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CONTENT

INTORDUCTION

In the past ten years the educational, research and applicative activities of the Faculty of Agriculture – Stip, Goce Delcev University – Stip, contributed to the development of agriculture sector in the country and broader region.

The Faculty of Agriculture organized the 1st International Meeting Agriscience & Practice (ASP 2018), giving an opportunity to the participants for presentation and discussion of original scientific and practical results in different fields of agriculture.

The 1st International Meeting Agriscience & Practice (ASP 2018), heled on 10-11 May at Faculty of Agriculture - Stip, was organized with intention to bring together all agricultural stakeholders for sharing their knowledge, experience and obstacles. One of the main aims was to link research and field work in agricultural sector in the country and broader, giving it an international dimension. All oral presentations as well as poster presentations at ASP 2018 were organized in several scientific sessions:

- Agricultural economics,
- Plant biotechnology,
- Plant production,
- Plant protection,
- Quality control and food safety,
- Soil science and hydrology,
- Viticulture, enology and fruit production.

The main goal of the Meeting was linking and promoting scientific achievements and practical knowledge, presented in different thematic areas, which were achieved in the Republic of Macedonia and wider in the region.

Journal of Agriculture and Plant Sciences Vol. 16, No. 1 contains the presented papers from the 1st International Meeting Agriscience & Practice (ASP 2018).

Editorial Board, **Editor in chief,**

August, 2018 **Prof. Liljana Koleva Gudeva, PhD**

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THE EFFECT OF BACKCROSS METHOD IN TOBACCO BREEDING

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Abstract

The paper encompasses investigations of four oriental tobacco varieties (P10-3/2, P-23, P-84 and YK 7-4/2) and their diallel F1, F2, BC1 (P1) and BC1 (P2) crosses in the course of 2016 and 2017, for the characters leaf number per stalk and dimensions of the middle belt leaves. The research was carried out on the experimental field of the Scientific Tobacco Institute - Prilep in randomized block system with four replications. The measurements were made in the stage of rapid growth (butonization), and the values obtained were processed by variational-statistical analysis. Heritability (h2) of the traits was calculated using the Mather and Jinks method.

The aim of the research is to study the effect of backcross breeding on improving the quantitative traits of tobacco, to open up the possibility of selecting individuals at the maximum number of crosses enabled by the diallel of parental genotypes and to offer directions for further selection.

The obtained results showed different modes of inheritance in all generations studied. Heritability of the crosses was over 95%, which is a sign of high heritability of the studied traits. The offered pattern for further successive selection can also be used for other plant species to improve a range of traits, including the resistance to diseases.

Key words: *tobacco (Nicotiana tabacum L.), backcross, diallel, heritability*

INTRODUCTION

Plant breeding is considered to be one of the oldest skills that man has occupied. It dates back 10,000 years ago with the cultivation of wild species, which will mean the beginning of agriculture. However, the creativity from this area of science is more recent and begins with gender studies in species. In Europe first explorations for the sexes of plants are published by Camerarius (1694). At that time the ever-present desire of the breeder to create varieties more superior than the existing ones is born. All actions in this direction are called breeding and they lead the selection forward towards ever-increasing success. According to Borojevic (1992) breeding is man's work for improvement and creation of new varieties of plants, in order to meet the needs of people and domestic animals.

Oriental tobacco, like the rest of the agricultural crops where breeding and selection is continuous process, is a strategic crop with a centuries-old tradition of production and high quality. Oriental tobacco is mainly grown in Prilep and Yaka types or Dzebel and Basmak on smaller areas which is due to the pleasant aroma and a high price on the international market.

The main aim of the breeders at the Scientific Tobacco Institute in Prilep is to create new varieties and improve the existing ones by applying appropriate methods, including the Backcross Method - a process that improves the existing commercial varieties with some desired traits, such as resistance to diseases.

The Back-crossing Method is defined by a large number of scientists including: Gornik (1973) – Backcross is a method for fast and secure improvement or addition of traits that are not present in the existing varieties or not sufficiently expressed; Matthew (2012) – Backcross breeding is a method that can be used to insert a particular trait, such as disease resistance, from one line, often inferior, to another that is usually an elite

line for improvements; Pavlica et al., (2013) – Backcross is crossing of hybrid (individual of the F1 generation) with one of the parents or individual of the parent genotype, and so on. In The Free Dictionary (2014) Back-cross means to cross (a hybrid) with one of its parents or with an individual genetically identical to one of its parents; while in Wikipedia (2017) – Backcrossing is a crossing of a hybrid with one of its parents or an individual genetically similar to its parent, in order to achieve offspring with a genetic identity which is closer to that of the parent. In this direction, several selection programmes were realized. For example, Korubin-Aleksoska (2007) in research for resistance to blue mould (Peronospora tabacina Adam) found dominant inheritance in the oriental variety Pobeda 2 and its application in the Backcross Method for transferring the resistance on to the current varieties. Dimitrieski et al. (2012) examined two varieties and eight lines of Oriental tobacco for resistance to blue mould and black shank as ones of the most dangerous tobacco diseases and discovered a line resistant to blue mould (P 123-65/82) and two lines resistant to black shank (YK 20-23/10 и P 65-54/09) which can be used in projects for breeding of oriental tobacco. Korubin-Aleksoska and Aleksoski (2015), in an experiment with 10 varieties, of which one flue-cured and nine oriental, found that YK 10-7/1 is characterized by the dominance in the inheritance of resistance to the black shank (Phytophthora parasitica var. nicotianae) and with the application of Backcross Method improved the large-leaf variety MV-1.

The aim of this paper is to explain the Backcross Method as one of the methods for breeding the plants, and to show the way and effect of its application.

MATERIAL AND METHODS

Breeding of tobacco is done in order to obtain new and more superior varieties that are homozygous with a stable inheritance of the traits: yield, dry leaf quality, chemical components, technological characteristics and resistance to diseases. Tobacco processing methods are those that apply to the selection of self-sufficient plants (Martincić & Kozumplik, 1996), such as: Mass Selection, Individual Selection, Pedigree Method, Bulk Method, Single seed descent Method and Baeckcross Method - subject to this work. This method is carried out with series of backcrossing with the variety to be improved. The efficiency of this method is greater if it is done for the breeding of only one or two traits where are one gene or one pair of major genes is responsible for the inheritance. The procedure is different, depending on the manner of inheritance.

As a working material we took three Oriental varieties of the type Prilep (P10-3/2, P-23 and P-84) and one Oriental variety of Yaka type (YK 7-4/2).

Prilep P 10–3/2 (Fig. 1) – Originated from the local tobacco variety Djumaj–bale from Gorna Djumaja – Bulgaria. Released in mass production by Gornik, in the 30-ies of the last century. Characterized by cup-like habitus, average stalk height 50cm, with 30-36 sessile leaves, dry mass yield averages 1200 kg/ha (Korubin – Aleksoska, 2004).

Prilep P–23 (Fig. 2) – created by Kostadin Nikoloski and Milan Mitreski, through hybridization and selection in Tobacco Institute – Prilep; recognized by the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia in 1995 (Korubin – Aleksoska A., 2004). It has elliptical–conical habitus, average stalk height 65 cm, with about 55 densely arranged leaves, dry mass yield 2000–2500 kg/ha (Korubin – Aleksoska, 2004).

Prilep P–84 (Fig. 3) – created by Kiril Naumovski and Ana Korubin–Aleksoska, through hybridization and selection; recognized in 1988 in former Yugoslavia, as one of the first varieties of the type Prilep. Characterized by cylindrical – elliptical habitus, average stalk height 65 cm, with approximately 40 – 42 sessile leaves, elliptical in shape, dry mass yield 2500–3200 kg/ha (Korubin – Aleksoska, 2004).

Yaka YK 7–4/2 (Fig. 4) – released in mass production in 1932 by Gornik. Originated from Xanthian Yaka originating from Xanthy – Greece; a plant with narrow, spindle shaped–elliptic habitus; average stalk height 100cm, with 26–32 sessile leaves, dry mass yield averages 1000 kg/ha (Korubin – Aleksoska, 2004).

Figure 1. Prilep, P 10–3/2

Figure 2. Prilep, P–23

Figure 3. Prilep, P–84

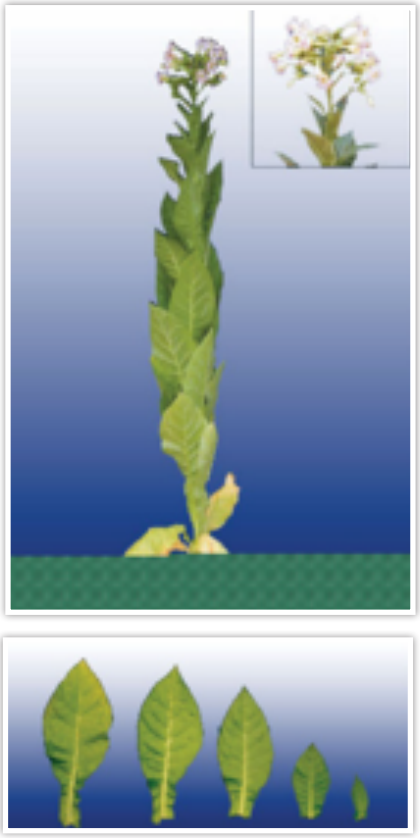

Figure 4. Yaka, YK 7–4/2

In the course of 2015, we made diallel crosses, and in 2016 we set up an experiment in a field with the parents and their 6 F1 hybrids. The paper presents data from the measurements for the number of leaves per stalk, as well as the length and width of the middle belt leaves. Besides measurements, we isolated the plants for the F2 generation and made two-way reciprocal crossings, with the first and the second parent. In 2017 we set up an experiment with parental varieties and the offspring of F1, F2, BC1 (P1) and BC1 (P2) generations. The experiment took place in the Experimental Field of Scientific Tobacco Institute – Prilep by random block system in four repetitions. During the vegetation common agrotechnical measures were applied. The measurements were carried out in the early stage of flowering. The obtained data is statistically processed through parameters of traits variability. The analysis of the variance was made according to the Griffing Method (1956).

RESULTS AND DISCUSSION

In order to get acquainted with the genetic stability of the selected varieties and their F1 hybrids, we made measurements in 2016 and 2017 and on the basis of the results obtained we analyzed the inheritance of the number of leaves per plant and the dimensions (length and width) of the middle-belt leaves.

