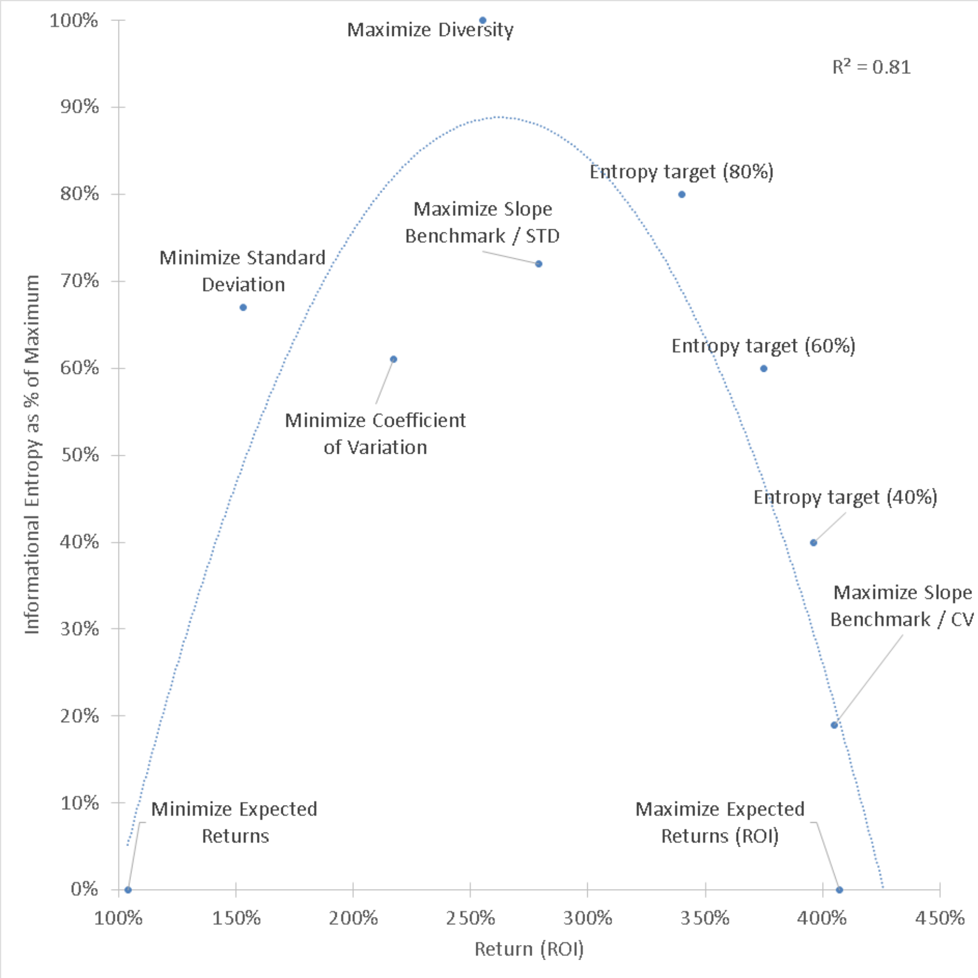


Graph 1. Portfolio Selection by Target Function.

These results corroborate the conclusion arrived at by others that useful in-kind incentives and access to new markets, as well as "intangible" benefits, such as training, may explain participation in conservation schemes better than opportunity cost analyses (Kosoya, et al. 2007; IIED 2007; Muradian et al. 2009; Bottazzi et al. 2018). Agricultural risk reduction provides a motivation to engage in a program such as watershed conservation and has merit in and of itself for household wellbeing.

One interesting result is that the relationship between ROI and H is curvilinear, best described by a polynomic curve of 2^{nd} order (R^2=0.81, see graph 2), in confirmation of the fact that misdirected diversification may *worsen* the exposure of smallholder farmers with detrimental impacts on income of smallholder farmers. As allocations improve, expected returns increase and risk is reduced, increased diversity of assets correlates positively with ROI and negatively with risk. Increase in diversity is associated with increase in allocation efficiency only on the inefficient side of the curve.

Once the turning point to the efficient side of the curve is passed (RI = 255% for “maximum diversity”) and the allocations improve in favor of ROI, diversity decreases with a negative correlation. Efficiency improvements increase both risks and return, showing how diversity per se is not the target of actions aimed at risk reduction. Agencies involved in agricultural risk reduction as an incentive to protect environmental services or as a goal in itself, can make use of portfolio optimization and apply it to relatively inexpensive cross-section data.



Graph 2. relationship between ROI and H is curvilinear.

CONCLUSIONS

In this and similar rural contexts misdirected diversification may have harmful impacts on both the environment and livelihoods, i.e. the most risk-averse allocation (min STD) relies heavily on annual agriculture and livestock, representing a local minimum trap and being associated with deforestation and forest degradation. Risk-aversion and constraints shape households´ preferences but not necessarily in their best interest. Public and private services may assess livelihoods in the target population to fine tune extension services.
While beekeeping is often a relatively new business for beneficiary households, orchards are known to generate returns without the annual investments (and risks) implied by annual agriculture. The entry barrier to get started with this business is given by the fact that the establishment of orchards requires at least three years until the first fruits can be harvested, an unaffordable use of land for most asset poor households. Natura's program incentivizes planting fruit trees on degraded land, providing the kick-start incentive to break annual agriculture long fallow cycle. The combination of local knowledge and preferences with environmentally friendly development options generated optimal choices to maximize social, economic and environmental goals at the same time. These tools can support and accelerate desired transformative change in a socio-ecological system, helping farmers and agricultural extensionists to make more informed investment decisions.

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SUBSTITUTION OF SOYBEAN MEAL WITH LOCAL PRODUCED LEGUME FORAGES IN EWES RATIONS

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ABSTRACT

Nutrition of intensive sheep farming in Greece is based mainly on feeding forages and concentrates in the stable, while grazing is provided in a low extend. Feeding cost is the major cost factor and from the protein supplementation side is based on alfalfa hay and soybean meal. The aim of the study was to investigate the substitution of soybean meal with local produced legumes forages. Thirty ewes of the “Karagouniko” breed were split in three groups of ten animals each. In the first group (control group), nutrition was based on alfalfa hay and soybean meal. In the second group, alfalfa hay and soybean meal were entirely substituted with vetch hay. In the third group, alfalfa hay was completely substituted with peas hay and the soybean meal inclusion was increased. Body weight was measured in the beginning and at the end of the trial period. Feed consumption and milk production were recorded daily and milk quality was analyzed weekly. No significant differences were found for all the parameters mentioned. Substitution of imported soybean meal with locally produced legumes is possible and will be an advantage in the differentiation of Greek sheep milk. The economic analysis showed that in farm, the cultivation of vetch and pea hay is more preferable than alfalfa.

Keywords: *Vetch, Peas, Karagouniko, Greece.*

INTRODUCTION

The nutrition of small ruminants reared in traditional farms in Greece is based mainly on soybean meal as a protein source. Among other protein sources, peas have been widely used as livestock feed (Loe *et al.* 2004; Van der Pol *et al.* 2008, Tufarelli *et al.* 2012). For legume seeds to be more efficiently utilized in ruminant

diets as alternative protein source, under certain circumstances, the rate and extent of degradation must be reduced without diminishing the extent of their intestinal digestion (Yu et al., 2002). Straw and hay, mainly originating from wheat, are utilized in Mediterranean countries for feeding small ruminants Hay from leguminous plants could be safely utilized as fiber source for sheep, provided at certain levels. However, its availability depends on climate and other factors (Awawdeh, 2011). Vetch (*Vicia sativa* L.) has been reported in some research works as a component in lamb diet (Gul *et al*., 2005) with satisfactory results, although it has been also shown to have low oil content (Aksoy, 2007). Haddad and Husein (2001) reported that vetch straw had a better nutritive value as fiber source for lambs in comparison to wheat straw, but less than alfalfa or lentil straw. Vicia could be used as a feed for livestock (Kaya *et al*., 2013). Some studies showed no differences of feeding vetch to sheep and that vetch has the potential to be used as an alternative source of high-protein fresh forage in spring to support lactating ewes and their lambs (Marley, 2016). Certain pre-processing methods are required in order to increase the efficiency and digestibility of the plant (Huang *et al.*, 2017). Liu *et al.*, (2018) reported the positive role on lamb nutrition of another legume, alfalfa (*Medicago sativa* L.) a common cultivated crop in many countries. Rong *et al.*, (2014).reported that lambs fed alfalfa had higher intake of dry matter, organic matter, crude protein and fiber than lambs fed only grass hay/crop straw.
The aim of the study was to estimate the substitution of soybean meal with locally produced legume forages based on alfalfa and vetch.