It can be seen from Table 1 that with the highest number of leaves among parental genotypes, in both years of investigations, is characterized P-23 (48.35 – 2016; 45.60 –2017), and with the lowest YK 7-4/2 (28.70 – 2016; 28.20 – 2017). Existence significantly differ between parents, which means that the genes are different. The highest number of leaves among diallel F1 hybrids has P-23 x P-84 (46.35 – 2016; 45.60 –2017), and the lowest P10-3/2 x YK 7-4/2 (31.05 – 2016; 30.15 –2017). The overall genotype set in 2017 has lower number of leaves in comparison with 2016, which is due to extremely drought conditions in the vegetation period of 2017, in spite of the three necessary watering. It is interesting to note that the biggest difference is in P-23 (approximately three leaves less in 2017, compared to 2016), which means that this variety is not resistant to drought. The other parents showed high resistance to dry conditions because the difference in the number of leaves between the two years is less than one leaf. Here, in the first place, it is P-84 (difference of 0.25), then YK 7-4/2 (difference of 0.50), then P 10-3/2 (difference of 0.60). Between F1 hybrids the slightest difference in the traits has in P 10-3/2 x P-23 (0.1) and P-23 x YK 7-4/2 (0.25). This data is important for the selection directed towards to stress drought resistance. From the visual inspection of the experiment, absence of the economically important diseases blue mould (Peronospora tabacina Adam) and black shank (Phytophthora parasitica var. nicotianae) was ascertained, which is important for further selection programs, obtaining resistant varieties, or improving resistance in the existing commercially important tobacco varieties. In parents and F1 hybrids the error of the mean value (S) is small and moves from 0.19 (P-23 x YK 7-4/2 and P-84 x YK 7-4/2) to 0.78 (P-23 x P-84) in 2016 and from 0.18 (P-84 x YK 7-4/2) to 0.63 (P-23 x P-84) in 2017. The standard deviation (σ) ranges from 0.87 to 3.48 in 2016 and from 0.80 to 2.82 in 2017 for the same combinations. The coefficient of variability (CV) ranges from 2.02 % (P-23 x YK 7-4/2) to 7.94 % (P 10-3/2) in 2016 and from 2.20 (P-84 x YK 7-4/2) to 6.29 % (P 10-3/2) in 2017. Low variability values are an indication of the high genetic homogeneity of the studied traits, i.e., stable homozygous varieties are selected and uniform heterozygous diallel F1 hybrids are obtained.

Table 1. Number of leaves per stalk in parents and F_1 hybrids.

Parents and F_1 hybrids	n	2016			2017		
		± S	σ	CV (%)	± S	σ	CV (%)
P 10-3/2	20	33.40 ± 0.59	2.65	7.94	32.80 ± 0.46	2.06	6.29
P 10-3/2 x P-23	20	40.30 ± 0.35	1.58	3.93	40.20 ± 0.34	1.54	3.82
P 10-3/2 x P-84	20	35.30 ± 0.26	1.14	3.24	34.35 ± 0.20	0.91	2.65
P10-3/2 x YK7-4/2	20	31.05 ± 0.28	1.24	4.01	30.15 ± 0.23	1.01	3.36
P-23	20	48.35 ± 0.44	1.96	4.05	43.55 ± 0.44	1.99	4.56
P-23 x P-84	20	46.35 ± 0.78	3.48	7.51	45.60 ± 0.63	2.82	6.18
P-23 x YK 7-4/2	20	43.20 ± 0.19	0.87	2.02	42.95 ± 0.28	1.24	2.90
P-84	20	42.45 ± 0.29	1.28	3.02	42.20 ± 0.31	1.37	3.26
P-84 x YK 7-4/2	20	37.50 ± 0.19	0.87	2.31	36.40 ± 0.18	0.80	2.20
YK 7-4/2	20	28.70 ± 0.28	1.27	4.42	28.20 ± 0.25	1.12	3.98
		$LSD_{0.05}$ = 1.52 $_{0.01}$ = 2.06			$LSD_{0.05}$ = 1.48 $_{0.01}$ = 1.99		

It can be seen From Table 2 that with the highest average value for the length of the middle-belt leaves among parental genotypes, in both years of research, is characterized P 10-3/2 (22.00 – 2016; 21.80 – 2017), and with the lowest number YK 7-4/2 (18 – 2016; 17.9 – 2017). There are significant differences between parents, which is a sign of genetic differentiation (with the exception of P-23 and P-84, among which there is no significant difference). Among diallel F1 hybrids the longest leaves have P10-3/2 x P-84 (21.90 – 2016; 21.70 – 2017), and the shortest P-84 x YK 7-4/2 (19.10 – 2016; 18.72 – 2017). In all investigated genotypes, the length of the leaves is lower in 2017 compared with that measured in 2016 as a result of the dry conditions during vegetation in 2017.

In parents and F1 hybrids the error of the mean value (S) is small and moves from 0.29 cm (P 10-3/2 x P-84) to 0.49 cm (P 10-3/2) in 2016 and from 0.27 cm (P-23 x P-84 and P-84 x YK 7-4/2) to 0.42 cm (P 10-3/2) in 2017. The standard deviation (σ) ranges from 1.32 cm (P10-3/2 x P-84) to 2.18 cm (P 10-3/2) in 2016 and from 0.27 cm (P-23 x P-84 and P-84 x YK 7-4/2) to 0.42 cm (P 10-3/2) in 2017. The coefficient of variability (CV) varies from 6.02 % (P10 3/2 x P-84) to 9.93 % (P 10-3/2) in 2016 and from 5.88 (P-23 x P-84) to 8.72 % (P 10-3/2) in 2017. Low values for variability in these traits are an indicator for existence of high genetic homogeneity.

Table 2. Length of the leaves from the middle belt in parents and F_1 hybrids (cm).

Parents and F_1 hybrids	n	2016			2017		
		± S	σ	CV (%)	± S	σ	CV (%)
P 10-3/2	20	22.00 ± 0.49	2.18	9.93	21.80 ± 0.42	1.90	8.72
P 10-3/2 x P-23	20	22.50 ± 0.39	1.73	7.70	22.04 ± 0.34	1.54	6.99
P 10-3/2 x P-84	20	21.90 ± 0.29	1.32	6.02	21.70 ± 0.29	1.28	5.89
P10-3/2 x YK7-4/2	20	20.04 ± 0.34	1.51	7.54	19.91 ± 0.34	1.52	7.64
P-23	20	20.50 ± 0.32	1.43	6.98	19.70 ± 0.33	1.46	7.42
P-23 x P-84	20	20.50 ± 0.31	1.40	6.81	20.20 ± 0.27	1.19	5.88
P-23 x YK 7-4/2	20	19.30 ± 0.31	1.37	7.11	19.20 ± 0.33	1.48	7.70
P-84	20	20.60 ± 0.35	1.56	7.58	20.50 ± 0.39	1.73	8.45
P-84 x YK 7-4/2	20	19.10 ± 0.32	1.44	7.52	18.72 ± 0.27	1.20	6.39
YK 7-4/2	20	18.00 ± 0.32	1.45	8.05	17.90 ± 0.32	1.45	8.11
		$LSD_{0.05}$ = 1.17 $_{0.01}$ = 1.57			$LSD_{0.05}$ = 0.96 $_{0.01}$ = 1.30		

The data on the width of the middle belt leaves shown in Table 3 shows that with the highest average values between parental genotypes, in both years of investigation has P-23 (10.60 – 2016; 10.50 – 2017), and with the lowest YK 7-4/2 (8.7 – 2016; 8.5 – 2017). The significance of differences between parental genotypes by 1% exists only in relation to YK 7-4/2. Among diallel F1 hybrids the widest leaves have P-23 x P-84 (10.30 – 2016; 10 – 2017), and the narrowest P 10-3/2 x YK 7-4/2 (9 – 2016; 8.8 – 2017).

In the parents and F1 hybrids standard deviation (σ) ranges from 0.42 cm (P 10-3/2 x P-84) to 0.93 cm (P-23 x YK 7-4/2) in 2016 and from 0.17 cm (YK 7 -4/2) to 0.87 cm (P 10-3/2 x P-84) in 2017. The coefficient of variability (CV) ranges from 4.18% (P 10-3/2 x P-84) to 9.85% (P-23 x YK 7-4/2) at 2016 and from 5.70 (P-23 x P-84) to 9.30% (P-84 x YK 7-4/2) in 2017. Coefficient of variability (CV) ranges from 4.18% (P 10-3/2 x P-84) to 9.85% (P-23 x YK 7-4/2) at 2016, and from 5.70 (P-23 x P-84) to 9.30% (P-84 x YK 7-4/2) in 2017. The low values for variability in this traits are indicative of the existence of high genetic homogeneity.

Table 3. Width of the leaves from the middle belt in parents and F_1 hybrids (cm).

Parents and F_1 hybrids	n	2016			2017		
		± S	σ	CV (%)	± S	σ	CV (%)
P 10-3/2	20	10.10 ± 0.13	0.60	5.98	10.00 ± 0.14	0.63	6.32
P 10-3/2 x P-23	20	10.20 ± 0.13	0.58	5.67	10.00 ± 0.17	0.74	7.42
P 10-3/2 x P-84	20	10.00 ± 0.09	0.42	4.18	9.70 ± 0.19	0.87	8.99
P10-3/2 x YK7-4/2	20	9.00 ± 0.18	0.79	8.78	8.80 ± 0.26	0.80	9.05
P-23	20	10.60 ± 0.17	0.78	7.40	10.50 ± 0.19	0.85	8.11
P-23 x P-84	20	10.30 ± 0.13	0.60	5.82	10.00 ± 0.13	0.57	5.70
P-23 x YK 7-4/2	20	9.50 ± 0.21	0.93	9.85	9.00 ± 0.16	0.71	7.86
P-84	20	10.00 ± 0.12	0.52	5.24	9.50 ± 0.14	0.63	6.66
P-84 x YK 7-4/2	20	9.80 ± 0.12	0.56	5.68	9.00 ± 0.19	0.84	9.30
YK 7-4/2	20	8.70 ± 0.17	0.78	8.98	8.50 ± 0.20	0.17	8.72
		$LSD_{0.05}$ = 0.51 $_{0.01}$ = 0.69			$LSD_{0.05}$ = 0.68 $_{0.01}$ = 0.92		

In order to give genetic knowledge for the number of leaves and dimensions of leaves from the middle belt in F2 – generation at the beginning of the selection activity, and BC1(P1) – generation obtained by backcross of F1 with the first parent, and BC1 (P2) – generation obtained by backcross of F1 with the second parent, we made measurements in 2017 and based on the obtained results, we made analysis of inheritance for traits. The aim of this investigation was to determine the selection material needed for the future selection programs in order to improve some traits in commercial varieties, as well as for obtaining resistance to drought stress and resistance to diseases of crucial nature.

The analysis of the number of leaves per plant in the offspring of F2 generation and those from backcross, points to a series of laws (Table 4). Thus, the number of leaves is greater in F2 offspring relative to F1 (with the exception of P-23 x P-84 where the F1 offspring has more leaves than both parents and the F2 generation, while in P-23 x YK 7-4/2, the F1 offspring has more leaves than the F2 generation, but the difference between the values is minimal). Values for the F2 property range from 31cm in P 10-3/2 x YK 7-4/2 to 43.2 cm in P-23 x P-84. The heterotic effect of P-23 x P-84 for this trait could not be confirmed with certainty because it is not in line with the 2016 results. It is necessary to repeat the experiment in 2018.

With the highest number of leaves per plant in offspring of the BC1 (P1) generation is characterized (P-23 x P-84) x P-23 (42.50) and with the lowest (P 10-3/2 x YK 7-4/2) x P 10-3/2 (32.20). The inheritance of the trait in this

generation depends from the leaves number of parent that backcrossed F1 population. In combinations where backcrossed with a parent with a lower number of leaves, BC1 (P1) offspring is with smaller size than F2, while in the examples where backcrossed with the parent with a higher number of leaves, BC1 (P1) offspring is with larger size than F2 generation. With the highest number of leaves per plant in offspring of the BC1 (P2) generation is characterized (P 10-3/2 x P-23) x P-23 (43), and with the lowest (P-84 x YK 7-4/2) x YK 7-4/2 (37). In BC1 (P2) generation, all crosses of BC1 (P2) where they are backcrossed with the better parent, better offspring are obtained in relation to BC1 (P1) and vice versa.

Table 4. Number of leaves per stalk in the parents, F_1, F_2, BC_1 (P_1) and BC_1 (P_2) offspring (2017).

Parents and hybrids	Number of leaves per stalk			
	P & F_1	P & F_2	P & BC_1 (P_1)	P & BC_1 (P_2)
P 10-3/2	32.80	32.80	32.80	32.80
P 10-3/2 x P-23	40.20	41.50	36.00	43.00
P 10-3/2 x P-84	34.35	38.00	35.00	41.00
P 10-3/2 x YK 7-4/2	30.15	31.00	32.20	30.20
P-23	43.55	43.52	43.52	43.52
P-23 x P-84	45.60	43.20	42.50	42.03
P-23 x YK 7-4/2	42.95	42.80	32.50	40.50
P-84	42.20	42.00	42.00	42.00
P-84 x YK 7-4/2	36.40	38.00	41.00	37.00
YK 7-4/2	28.20	28.20	28.20	28.20
	$LSD_{0.05}$ = 1.48 $_{0.01}$ = 1.99	$LSD_{0.05}$ = 2.01 $_{0.01}$ = 2.72	$LSD_{0.05}$ = 2.18 $_{0.01}$ = 2.94	$LSD_{0.05}$ = 1.90 $_{0.01}$ = 2.57

The length of the middle-belt leaves is greater in F2 offspring relative to that of F1 (with the exception of P 10-3/2 x P-23 where the difference between the values is minimal). Values for the traits in F2 range from 18.9 cm to P-84 x YK 7-4/2 to 21.75 cm in P 10-3/2 x P-84 (Table 5).

The largest length of the leaves in the BC1 (P1) generation is characterized in the cross (P 10-3/2 x P-84) x P 10-3/2 (21.75 cm), and the shortest in (P-23 x YK 7-4/2) x P-23 (19 cm). In this case the inheritance of the trait depends on the length of the parent's leaves that backcrossed the F1 population. In BC1 (P2) generation with the longest leaves is the cross (P 10-3/2 x P-23) x P-23 (21.24 cm), and with the smallest (P 10-3/2 x YK 7-4/2) x YK 7-4/2 (18.03 cm). In all BC1 (P2) crossings where backcrossed with the better parent, better offspring are obtained in relation to BC1 (P1) and vice versa.

Table 5. Length of the leaves from the middle belt in the parents, F_1, F_2, BC_1 (P_1) and BC_1 (P_2) offspring (2017).

Parents and hybrids	Length of the leaves from the middle belt (cm)			
	P & F_1	P & F_2	P & BC_1 (P_1)	P & BC_1 (P_2)
P 10-3/2	21.80	21.80	21.80	21.80
P 10-3/2 x P-23	22.04	21.30	21.73	19.85
P 10-3/2 x P-84	21.70	21.75	21.75	21.24
P 10-3/2 x YK 7-4/2	19.91	20.50	20.50	18.03
P-23	19.70	19.70	19.70	19.70
P-23 x P-84	20.20	20.40	20.50	19.90
P-23 x YK 7-4/2	19.20	19.50	19.00	18.50
P-84	20.50	20.50	20.50	20.50
P-84 x YK 7-4/2	18.72	18.90	19.50	18.25
YK 7-4/2	17.90	17.90	17.90	17.90
	$LSD_{0.05}$ = 0.96 $_{0.01}$ = 1.30	$LSD_{0.05}$ = 1.37 $_{0.01}$ = 1.84	$LSD_{0.05}$ = 1.35 $_{0.01}$ = 1.82	$LSD_{0.05}$ = 1.28 $_{0.01}$ = 1.73

The width of the middle belt leaves is a property whose values do not have an overall significance in the differences between the parents and, therefore, between the crosses. Therefore, lawfulness in the inheritance of the generations studied is not fully expressed. Values for the F2 traits are ranged from 8.8 cm in P 10-3/2 x YK 7-4/2 to 10.3 cm in P-23 x P-84 (Table 6). The highest width of the leaves in the BC1 (P1) generation is characterized by the cross (P-23 x P-84) x P-23 (10.5 cm) and the smallest (P-84 x YK 7-4/2) x P-84 (9.3 cm). The broadest middle belt leaves in the BC1 (P2) generation has the cross (P 10-3/2 x P-23) x P-23 (10.5 cm), and the narrowest (P-84 x YK 7-4/2) x YK 7-4/2 (8.7 cm).

Table 6. Width of the leaves from the middle belt in the parents, F_1, F_2, BC_1 (P_1) and BC_1 (P_2) offspring (2017)

Parents and hybrids	Width of the leaves from the middle belt (cm)			
	P & F_1	P & F_2	P & BC_1 (P_1)	P & BC_1 (P_2)
P 10-3/2	10.00	10	10	10
P 10-3/2 x P-23	10.00	10.2	10.1	10.5
P 10-3/2 x P-84	9.70	9.7	9.8	9.4
P 10-3/2 x YK 7-4/2	8.80	8.8	9.8	8.8
P-23	10.50	10.5	10.5	10.5
P-23 x P-84	10.00	10.3	10.5	9.3
P-23 x YK 7-4/2	9.00	9.5	10.4	9
P-84	9.50	9.5	9.5	9.5
P-84 x YK 7-4/2	9.00	9.4	9.3	8.7
YK 7-4/2	8.50	8.5	8.5	8.5
	$LSD_{0.05}$ = 0.68 $_{0.01}$ = 0.92	$LSD_{0.05}$ = 0.66 $_{0.01}$ = 0.89	$LSD_{0.05}$ = 0.78 $_{0.01}$ = 1.05	$LSD_{0.05}$ = 0.61 $_{0.01}$ = 0.82

CONCLUDING REMARKS

The greatest number of leaves among parental genotypes in both years of investigation gave P-23 (48.35 – 2016; 45.60 – 2017), and the smallest YK 7-4/2 (28.7 – 2016; 28.2 – 2017). The largest length of the middle-belt leaves was in P 10-3/2 (22 cm – 2016; 21.8 cm – 2017), and the smallest in YK 7-4 / 2 (18 cm – 2016; 17.9 cm – 2017). With the greatest width of the middle belt leaves is characterized P-23 (10.6 cm – 2016; 10.5 cm – 2017), and with the smallest width YK 7-4/2 (8.7 cm – 2017; 8.5 cm – 2017). Differences in values between the two years in the varieties are minimal, which is a sign of their ecological stability. An exception is made by P-23, which in 2017 (extremely drought, with three irrigations trough the season) gave about 3 leaves less than 2016, and thus it is characterized by poor resistance to drought stress.

In F1 hybrids P-23 x P-84 (46.35 – 2016) with the highest number of leaves is characterized, and with the smallest P 10-3/2 x YK 7-4/2 (31.05 – 2016). The longest leaves had P 10-3/2 x P-84 (21.9 cm – 2016), and the shortest P-84 x YK 7-4/2 (18.72 cm – 2017). The widest leaves had P-23 x P-84 (10.3 cm – 2016), and the narrowest P 10-3/2 x YK 7-4/2 (8.8 cm – 2017). The low differences between the years of research are another confirmation that these are high-heritable traits on which the influence of environmental factors is weak and limited.

Two years of investigations on the variability of the traits of parental genotypes and their diallel F1 crosses showed low values. The coefficient of variability (CV) for the number of leaves per stalk ranges from 2.02% (P-23 x YK 7-4/2 – 2016) to 7.94% (P 10-3/2 – 2016), for the length of the leaves of the middle belt from 5.88% (P-23 x P-84 – 2017) to 9.93% (P 10-3/2 – 2016), and for the width by 4.18% (P 10-3/2 x P -84 – 2016) to 9.85% (P-23 x YK 7-4/2 – 2016). The results indicate high genetic homogeneity, i.e. stability of homozygous parents and uniformity

of heterozygous hybrids.

With the highest number of leaves in the F2 generation is characterized P-23 x P-84 (43.2), and with smallest P 10-3/2 x YK 7-4/2 (31). The longest leaves had P 10-3/2 x P-84 (21.75 cm), and the shortest P-84 x YK 7-4/2 (18.9 cm). The widest are the leaves of P-23 x P-84 (10.3 cm), and the narrowest are of P 10-3/2 x YK 7-4/2 (8.8 cm). The values obtained from F2 offspring are higher than those of the F1 generation.

In the offspring of the BC1 (P1) generation with the highest number of leaves per stalk is characterized (P-23 x P-84) x P-23 (42.5), and with the lowest (P10-3/2 x YK 7-4/2) x P10-3/2 (32.2). With the largest length of the leaves is (P10-3/2 x P-84) x P10-3/2 (21.75 cm), and with the smallest (P-23 x YK 7-4/2) x P-23 (19 cm). The highest width of the leaves has (P-23 x P-84) x P-23 (10.5 cm), and the smallest (P-84 x YK 7-4/2) x P-84 (9.3 cm). The inheritance of the traits in this backcross generation depends on the parent back-crossed with the F1 offspring.

The highest number of leaves in the offspring of the BC1 (P2) generation is characterized by (P-10-3/2 x P-23) x P-23 (43), and the lowest by (P-84 x YK 7-4/2) x YK 7-4/2 (37). The largest length has the leaves of (P-10-3/2 x P-23) x P-23 (21.24 cm) and the smallest of (P10-3/2 x JK 7-4/2) x JK 7-4/2 (18.03 cm). With the highest width of the leaves is (P-10-3/2 x P-23) x P-23 (10.5 cm), and with the smallest width is (P-84 x YK 7-4/2) x YK 7-4/2 (8.7 cm). And here the inheritance of the traits depends on the parent back-crossed with the F1 offspring.

From the results shown in the paper, we can see, among other things, the effects of the backcrossing are obtaining resistance to stress from drought – an increasing world problem. In the breeding directed to this aim, the varieties P 10-3/2, P-23 and P-84 are distinguished, and they can be successfully integrated in selection programmes. From the crosses most suitable for the given purpose is P-23 x P-84.

REFERENCES

Beljo, J. (2006). Oplemenivanje bilja: Metode oplemenjivanja. Sveučilište Mostar.

Borojevic, S. (1992). Principi i metodi oplemenjivanja bilja: Metodi selekcije. Radivoj Cirpanov, Novi sad.

Camerarius, R.J. (1694). Ueber das geschlecht der pflanzen. W. Engelmann, Leipzig, (Series: Ostwalds Klassiker der exakten Wissenschaften, nr. 105.) xiii, p.78. (available 1899). Retrieved from www.worldcat.org/title/ueber-das-geschlecht-der-pflanzen

Collins English Dictionary (2014 – 12th Edition). Definition Backcross. Harper Collins Publishers. p.1583. Retrieved from https://www.thefreedictionary.com/backcross

Dimitrieski, M., Miceska, G., & Taskoski, P. (2012). Investigation of the resistance to Blue mold (Peronospora tabacina Adam) and Black shank (Phytophthora parasitica var. nicotianae) in some Oriental tobacco cultivars and lines. Tutun / Tobacco, 62(1-6), 30–35.