MATERIALS AND METHODS

The study was carried out in the farm of TEI of Thessaly, in Larissa, Greece. Thirty lactating ewes of the "Karagouniko" breed were split in three groups of ten animals each. TMR rations were provided to all experimental animals in the quantity presented in table 1 per animal. In the first group (control group), nutrition was based on alfalfa hay and soybean meal. In the second group, alfalfa hay and soybean meal were totally substituted with vetch hay. In the third group, alfalfa hay was totally substituted with peas' hay and soybean meal. Analysis of diets is presented in Tables 1. Vetch hay and pea hay were analyzed for their moisture content, total nitrogenous substances, total fat, fiber and ash. Nutritional value for each diet is presented in Table 2. Body weight was measured in the beginning and at the end of the trial period. Feed consumption and milk production were recorded daily and milk quality was analyzed weekly. Analysis of milk samples took place in the Laboratory of Milk Quality Control of Larissa, of the Hellenic Agricultural Organization (ELGO – DEMETER), using a Milkoscan 4000 (A/S N. Foss Electric, Hillerod, Denmark). All samples were conserved in potassium dichromate solution and analyzed within 5 days after collection. All ewes were lactating and each group was kept in a pen of 20 m^2 area. Daily feed was given in two meals and water was available *ad libitum*. Records for not-consumed-feed were kept throughout the experimental period. Additionally, barley straw and alfalfa hay were given every day. General linear model was used to statistically analyse the data through SPSS ver. 17 according to Steel and Torrie (1980).

Table 1. Feed composition (kg per feedstuff)

	Group A Control	Group B (Vetch)	Group C (Peas)
Balancer	0.0230	0.0230	0.0230
Barley straw	0.5000	0.1345	0
Corn	0.5000	0.4265	0.2010
Barley	0.2000	0.2000	0.1400
Marble mill	0.0115	0	0
Wheat bran	0.1833	0	0.2700
Soybean meal 45%	0.1430	0	0.2900
Phosphate mono-calcium	0.0081	0.0276	0.0570
Alfalfa hay	0.6031	0	0
Vetch hay	0	1.5413	0
Pea hay	0	0	1.1791
Cost (per Kg)	0.406€	0.401€	0.402€

Table 2. Nutritional value for each diet

	Group A Control	Group B (Vetch)	Group C (Peas)
Dry Matter (DM) (Kg)	1.97	2.04	1.94
Ash (g)	150.56	188.32	208.72
Total nitrogen (g)	300.00	322.30	301.18
Total fat (TF) (g)	48.38	57.43	46.84
Crude Fibre (CF) (Kg)	0.43	0.45	0.44
Neutral detergent fibre (NDF) (Kg)	0.76	0.73	0.78
Metabolizable energy(ME) (MJ)	11.98	11.98	11.97
Ca (g)	23.30	27.89	40.04
Mg (g)	3.31	3.68	4.33
P (g)	12.26	13.76	19.36
Na (g)	3.80	5.22	3.18
TF (g) : DM (Kg)	24.59	28.17	24.16
CF (Kg) : DM (Kg)	0.22	0.24	0.23
Ca (g) : P (g)	1.90	2.03	2.07
Mg (g) : P (g)	0.27	0.27	0.22

RESULTS AND DISCUSSION

Problems with feed intake and consumption of the daily rations were not recorded in any experimental group. Reed *et al.* (1990) also reported that vetch hay had been consumed fully by male sheep. For the other parameters recorded the results are as follows:

a) Body weight

The body weight of the animals was the same for all groups at the beginning of the study, as well as at the end. There was no statistically significant difference noted, at significance level of 5%.

The body weight loss during the experimental period was 2.98 ± 0.80 kg for the control group, 2.72 ± 0.95 kg for group B (vetch) and 3.04 ± 1.00 kg for group C (pea), again without statistically significant difference at a 95% confidence level.

Table 3. Average body weight

Group	Beginning	End
Group A (Control)	58.17 [a] ± 1.01	55.19 [a] ± 1.15
Group B (Vetch)	57.84 [a] ± 0.74	55.12 [a] ± 1.25
Group C (Peas)	57.96 [a] ± 0.89	54.92 [a] ± 1.03

There is not a significant difference among different groups.

b) Milk yield and composition

The average daily milk yield throughout the period was: 1.42 ± 0.17 kg for the control group, and 1.41 ± 0.18 kg for group B (vetch hay) and 1.39 ± 0.18 kg for group C (pea hay). Tables 3 and 4 present the average daily milk yield on a weekly basis and milk composition, respectively.