Gornik, R. (1973). Tobacco breeding: Crossing as a breeding method. Tobacco factory – Prilep.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing system. Aust. J. Biol. Sci., (9), 463–493.

Korubin – Aleksoska, A. (2004). Tobacco varieties from Tobacco Instiute - Prilep. NITP.

Korubin – Aleksoska, A. (2007). Developing a resistance to Blue Mold disease (Peronospora tabacina Adam) in tobacco by the use of Back–cross hybridization. Plant Science, XLIV(1), 63–87.

Korubin – Aleksoska, A., & Aleksoski, J. (2015). Detection of Resistance to Black Shank (Phytophthora parasitica var. nicotianae) in a Trial with Diallel Crosses of Tobacco. International Journal of Agriculture Innovations and Research, 3(4),1114–1118.

Manjit S. Kong – editor (2002). Quantitative Genetics, Genomics and Plant Breeding. CABI Publishing.

Martinčić, J., & Kozumplik, V. (1996). Oplemenjivanje bilja: Metode oplemenjivanja samooplodnog bilja. Poljoprivredni fakultet Osijek & Agronomski fakultet Zagreb.

Matthew, R. (2012). Backcrossing, Backcross (BC) Populations, and Backcross Breeding. Plant Breeding and Genomics.

Mulekano, O.L.P. (2013). Evaluation of elite breeding lines of flue-cured tobacco for field and market performance. The conventional method of tobacco cultivar development). University of Pretoria. National ETD portal (Sauth African theses and dissertations). Retrieved from http://hdl.handle.net/2263/30318

Pavlica, M., Zoldos, V., Horvat, T., Malenica, N., Mlinarec, J., & Vicic V. (2013). Praktikum iz genetike. Bioloski odsjek PMF-Sveuciliste u Zagrebu, p.136.

Wikipedia – the free encyclopaedia (2017, 28 November – last edit on the page). Definition: Backcrossing. Retrieved from https://en.wikipedia.org/wiki/Backcrossing

ЕФЕКТ ОД ПОВРАТНОТО ВКРСТУВАЊЕ ВО ОБЛАГОРОДУВАЊЕТО НА ТУТУНОТ

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Резиме

Трудот опфаќа испитувања на четири сорти од ориенталски тип (П10-3/2, П-23, П-84 и ЈК 7-4/2, и нивните дијалелни F1, F2, BC1(P1) и BC1 (P2) крстоски, во текот на 2016 и 2017 година за бројот на листови по страк и димензиите на листовите од средниот појас. Истражувањата беа направени на опитното поле при Научниот институт за тутун – Прилеп во опит поставен по случаен блок-систем во четири повторувања. Мерењата беа направени во периодот на буен пораст (фаза на бутонизација), а добиените вредности беа варијационо статистички обработени. Херитабилноста (h2) на својствата беше пресметувана по метод на Mather и Jinks.

Целта на испитувањата е да се проучи ефектот од повратното вкрстување во подобрувањето на квантитативните својства кај тутунот, да се даде визија за можноста на избор на единки кај максималниот број крстоски кои ги овозможува дијалелот на родителските генотипови и да се понудат насоки за понатамошна селекција.

Добиените резултати покажаа различен начин на наследување во сите анализирани генерации. Херитабилноста кај сите крстоски е повисока од 95%, што е знак за висока наследност на проучуваните својства. Понудената шема за понатамошна сукцесивна селекција може да се користи и кај други растителни видови за подобрување на голем број особини, меѓу кои и добивање на отпорност кон болести.

Клучни зборови: *тутун* (*Nicotiana tabacum* L.), *повратно вкрстување* (backcross), *дијалел, херитабилност*

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EXAMINATION ON YIELD AND SOME YIELD ASSOCIATED PARAMETERS IN DIFFERENT RICE GENOTYPES

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Abstract

The aim of this paper was to evaluate the grain yield and some yield related traits in different rice genotypes grown in the Republic of Macedonia. Nine rice genotypes (Prima Riska, 78/12-3-1, 79/22-2, Ronaldo, Onice, Opale, Gloria, Pato and San Andrea) were used for this research. All rice genotypes were from Italy except Prima Riska variety and two promising lines (78/12-3-1 and 79/22-2) which have Macedonian origin. The field experiments were carried out during 2014 and 2015 on the field areas in Kocani, Republic of Macedonia. Each genotype was set up on 5 m2 test area in three repetitions in randomized block design. Grain yield and some important yield associated traits, like plant height, panicle length, 1 000 grain weight and the number of plants per m2 were evaluated. This study investigated the effect of genotype and environment, but also their interaction on the grain yield. Besides the importance of genotype, the analysis of variance showed that the grain yield was strongly affected by environmental conditions (over 87 %). The average grain yield for all tested genotypes during the period of study was 6 100 kg/ha.

Key words: *grain yield*, *rice*, *agronomic traits*, *genotype*

INTRODUCTION

Rice (*Oryza sativa* L.) is a member of *Graminaeae* family and it is one of the most important cereals cultivated worldwide, constituting the basic food for large number of human beings, sustaining 2/3 of the world population (Zhout et al., 2002). The world's farmers have to produce about 60 % more rice than at present to meet up the food demands of the expected world population by 2025 (Fageria, 2007).

In the Republic of Macedonia, rice production has an important role in economy, because it provides sufficient rice for domestic needs and a significant part of rice production is export-oriented. Among the production factors, selection of variety plays an important role in the productivity of rice in any location (Alam et al., 2009).

In recent years, the main varieties in rice production in Macedonia are the Italian varieties San Andrea and R-76/6, which account for more than 90 % of the total production. The remaining part is represented by newly introduced varieties from Italy (Opale, Onice), Turkey (Halilbey) and selected domestic varieties (Prima riska and Biser-2).

In general, the productivity of this crop can be influenced by environmental conditions such as solar radiation, temperature and water availability during growth and development (Fageria, 2007). Grain yield depends on genotype, environment and management practices and their interaction with each other (Messina et al., 2009). Under the same management conditions, the variation of grain yield is principally explained by the effects of genotype and environment (Dingkuhn et al., 2006). Interaction between these two explanatory variables gives an insight in identifying genotype suitable for specific environments (Suchit et al., 2011).

The low heritability of grain yield characters made selection for high yielding varieties possible usually using various components

traits associated with yield (Atlin, 2003). Many researches confirmed that rice yield depends on a lot of number of traits, such as: the number of plants (the number of panicle per unit area), plant height, panicle length, the number of grains per panicle, the weight of the grains in panicle and 1 000 grain weight. The significance of each of these properties depends on climatic conditions and the water regime during the vegetation, as well as applied agro-technical measures.

Today, rice selection is mainly aimed at increasing the grain yield potential, improving the chemical and technological properties, good adaptability, shorter vegetation and shorter stem. Complex interactions between these indicators are exiting, because when increasing the value of one parameter, the value of another is often reduced.

The aim of this paper is to examine and evaluate the grain yield and some important yield associated properties in rice varieties which are dominate in rice production in Macedonia, in varieties that are recently recognized and in varieties and genotypes that according to the preliminary results represent a perspective for rice production in the Republic of Macedonia.

MATERIAL AND METHODS

Plant material and experimental design

During two years (2014 – 2015), nine rice varieties were cultivated and evaluated in agro-ecological conditions in Republic of Macedonia. Five of them were newly introduced varieties from Italy (Ronaldo, Opale, Onice, Gloria and Pato), one variety is domestic selection (Prima riska) and two are domestic prospective lines (78/12-3-1 and 79/22-2). San andrea, also Italian variety, was used as an experimental material and this cultivar for a long period is main variety in commercial rice production in Macedonia. The experiment was performed on alluvial soil type in the region of Kocani. Each test area was 5 m^2 set in three repetitions in randomized block method. In both years of research, rice was pre-crop. In 2014, the sowing was conducted on 25 April and in 2015 on 7 May. During the vegetation, standard agronomic practices were used. Before sowing, 500 kg/ha of NPK fertilizer (15:15:15) and 200 kg/ha Urea (46 %) were applied and during vegetation 100 kg/ha ammonium nitrate (34 %), of which 50 kg/ha during tillering and 50 kg/ha during heading was used. For weeds control, Basagran (4 l/ha), Rainbow (1.5 l/ha) and Clincher duo (1.5 l/ha) were applied.

Data collection

Ten randomly selected plants from each repetition have been analyzed for the plant height (cm) and panicle length (cm). The number of plants per m^2 was determined by counting the plants from m^2 of each repetition. 1 000 grains weight, has been determined to measure 1 000 grains of each repetition. Grain yield obtained from 5 m^2 was calculated in kg/ha. The moisture content was reduced in 14 %, when was calculated the 1 000 grains weight and grain yield.

Statistical analysis

For analysis of variance (ANOVA) the statistical package SPSS (2010) was used. Least significant difference (LSD) was calculated using Statistical analysis system software JMP (2002).

RESULTS AND DISCUSSION

The results of mean values for grain yield and some yield associated traits are shown in Table 1. Significant differences were found between the tested varieties for all analyzed properties.

Yield superiority was shown by Ronaldo variety (7 082 kg/ha) and Prima riska (7 057 kg/ha) but the lowest grain yield was obtained from Gloria variety (4 766 kg/ha). The paddy yield obtained from genotype 78/12-3-1 (6 753 kg/ha) statistically does not differ significantly from the yield received from Ronaldo and Prima riska. The average value for grain yield for all tested varieties during the period of study was 6 100 kg/ha.

Table 1. Mean values for yield and some yield related traits in rice varieties (2014-2015).

Variety	Plant height (cm)	Panicle length (cm)	1000 grain weight (g)	Number of plants per m^2	Grain yield (kg/ha)
Prima riska	92a	19a	38,82b	449bc	7 057a
78/12-3-1	88b	19a	35,09c	502ab	6 753a
79/22-2	86b	17b	41,21a	468bc	6 540ab
Ronaldo	54b	16cd	32,21d	446bc	7 082a
Onice	82c	12g	32,58d	464bc	5 885bc
Opale	62d	14f	32,44d	432c	5 768c
Gloria	57e	15de	41,12a	443bc	4 766d
Pato	63d	14ef	42,39a	438bc	5 234cd
San Andrea	88b	16bc	39,49b	538a	5 815c
Mean	**75**	**16,0**	**37,26**	**465**	**6 100**
Minimum	**43**	**9**	**28,34**	**202**	**1 972**
Maximum	**107**	**25**	**48,50**	**720**	**11 400**
$LSD_{0.05}$	**3,60**	**1,09**	**1,39**	**68,92**	**659,40**
CV (%)	**2,81**	**4,05**	**2,18**	**8,65**	**6,30**

The results of many researches show that the grain yield is determined by the three main components such as: the number of panicles per unit area, the number of grains per panicle and 1 000 grain weight. San Andrea has the largest number of plants per m^2 (538), followed by genotype 78/12-3-1 (502). Opale variety showed the lowest number of plants (432).

1 000 grain weight varies from 32.21 g in variety Ronaldo to 42.39 g in Pato cultivar.

Significant impact on grain yield has panicle length. Longer panicle length usually is associated with a larger number of grains. Plants which have longer panicle, can serve as a source of assimilates because of more active photosynthesis. From this study Prima riska and 78/12-3-1 have the longest panicle (19 cm) while the shortest was recorder by Onice variety (12 cm). The average value for this trait was 16.0 cm for all tested varieties.

Prima riska variety also was the highest (92 cm) from all tested varieties.

Significant differences between the varieties show the presence of genetic variability among them and give a great opportunity to improve the yield. The obtained results for the tested properties show that all investigated genotypes are significant and have great potential for yield. Their inclusion in selection programs to improve these properties can be effective for further rice improvement.

The results from ANOVA obtained from the research are given in Table 2.

Grain yield was significantly affected by the year, while the influence of the variety and the interaction of variety and year have not shown significance. The impact of the year on yield formation was 87.95 % while from the variety 7,61 %. The least influence on paddy yield has the interaction between variety and year (4.44 %).

Table 2. The influence of variety, year and their interaction on grain yield.

Factor	Sum of Squares	df	Mean Squere	F	η
Total	424,949	53			
Factor (A) - variety	31,600	8	3,950	14,512	7,61
Factor (B) - year	365,134	1	365,134	1341,501	**87,95***
A x B	18,417	8	2,302	8,458	4,44
Error	9,799	36	0,272		

The vegetation period in both years of study differed in ration of air temperatures, amount and schedule of precipitations. Deviations in temperatures were more pronounced during the blooming and spraying the grains. Negative impact on grain yield in the second year of the trials has the precipitations during grain spraying and ripening. Frequent and heavy rainfall prolonged the harvest. Additional and the worst negative impact on the research has unfavourable water regime during and after the treatment with herbicide in the second testing year. According to this, the destruction of the weeds was not timely and fully effective.

CONCLUDING REMARKS

The results from the study had proved significant differences between tested rice genotypes for all analyzed traits.

Genotypes Ronaldo, Prima riska and 78/12-3-1 have shown the highest yield potential. Prima riska is already recognized domestic variety, present in rice production but with better agro technology measurements can be much better ranked in rice assortment. Ronaldo, Pato, 78/12-3-1 and 79/22-2 with additional researches may also be more popular among rice manufactures. For a longer time in rice production, San Andrea variety has the dominant role and this study shows that it is still justifies the "backbone" of rice production. The presence and use in rice production of genotypes Onice, Opale, Pato and Gloria is also justified. All genotypes, used in this research, under favourable external conditions and application of more intensive modern agro-technology, can further exploit their potential. All genotypes can also be used as parents in breeding programs for creation of new rice genotypes, in order to get the new high yielding varieties.

REFERENCES

Alam, M.M., Hasanuzzaman, M., & Nahar, K. (2009). Growth pattern of three high yielding rice varieties under different phosphorus levels. Advances in Biological Research, 3 (3-4), 110-116.

Atlin, G. (2003). Improving drought tolerance by selecting for yield. Breeding rice for droughtprone environments. Los Banos, Philippines, 14-22.

Dingkuhn, M., Luquet, D., Kim, H., Tambour, L., & Clement-Vidal, A. (2006). EcoMeristem, a model of morphogenesis and competition among sinks in rice. 2. Simulating genotype responses to phosphorus deficiency. Functional Plant Biology 33, 325-337.

Fageria, N.K. (2007). Yield physiology of rice. Journal of Plant Nutrition, 30, 843-879.

JMP (2002). Version 5.0 1a, A Business Unit of SAS 1989 - 2002 SAS Institute Inc.

Messina, C., Hammer, G., Dong, Z., Podlich, D., & Cooper, M. (2009). Modelling crop improvement in a GxExM framework via gene-trait-phenotype relationships. In: Sadras, V.O., Calderini, D. (Eds.), Crop physiology: Applications for Genetic Improvement and Agronomy, Elsevier, Netherlands, 235-265.

SPSS Statistics 19 (2010). SPSS Inc., an IBM Company.

Suchit P.S., Folkard, A., Holger, B., Julie, D., & Alain, R. (2011). Yield stability and genotype x environment interactions of upland rice in Altitudinal gradient in Madagascar. Tropentag, University of Bonn, October 5 - 7, 2011. Conference on International Research on Food Security, Natural Resource Management and Rural Development, 1-4.

Zhout, Z., Robards, K., Heliwell, S., & Blanchard, C. (2002). Ageing stored rice: changes in chemical and physical attributes. Journal of Cereal Science, 35, 65-78.

ИСПИТУВАЊЕ НА ПРИНОСОТ И НЕКОИ КОМПОНЕНТИ НА ПРИНОС КАЈ РАЗЛИЧНИ ГЕНОТИПОВИ НА ОРИЗ

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Резиме

Целта на овој труд беше да се испита приносот на зрно и некои компоненти на принос кај различни генотипови на ориз одгледувани во Република Македонија. Девет генотипови на ориз (*Prima Riska*, *78/12-3-1*, *79/22-2*, *Ronaldo*, *Onice*, *Opale*, *Gloria*, *Pato* и *San Andrea*) беа употребени како епксперименталeн материјал. Сите генотипови имаат италијанско потекло, со исклучок на *Prima Riska* и две перспективни селекциски линии кои имаат македонско потекло. Експерименталните опити беа поставени во текот на 2014 и 2015 година на опитните површини во Кочани. Секој генотип беше поставен на опитни површини од 5 m^2 во три повторувања во рандомизиран блок систем. Во текот на двете години од истражувањата беа анализирани следниве својства: принос на зрно, како и некои важни компоненти на приносот, висина на растение, должина на метличка, маса на 1000 зрна и број на растенија на m^2. Истражувањето имаше за цел да го утврди и влијанието на генотипот, годината како и нивната интеракција врз приносот на зрно. Освен значајноста на генотипот, со анализа на варијанса се покажа дека условите на надворешната средина (годината) силно влијаат врз приносот на зрно (над 87 %). Просечната вредност за принос на зрно за сите испитувани генотипови беше 6100 kg/ha.

Клучни зборови: *принос на зрно*, *ориз*, *компонети на принос*, *генотип*

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Review paper

EVALUATION OF THE CURRENT STATUS IN ORGANIC AGRICULTURAL PRODUCTION IN REPUBLIC OF MACEDONIA AND EUROPEAN COUNTRIES

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Abstract

Implementation of organic agriculture and development of sustainable agriculture production gives a new lifestyle quality in local communities and country in general. Intensive agriculture is unsustainable and it cannot support production of enough food longtermly because of compromising the basic settings for food production.

The modern agriculture trends involve favorizing of new high yielding varieties produced with high input consumption which is a direct threat for genetic diversity and agriculture. Ingenious genotypes have enormous gene poll of different desirable characteristics, but they are permanently endangered because of utilization of commercial varieties.

Introduction of organic farming is a hope for survival of endangered and ingenious varieties and genotypes. The positive role of organic farming is foreseen in growing crops in their natural environment which contributes to conservation of existing genetic diversity. Utilization of old varieties and local genotypes in organic farming is one of the ways to increase genetic diversity and biodiversity in agricultural system and it gives to the organic farming broader and durable meaning regarding protection of ecosystem and environment.

Environmental conservation and protection are important to the politics of rural development in EU. Following this approach, there is need for implementation of instruments which will create conditions for development of sustainable, highly harmonized agriculture with the principles of environmental protection.

Key words: *sustainable agriculture, intensive agriculture, biodiversity, organic farming*

INTRODUCTION

Conventional agriculture in the last century achieved significant results in the field of food production. Conventional agriculture is characterized by the use of advanced technological solutions, significant investments, enlargement of agricultural land, cultivation of monocultures, uniform high-yield hybrids and varieties, using large amounts of pesticides and fertilizers, using external energy inputs, high efficiency of workforce, etc. But, on the other hand, in conventional farming, soil processing is intense and as such destroys its quality in different ways. First of all, it reduces the amount of organic matter in the soil, and therefore it reduces its fertility and disrupts its structure. Also, other agrotechnical measures applied in intensive agricultural production contribute to environmental degradation and pose a threat to the sustainability of the agroecosystem in the long run.

In response to the vicious disruption of agro-biocenosis and environmental pollution, as well as the decrease in the quality of agricultural products and the deterioration of the structure and fertility of the soil, the need and necessity for sustainable agriculture will be carried out under conditions of reasonable comfort within the framework of natural boundaries of natural communities.

Sustainable agriculture is based on sustainable agricultural practices with adapted soil treatment systems, with the application of which produces positive effects on the health and productivity of mutually

dependent communities, the life of the land, plants, animals and humans. At the core of these agricultural practices is the avoidance of the use of artificially synthesized materials in the production and promotion of exclusively natural materials used as fertilizers, plant protection products, that is, pesticides, plant growth and development stimulators, as well as additives in the production and processing of food. The application of the crop rotation, plays a key role in the achievement of the objectives of sustainable agriculture (Lampkin, 1994; Wijnands, 1999; Milošev and Šeremešić, 2004) and improvement of soil quality (Jaenicke and Drinkwater, 1999; Olesen et al., 1999; Lampkin and Measures, 2001). In fact, the key principle of sustainable food production is the interaction of all components involved in the food production cycle.

Organic farming is a sustainable food production system aimed at reducing harmful effects on the environment, soil fertility conservation, using natural mechanisms in ecosystems and improving natural resources. Today, the organic farming sector is the fastest growing food sector. Consumer interest grows in response to the ever-present fear of food safety and animal welfare, as well as the impact of conventional agriculture on the environment (Bavec and Bavec, 2006).

The Republic of Macedonia has natural resources that enable the development of organic agricultural practice and environmental production management systems. Organic farming is separated as a system of agricultural production that has been established on the agricultural land in the Republic of Macedonia in recent years. Although this system is introduced to only about 1% of the total arable land, the trend for introducing organic farming is increasing and it gives the right to expect organic farming to be the carrier and basis for the further development of other systems of sustainable agriculture in Macedonia (National Plan for Organic Production, Ministry of Agriculture, Forestry and Water Economy, 2013 - 2030). The activities and measures undertaken in this area are in line with the National Agricultural Strategy and Action Plans that the Ministry of Agriculture, Forestry and Water Economy creates for certain programme periods, with the aim of increasing the development of organic farming in the Republic of Macedonia.

In the past few years, organic farming in Macedonia has seen a certain increase, which is perceived through the increased total production capacities and the increased number of organic producers. However, despite the favourable conditions and resources available in the Republic of Macedonia, it can be pointed out that they are underutilized, especially in terms of arable land.

MATERIAL AND METHODS

This paper presents the overall situation in the organic agricultural production in the Republic of Macedonia in the period of 2013-2017. During the preparation of the paper, relevant literature and data were obtained from public, state and scientific institutions. Also, certain consultations have been made personally with manufacturers, control and certification bodies and advisory services.

RESULTS AND DISCUSSION

The potential of the natural resources of the Republic of Macedonia for the development of organic production as part of sustainable agricultural practice and as an environmentally friendly production management system is necessary to be used at a higher level. This is necessary in order to achieve a reasonable relation of man to the environment, thereby supporting and strengthening the health of ecosystems as a whole. Proper targeting of the Macedonian agro-food sector to the process of approximation of the standards defined by the Common Agricultural Policy of the EU is necessary.