Table 3. Average daily milk yield per week

Group	Week 1	Week 2	Week 3	Week 4
Group A (Control)	1.64 [a] ± 0.10	1.46 [a] ± 0.11	1.33 [a] ± 0.11	1.27 [a] ± 0.05
Group B (Vetch)	1.67 [a] ± 0.07	1.40 [a] ± 0.09	1.30 [a] ± 0.07	1.26 [a] ± 0.07
Group C (Peas)	1.65 [a] ± 0.07	1.41 [a] ± 0.08	1.29 [a] ± 0.08	1.23 [a] ± 0.06

There is not a significant difference among different groups.

Table 4. Milk composition

		Week 1	Week 2	Week 3	Week 4	Total
Fat	Group A Control	6.48 [a] ± 0.28	6.61 [a] ± 0.26	6.86 [a] ± 0.23	6.93 [a] ± 0.30	6.72 [a] ± 0.32
	Group B (Vetch)	6.44 [a] ± 0.27	6.60 [a] ± 0.24	6.83 [a] ± 0.29	6.82 [a] ± 0.21	6.67 [a] ± 0.29
	Group C (Peas)	6.46 [a] ± 0.25	6.61 [a] ± 0.25	6.91[a] ± 0.25	6.92 [a] ± 0.26	6.72 [a] ± 0.31
Protein	Group A Control	5.23 [a] ± 0.27	5.31 [a] ± 0.31	5.46 [a] ± 0.31	5.56 [a] ± 0.37	5.39 [a] ± 0.30
	Group B (Vetch)	5.18 [a] ± 0.28	5.21 [a] ± 0.24	5.42 [a] ± 0.28	5.51 [a] ± 0.29	5.33 [a] ± 0.25
	Group C (Peas)	5.14 [a] ± 0.26	5.20 [a] ± 0.29	5.37 [a] ± 0.30	5.45 [a] ± 0.35	5.29 [a] ± 0.28

Lactose	Group A Control	5.02 [a] ± 0.27	5.01 [a] ± 0.31	4.88 [a] ± 0.31	4.81 [a] ± 0.37	4.93 [a] ± 0.32
	Group B (Vetch)	4.91 [a] ± 0.28	4.84 [a] ± 0.24	4.77 [a] ± 0.28	4.67 [a] ± 0.29	4.80 [a] ± 0.28
	Group C (Peas)	5.00 [a] ± 0.23	4.91 [a] ± 0.28	4.91 [a] ± 0.29	4.91 [a] ± 0.32	4.93 [a] ± 0.28

There is not a significant difference among different groups.

Weight loss, milk yield and quality characteristics of the milk produced, although showing small numerical differences in means, were not statistically significant at a 95% confidence level. Therefore, neither vetch hay or pea hay addition, in order to substitute soybean meal in ewes' diet, affected those characteristics. The results from the present study are in accordance with the results obtained by Marley et al. (2016) for grazing ewes, although in our case the animals were kept under intensive conditions and in contrast with the results obtained from lamps or goat trials. Abbeddou et al., (2011) found that vetch hay was the most valuable forage in terms of energy and protein supply in fat-tailed sheep, concerning the nutritional composition. Berhane and Eik (2006), found a positive effect of vetch hay supplementation that increased milk yield by up to 50%, but decreased percent fat and total solids in the milk of both Begait and Abergelle goats.
The results show that there were no differences between the experimental groups, with regard to the mean body weight of the ewes, and the daily milk yield. This was expected, since the diets of the experimental groups were about of the same nutritional value, due to the study design. In group B (vetch hay), it was possible to completely replace both alfalfa and soybean meal and even offer a larger amount of nitrogenous substances in the final ration. In the case of pea hay, replacement of alfalfa hay has led to increased participation of the soybean meal in the final ration.

CONCLUSION

Based on the current hay market prices for sheep farmers, the cost of rations for all experimental groups is about the same. In the case of self-production, however, it appears that the cultivation of vetch and peas as hay is more advantageous than that of alfalfa.
The substitution of imported soybean meal with local vetch hay may be promising for the "identity" of local production and especially for the cheese factory, as it can enhance the originality and marketing of products.

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- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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