Organic production in the Republic of Macedonia is regulated by the Law on Organic Agricultural Production (Official Gazette of the Republic of Macedonia, No. 146/2009, 53/2011, 149/2015, 39/2016, 132/2016), which is fully harmonized with the European Regulations for Organic Production, No. 834/2007 and 889/2008. In order to protect organic producers from unfair competition and at the same time to protect consumers of organic products, it is necessary to guarantee that the product is produced in accordance with the principles of organic farming. Professional control and certification are a guarantee for consumers that the organic product is produced in accordance with all the criteria and requirements of the law and the by-laws for organic agricultural production.

The certification of organic production in Macedonia is carried out by two national control / certification bodies - Balkan Biosert and Pro Cert. These bodies are authorized by the Ministry of Agriculture, Forestry and Water Economy (MAFWE) on the basis of accreditation received by the Accreditation Institute of the Republic of Macedonia, which is a full member of the European Accreditation and is a signatory of the EA MLA - Multilateral Agreement, which is an agreement between European accredited certification bodies for mutual recognition of certificates.

Based on the data from the procedure for expert control, the authorized control / certification bodies prepare a report and make a decision for certification of organic production, and for that they issue a certificate to the applicants for the certification. The certificate shall be valid until the next control, generally one year from the date of issue. By acquiring a certificate for organic production, the right to use the national organic product label is acquired.

a b

Figure 1. National label for organic product (a) and organic product in conversion (b).

The Government of the Republic of Macedonia is making great efforts to increase the production of organic food through various support measures, harmonizing the legislative basis with the European regulations for organic production and implementing various measures for encouraging the development of this food production system.

In order to raise the national awareness of the value of organic food, as well as to promote the national organic product label, the MAFWE, in cooperation with the Federation of Organic Producers of the Republic of Macedonia (FOPM), implemented various promotional activities and campaigns for raising the awareness of consumers for organic foods, on the basis of which is the need for additional awareness raising and information regarding organic agricultural production. Among many promotional activities, MAFWE also promotes plant protection products approved for use in organic production and present on the market in Macedonia.

All activities and measures undertaken in the direction of better development of organic production in the Republic of Macedonia are in accordance with the National Plan for Organic Production (2013-2020). According to this strategic document, a national strategic goal for increasing the competitiveness of organic production in the Republic of Macedonia has been set up, for the successful placement on the domestic and foreign markets, for which the realization has determined several specific goals and measures in both primary and secondary organic production, in trade, control and certification, etc. One of the most significant specific goals to be achieved by 2020 in the area of primary agricultural production is to identify and support strategically important organic products, improve the availability of raw materials authorized for use in organic production, the arable land under organic production to have a share of 4% in the total arable agricultural land in Macedonia and 4% of the total livestock in the country to be certified as organic livestock (including beekeeping and fisheries).

Bearing in mind that organic production is more complex in terms of conventional production, the Government of the Republic of Macedonia financially supports the development of organic production each year with 30% to 100% increased financial support in terms of conventional production (MAFWE, Decree on the detailed criteria for direct payments, users of funds, maximum amounts and the manner of direct payments, 2018).

The total National Budget, as well as the measures for financial support of organic production are created annually and are suitable to the specific needs of the sector.

The number of farmers and other operators of organic products has been increased in the last few years (Fig. 2),while organically certified arable land is decreasing (Fig. 3).

Specifically, although in 2014 as compared to the previous year, the number of farmers, processors and traders with organic products decreased from 400 (2013) to 344 (2014), their number from 2015 shows an increasing trend, so the number of operators in 2015 is 481, 533 in 2016 and 654 in 2017.

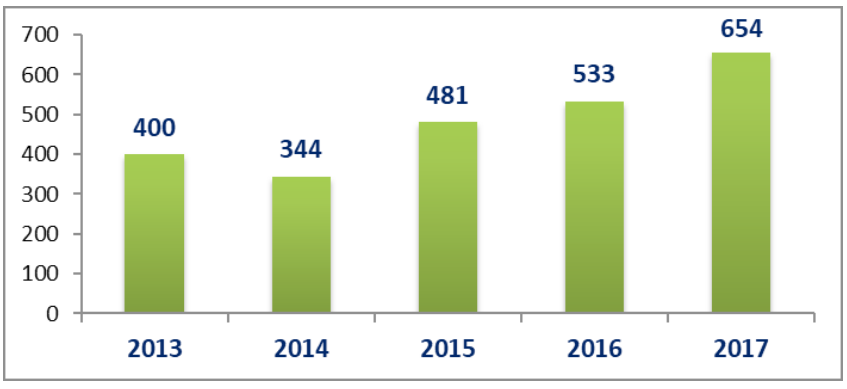

Figure 2. Number of operators involved in organic production (2013 – 2017)

Despite the increased number of operators, the situation with the production capacities under plant organic production in the investigated period registered changes of 2,359 ha in 2014 to 3,240 ha in 2016. In 2017 organic farming was established on 2,900 ha. With the measures of support undertaken by the MAFWE in the forthcoming period even greater development and increase of the production capacities is expected.

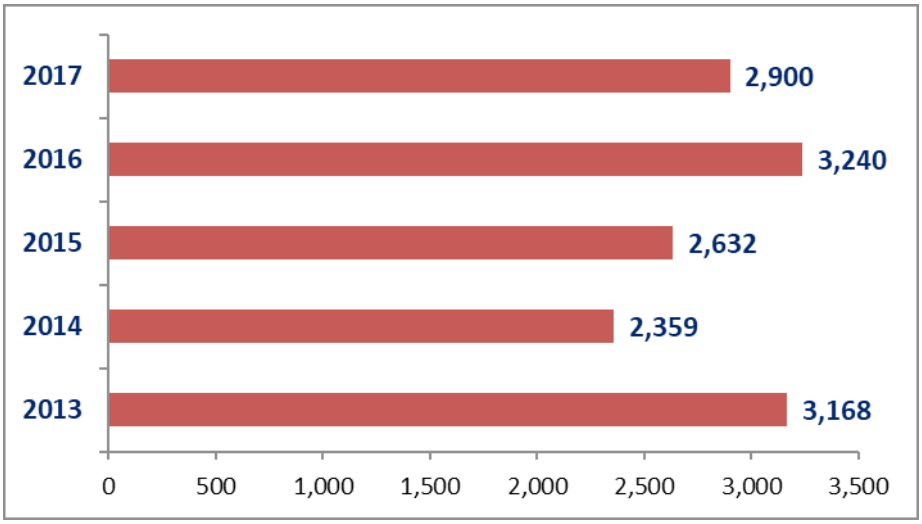

Figure 3. Organically certified arable area in the Republic of Macedonia (2013 – 2017)

According to FiBL and IFOAM data, the situation in the countries of the region and some EU countries shows a gradual increase in agricultural land certified for the organic production. There were 515 ha (2013) and were 662 ha (2016) certified arable areas in Albania. In Bosnia and Herzegovina and Serbia, there is also a trend of increasing the area. Serbia in 2013 had 8,228 ha, 9,548 ha (2014), 15,298 ha (2015) and 14,358 ha (2016), while in Bosnia and Herzegovina this trend is even more noticeable because the certified arable land increased from 292 ha in 2013 to the 992 ha in 2016.

In some EU countries, organic farming areas are also increasing. Detailed list organic agriculture land is given in Table 1.

Table 1. Organic agriculture land in some EU and courtiers from the region (FiBL & IFOAM, 2018).

Country	Year / ha			
	2013	**2014**	**2015**	**2016**
Albania	515	662	662	662
Bosnia and Herzegovina	292	353	576	992
Serbia	8,228	9,548	15,298	14,358
Montenegro	3,068	3,289	3,213	3,470
Turkey	461,396	491,977	486,069	523,777
Greece	383,606	362,826	407,069	342,485
Bulgaria	56,287	74,351	118,552	160,620
Slovenia	38,665	41,237	42,188	43,579
Croatia	40,641	50,054	75,883	93,593

Republic of Macedonia has favourable agro-ecological conditions for the implementation of organic plant production since there are large unpolluted areas suitable for crop production and favourable agro-ecological conditions and possibilities for optimal and successful organic cultivation of a wide assortment of different plant crops (cereal, industrial, fodder, horticultural and viticulture-orchard production). In plant production, the most producers are certified for organic production of various cereals, forage and fruit crops,

In the research period, the biggest area of 1557.97 ha certified for organic cereals was accomplished 2013, as opposite to 2014 and 2015, when the area under organic cereals was reduced by about 600 ha and 950 ha, respectively. The areas certified for the production of organic cereals have been gradually increased in 2016 (938.40 ha) and in 2017 (939.59 ha).

Certified areas under organic orchards have been increased from 321.55 ha (2013) to 559.20 ha (2017). There is a certain trend of growth in organic production of vegetable crops represented with 83.88 ha in 2015, 93.17 in 2016 and 174.38 in 2017 (Tab.2).

Farmers also have experience in the cultivation of crops in a traditional-extensive way, and there are opportunities for the application of appropriate crop rotation systems in plant production in the regions with the possibility of irrigation, which is considered as a strength according to the National Plan for Organic Production (2013 - 2020), (MAFWE, 2013).

Table 2. Organic plant production in the Republic of Macedonia (2013 – 2017).

Crop	Year / ha				
	2013	2014	2015	2016	2017
Cereals	1557.97	896.40	604.42	938.40	939.59
Forage	691.26	523.99	977.33	748.98	681.18
Industrial	34.59	-	-	-	-
Oilseed	73.44	119.53	103.56	42.84	32.78
Orchards	321.55	96.54	400.19	422.14	559.20
Viticulture	41.92	52.41	76.39	17.54	24.03
Vegetables	71.57	243.20	83.88	93.17	174.38
Fallow land	304.26	204.22	642.29	402.14	192.21

According to FiBL and IFOAM data, in Europe and the European Union the largest areas under the organic agriculture are certified for production of cereals and green fodder (FiBL & IFOAM, 2018). In 2016, 2.279.155 ha in Europe and 1.889.408 ha in the EU were certified for cereals, while 2.255.059 ha in Europe and 2.066.861 ha in the EU for green fodder. The same year, there were 148.088 ha (Europe) and 135.684 ha (EU) under organic vegetable production. Organic production of temperate fruits and (sub) tropical fruits was conducted on 158.182 ha in Europe and 117.276 ha in the EU. Italy (approx. 300.000 ha, including large areas of durum wheat), Germany (approx. 242.000 ha) and France (approx. 217.000 ha) have the largest organic cereal production. The largest certified areas for organic production of vegetables were registered in Italy (43.648 ha), France (18.064 ha) and Spain (17.013 ha) (Willer et al., 2016).

In the last few years, a significant increase in Republic of Macedonia occurs in organic livestock production. The country has favourable conditions for the development of organic farming of livestock, unpolluted natural meadows and pastures. Livestock producers have many years of experience and apply traditional animal husbandry practices, very close to the organic way of raising animals. Also, the interest of the processing sector for organic dairy products and meat products has increased, and various organic livestock products in the country's trade chains are more likely to be found.

The most certified animals in organic farming are sheep, but also the interest for certifying cattle and goats is increasing. The highest number of organically certified sheep (92.386) and cattle (8.565) was registered in 2017. The number of organically certified cattle in 2017 was two and a half times more as compared to 2016 and almost four times more as compared to 2013 and 2014. A slight decrease in the number of goats registered for organic production was registered in 2017, compared to 2015 (4.012 goats) and 2016 (4.142) (Tab. 3).

Table 3. Organic animal farming in the Republic of Macedonia (2013 – 2017).

Animal	Year / number of animals				
	2013	**2014**	**2015**	**2016**	**2017**
Cattle	2.736	2.136	3.180	3.317	8.565
Sheep	64.301	53.484	70.007	78.664	92.386
Goats	2.946	2.276	4.012	4.142	3.833

Taking into account currently available information, the organic animal sector is developing at a fast pace in the European countries. In 2016 in Europe, 3.9 million bovine animals, 1 million pigs and 46 million poultry were breed as organically certified (Willer et al., 2016).

The conditions in Republic of Macedonia are also exceptionally favourable for organic beekeeping production. Many beekeepers in the past have been converting their production into organic, which also gives them higher added value for bee products that are increasingly demanded on the market. The overview of the number of bee families in the period 2013 - 2017 is given in Figure 4. The average number of bee hives in the research period is around 7,000. In 2017, this number is 7.676; in 2016, it is 7.760, in 2015, it is 6.932, in 2014, it is 6.285 and in 2013 it is 6.363.

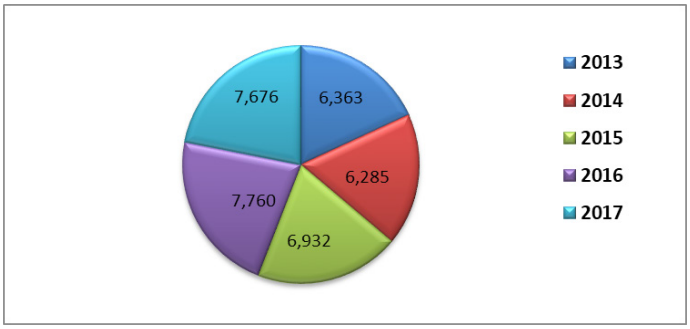

Figure 4. Number of certified organic bee hives in the Republic of Macedonia (2013 – 2017)

According to FiBL and IFOAM data, it is expected that organic beekeeping will continue to grow worldwide because to the increasing demand for organic honey and bee products. One of the main challenges for new organic beekeepers is the conversion process due to the luck of knowledge on organic beekeeping practices and the organic certification process. The country with the largest number of organic beehives is Brazil (537.014), followed by Mexico (368.000) and Bulgaria (236.462) (FiBL & IFOAM, 2018).

CONCLUDING REMARKS

The main goal of sustainable development is to create economically viable and environmentally acceptable agricultural production that would be the basis for rural development and the basis for rural livelihoods, which would create opportunities for a breakthrough in the European market.

Organic production is separated as a system that has been established in the last few years on larger agricultural areas in the Republic of Macedonia. In parallel, national agricultural policies for supporting sustainable agriculture, as well as the implementation of agroecological measures, are adapted. These policies are aimed at efficient and market-oriented agricultural production, where issues related to food safety, environmental protection and animal welfare, and which contribute to the general development of rural society, are an important place.

In fact, the main task of agricultural policies is defining strategies for the production of healthy and safe food, and the decision to support and develop the organic sector actually created the conditions for obtaining high quality and safe products for which with a well-planned and meaningful performance the markets would ensure the competitiveness of these products in the markets.

The European market offers numerous opportunities for exporters from developing countries, especially for organic and healthy foods. For that reason, Macedonia must use its potentials to produce such food, promote it and become recognizable and competitive on international markets.

REFERENCES

Bavec F.& Bavec M. (2006). Organic production and use of alternative crops. RC Press, Taylor & Francis Group, Boca Raton, FL, ISBN-13:978-1574446173.

FiBL & IFOAM, Organic International (2018). The world of organic agriculture. Statistics & Emerging Trends 2018. Available at https://shop.fibl.org/CHde/mwdownloads/download/link/id/1093/?ref=1

Jaenicke, E.C., & Drinkwater, L.E. (1999). Sources of productivity growth during the transition to alternative cropping systems. Agricultural and Resource Economics Review, 28 (2),169-181.

Lampkin, N.H. (1994). Organic farming. Farming Press, 540, Ipswich.

Lampkin, N.H., & Measures, M. (2001). Organic farming management handbook. Welsh Institute of Rural Studies. University of Wales, 1-100, Aberystwyth.

Milošev, D. & Šeremešić, S. (2004). Značaj plodoreda u organskoj proizvodnji ratarskih biljaka. Zbornik radova sa III Međunarodne Eko konferencije, Ekološki pokred grada Novog Sada, Novi Sad 413-418.

Ministry of Agriculture, Forestry and Water Economy (2013). National plan for organic production, Ministry of Agriculture. Forestry and Water Economy, (2013 – 2030).

Olesen, J. E., Rasmussen, I. A., Askegaard, M. & Kristensen, K. (1999). Design of the Danish crop rotation experiment. In: Designing and testing crop rotations for organic farming (Eds. J.E. Olesen, R. Eltun, M.J. Gooding, E.S. Jensen & Kopke U.), DARCOF Report No.1, 49-62.

Wijnands, F. G. (1999). Crop rotation in organic farming – theory and practice. In: Designing and testing crop rotations for organic farming (Eds. J.E. Olesen, R. Eltun, M.J. Gooding, E.S. Jensen & Kopke U.), DARCOF Report No.1, 21-35.

Willer, H., Lernoud, J. & Kilcher, L. (2016). The world of organic agriculture. statistics and emerging trends 2013. FiBL and IFOAM Report.

Willer, H., Lernoud, J. & Zanoli, R. (2016). The challenge of collecting and publishing data on organic agriculture worldwide. Proceedings ICAS VII Seventh International Conference on Agricultural Statistics, Rome.

СПОРЕДБА НА СОСТОЈБАТА СО ОРГАНСКОТО ЗЕМЈОДЕЛСКО ПРОИЗВОДСТВО ВО РЕПУБЛИКА МАКЕДОНИЈА И ЕВРОПСКИТЕ ЗЕМЈИ

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Резиме

Имплементацијата на органското земјоделство и унапредувањето на развојот на одржливото земјоделско производство претставува еден нов квалитет во животот на локалните заедници и земјата во целина.

Интензивното земјоделство е неодржливо и нема да може да продолжи со производство на доволни количини на храна во подолг временски период поради загрозувањето на суштинските услови од кои директно зависи земјоделското производство.

Трендот на модерното земјоделство исто така форсира нови сорти и видови кои даваат високи приноси при големи вложувања. Тоа претставува директна опасност за генетскиот диверзитет и земјоделството. Автохтоните популации имаат во изобилство гени за отпорност на болести и гени за квалитет и истите се сериозно загрозени трајно да исчезнат заради употребата на генотипови на комерцијални сорти и видови.

Воведувањето на органското земјоделство претставува надеж за опстанок на загрозените видови. Позитивната улога на органското земјоделство се согледува во тоа што културите се одгледуваат во нивното природно опкружување, со што се конзервира постоечкиот генетски диверзитет.

Користењето на стари сорти и локални популации во органското производство е еден од начините за зголемување на генетската дивергенција на одгледуваните растенија и нивно унапредување. Зачувувањето на генетската разновидност и биодиверзитетот во земјоделскиот систем на органското производство му дава пошироко и трајно значење во однос на заштитата на екосистемот.

Зачувувањето и заштитата на човековата околина заземаат значајно место во политиките за рурален развој на Европската Унија. Следејќи го овој пристап, потребно е да се применат инструментите кои ќе овозможат развој на одржливото земјоделство кое е максимално усогласено со принципите за заштита на животната средина.

Клучни зборови: *одржливо земјоделство, интензивно земјоделство, генетски диверзитет*

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CHARACTERIZATION OF SOME DOMESTIC AND INTRODUCED VARIETIES OF COTTON IN THE AGRO-ECOLOGICAL CONDITIONS OF STRUMICA REGION

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Abstract

During 2015-2016, research with 10 cotton varieties (lines *5136*, *5140* and *5141*, created at the Institute in Strumica and Bulgarian varieties *Chirpan 539*, *Veno*, *Perla 267*, *Avangard 264*, *Colorit 409*, *Helius 288* and *Natalia 361*) was done in the agro-ecological conditions of Strumica region. The aim was to study the biological and agricultural characteristics of cotton varieties. The experiments were done in three repetitions following a randomized block system and with size of experimental field parcel of 14m^2. All examined varieties in agro-ecological conditions in Strumica have fallen in medium early-matured varieties, with a vegetation period of 125-130 days. The lines belonging to the group of early-matured varieties had a vegetation period of 116-118 days. The seed cotton yields in the years of research were from 2853 kg/ha at the Bulgarian variety *Colorit* to 5158 kg/ha at the variety *Veno*. The highest lint percentage from the domestic genotypes was found for the line *5141* (42.6%), and for the Bulgarian genotypes *Chirpan 539* (45.4%) and *Helius 288* (43.8%).

Key words:*Gossypium hirsutum* L., *cotton* lint percentage, *yield, fibre length*

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the most important fibrous culture in the world. It is grown in tropical and subtropical regions in more than 80 countries around the world. Of the 39 species of cotton plants, only four species are grown in order to obtain fibre. These are *Gossypium hirsutum*, *Gossypium barbadense, Gossypium herbaceum and Gossypium arboretum*. Virtually all the commercial cotton that is grown today are the varieties of American species *Gossypium hirsutum* and *Gossipyum barbadense*. *Gossypium hirsutum* covers more than 90% of cotton grown in the world and is the main source of textile fibres and is also produced for the production of oil. The quality of cotton fibre, such as hygroscopic, softness and electro-neutrality, in many cases makes it indispensable from artificial fibres. Cotton continues to be a culture of great economic significance in many developing and some developed countries (Rathore et al., 2006). Today, cotton covers for less than 3% of arable land in the world. Long cotton fibres are spun into thread for textiles, towelling, paper, banknotes, fishing nets, tents, nappies, wallpaper, bandages, surgical sutures, rope and sheets. Short cotton fibres, or linters, provide cellulose used for dynamite, sausage skins, lino, cellophane, rayon, photographic film, nail polish, etc. From the crushed cotton seed you get useful vegetable oil and the meal from crushed seeds is used for cattle feed, fish bait and organic fertilizer. Global cotton seed production can potentially provide a protein requirement for half a billion people per year. The cotton production in Macedonia depends a lot on early maturity of the cotton (Spasova et al., 2010, 2016).

The selection of varieties is one of the most important decisions in the selection of cotton. The various features depend heavily on the environment. Environmental conditions do not change only with geography but also from season to season in a particular area. It is important for manufacturers to know what the limiting factors are and to choose the appropriate variety.

The main objective of the study was to assess the new Bulgarian and Macedonian varieties of cotton in agro-ecological conditions in Strumica region, and to determine which of them are best for introduction into production or effective use in the breeding work. The research in our country and in the world is directed towards the following goals: Improving the quality of the fibre and seeds, developing varieties resistant of drought and diseases and early maturing.

MATERIAL AND METHODS

During 2015-2016, research with 10 cotton varieties (lines 5136, 5140 and 5141, created at the Institute in Strumica, Macedonia and Bulgarian genotypes Chirpan 539, Veno, Perla 267, Avangard 264, Colorit 409, Helius 288 and Natalia 361 created at the Field Crops Institute in Chirpan - Bulgaria) was done, in the agro-ecological conditions of Strumica region.

In both years of examination, basic soil treatment was carried out at a depth of 30cm from the autumn, and in the spring only additional processing was carried out.

The trials were set in three repetitions following a randomized block system, with each experimental parcel occupying an area of 14m^2. Sowing of cotton in the years of examination was performed 13 to 17 May.

The sowing was performed manually with 4-5 seeds in the nest, at a distance of 70 cm between rows and 20cm in row, leaving two plants in the nest.

During the vegetation, surveys and biometric measurements for the growth, development, and the birth of plants were performed. Before harvesting, 10 bolls were taken from each repetition, of each variety, that was 30 bolls of each variety. In the laboratory the mass of one boll, the lint percentage and the length of the fibre were determined.

The results were statistically processed by the method analysis of variance, and tested by LSD - test.

The soil and climate conditions in the area of research

The Strumica valley is situated on 200-300m altitude and it is under the influence of the Sub-Mediterranean and Eastern-continental climate. Precipitation is characterized by Mediterranean regime with a maximum in November and a minimum in the summer months (July or August).

The type of soil where the tests were carried out is alluvial, poorly supplied with humus and nitrogen, intermediately supplied with physiologically active phosphorus and well supplied with active potassium.

The weather conditions in the years of examination were different in terms of temperature and precipitation (Tab. 1 and 2).

Table 1. Average monthly temperatures in Celsius

Year	Month												Annual amount of temp.	Average annual temp
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2015	2.,8	5.3	7.2	12.4	19.8	21.4	26.7	24.9	20.1	13.8	8.8	3.0	5052.2	13.8
2016	1.4	9.4	9.5	15.5	16.9	23.5	25.5	24.2	19.1	13.4	7.2	1.3	5073.5	13.9
2004-2014	2.4	4.1	8.8	13.5	18.2	22.4	25.1	24.9	19.4	12.6	7.8	3.4	4927.5	13.5

Table 2. Amount of monthly precipitation in mm

Year	Month												Annual amount of precipitation in mm
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2015	50.4	81.4	83.0	16.6	16.1	40.1	6.6	65.6	95.0	102.9	54.4	0.0	612.1
2016	61.3	23.5	135.5	52.5	96.2	38.3	18.7	18.7	31.4	64.3	53.3	0.0	593.7
2004 - 2014	43.5	51.5	50.5	44.0	66.3	60.1	52.0	39.9	61.0	80.1	48.3	66.9	663.9

In terms of temperature, 2015 was the most suitable for growing cotton throughout the whole vegetation. The high rainfall in August and September (160.6mm), which is 59.7mm above the perennial average, contributed to the fact that many fruiting elements remained unresolved. The temperature amount for the same period was 1.5°C above the perennial average, which contributed to the formation of high yield.

In 2016, the low amount of precipitation (37.4mm) in the months of July and August (during flowering and boll formation), which is 91.8mm below the multiyear average, contributed to the formation and retaining of less fruiting elements.

The temperature in both years was within the average value or slightly below the ten-year average.

RESULTS AND DISCUSSION

Phenological observations

Phenological observations are presented in Table 3. The germination in both years of research was from May 23 to May 29. The phase of budding of all examined varieties occurred in the second half of June. The blooming began from July 17-25. The biggest cracking of bolls was in the third decade of September. The bolls from the lines *5136* and *5140* cracked first, which is five to nine days earlier than the Bulgarian varieties.

All examined varieties in the agro-ecological conditions in Strumica region belong to the group of medium early-matured varieties with a vegetation period of 123-130 days, while the lines belonging to the group of early-matured varieties had a vegetative period of 119-122 days.

Table 3. Phenological observations and interphase period in days and height of plants per years.

Genotypes	Date of				Interphase period			
	Germination	Butonization	Blooming	Cracing of bolls	Germination Butoniz.	Butoniz. blooming	Blooming cracking of bolls	Germination cracking of bolls
	2015							
5136	23.05	24.06	17.07	19.09	32	24	63	119
5140	23.05	24.06	17.07	20.09	32	23	65	120
5141	23.05	24.06	18.07	22.09	32	24	66	122
Chirpan-539	24.05	25.06	19.07	24.09	33	24	67	123
Veno	24.05	25.06	18.07	25.09	33	23	68	125
Perla-267	24.05	25.06	19.07	28.09	33	24	68	127
Avangard-264	23.05	25.06	20.07	29.09	34	24	70	129
Colorit-409	23.05	24.06	18.07	29.09	31	24	72	130
Helius-288	23.05	24.06	18.07	26.09	32	24	67	125
Natalia-361	23.05	25.06	19.07	25.09	33	24	68	126

2016								
5136	28.05	29.06	24.07	27.09	32	25	65	121
5140	28.05	29.06	24.07	29.09	32	25	66	123
5141	28.05	29.06	24.07	28.09	32	25	64	122
Chirpan-539	29.05	29.06	25.07	29.09	31	26	66	123
Veno	29.05	30.06	25.07	02.10	32	25	67	127
Perla-267	28.05	30.06	24.07	03.10	33	24	69	128
Avangard-264	29.05	29.06	25.07	05.10	31	26	66	129
Colorit-409	28.05	29.06	25.07	04.10	32	26	68	129
Helius-288	28.05	30.06	24.07	30.09	33	24	67	125
Natalia-361	28.05	29.06	25.07	04.10	32	26	65	129

The number of fruiting elements of a plant is given in Table 4. The examined varieties differed among themselves on both the total number of young bolls and the number of detained or not fallen bolls per plants. In domestic lines, the number of young bolls ranged an average of 14.2 in the line 5136 to 20.7 in line *5141*. In Bulgarian varieties, the number of younger bolls ranged from 20.2 in Helius-288 to 28.2 in Veno.

Table 4. Number of young bolls per plant per year.

Variety	Young bolls number	Untouched	
		Number	%
2015			
5136	14 .0	12.0	85.7
5140	19.5	17.0	87.2
5141	25.0	21.0	84.0
Chirpan-539	39.0	33.0	84.6
Veno	44.0	34.0	77.3
Perla-267	25.5	22.5	88.2
Avangard-264	40.5	35.0	86.4
Colorit-409	38.0	32.0	84.2
Helius-288	27.5	23.0	83.6
Natalia-361	30.5	28.5	93.4
2016			
5136	14.5	5.5	37.9
5140	16.0	10.0	62.5
5141	16.5	7.5	45.4
Chirpan-539	12.5	7.5	60.0
Veno	12.5	7.5	60.0
Perla-267	15.5	7.5	48.4
Avangard-264	11.5	6.5	56.5
Colorit-409	11.0	7.0	63.6
Helius-288	13.0	7.0	53.8
Natalia-361	11.5	7.0	60.8

Productive characteristics of the varieties

In 2015, the variety Veno showed the highest yield – 5150 kg/ha, and exceeded the standard variety Chirpan-539 by 54.6% (Table 5). Very high yields of 4369-5019 kg/ha or 31.2-50.7% above the standard were obtained from Macedonian lines 5140, 5141 and Bulgarian varieties Helius-288, Natalia-361 and Perla-267.

The highest weight of the bolls were found for the genotypes 5136 – (7.4g) and Colorit-409 (7.4g), followed by 5140 and 5141 which had the same weight (7.2g), by 0.3-0.5g over the standard. The longest fibre was found for the Bulgarian varieties Colorit-409 (28.2mm) and Natalia-361 (28.1mm), whilst the shortest fibre

was found for lines 5140 (25.4mm) and 5136 and 5141 (25.5mm). The results for the fibre length were approximately equal in research (Spasova et al., 2009, 2016). As for the fibre lint percentage the varieties Veno and Perla-267 had the highest values – 46.2-46.9%, followed by Chirpan and Helius – 44.9%.

In 2016 also the variety Veno showed the highest yield of 5167 kg/ha and exceeded the standard Chirpan-539 by 40%. Except line 5141, all other varieties had a lower yield than the standard. Veno variety and line 5141 had the biggest bolls - 7.4g and 7.2g. The other varieties had boll weight of 6.1-7.1g. The longest fibre was found for Colorit-409 (27.6mm) and Natalia-361 (27.5mm), followed by Veno and Avangard-264 (27mm), while the lines had a shorter fibre. The highest lint percentage was obtained for Chirpan-539 - 46% and Avangard-264 - 43.9%.

The highest yield of 5158 kg/ha for two years was found for the genotype Veno which exceeded the standard Chirpan-539 by 47.3%. High yields of 4573 kg/ha and 4238 kg/ha by 30.7% and 22.2% over Chirpan-539 were obtained from line 5141 and variety Perla-267. The lowest yield of 2853 kg/ha was found for the variety Colorit-409 which is 18.2% below the standard. The highest bolls weight was found for lines 5136 - 7.3g, 5140 and 5141 - 7.2g, the smallest for Chirpan-539 - 6.5g. The others genotypes had boll weight of 6.7-7.1g. Similar results were obtained from other authors (Stoilova et al., 2014a; Stoilova et al., 2014b).

The fibre lint percentage ranged from an average of 41.6% in the line 5140 to 45.4% in Chirpan-539. The length of the fiber ranged from 25.1mm in 5140 and 5141 to 27.9mm in Colorit-409 and 27.8 in Natalia-361. In research by other authors, similar length of fiber was obtained (Spasova et al., 2016). In the both years of the examination, the highest total seed cotton yield was realized in the line 5141 (2735kg/ha) and the lowest in genotype Avangard-264 (1737kg/ha).

The Macedonian lines had shorter fibres than the Bulgarian genotypes.

Table 5. Productive and quality properties of varieties by years.

Variety	Row cotton yield kg/ha	In % to Chirpan-539	Boll weight G	Fiber length mm	Lint percentage%	Seed kg/ha	Plant height cm
	2015						
5136	3912	117.4	7.4	25.5	42.0	2321	104.0
5140	4492	134.8	7.2	25.4	42.4	2643	110.5
5141	4576	137.4	7.2	25.5	43.0	2643	119.0
Chirpan -539	3331	100.0	6.9	26.6	44.9	1833	104.5
Veno	5150	154.6	6.4	27.6	46.2	2770	116.5
Perla - 267	5019	150.7	6.7	27.6	46.9	2607	99.5
Avangard - 264	3264	97.9	7.0	27.7	43.6	1857	106.5
Colorit - 409	3007	90.3	7.4	28.2	45.7	1619	111.0
Helius - 288	4369	131.2	6.9	27.4	44.9	2405	104.0
Natalia - 361	4509	135.4	6.7	28.1	43.6	2571	103.0
LSD 5%	0.24	31.9	1.3	2.1	3.8	0.69	4.2
LSD 1%	0.34	43.6	1.8	2.8	5.2	0.95	5.7
	2016						
5136	3542	95.9	7.1	25.1	42.8	2038	109.3
5140	2614	70.8	7.2	25.1	40.8	1562	102.3
5141	4571	123.9	7.1	25.0	42.2	2828	118.3
Chirpan -539	3690	100.0	6.1	25.9	46.0	2680	106.0
Veno	5167	140.0	7.4	27.0	41.4	2524	113.0
Perla - 267	3457	93.7	6.9	26.9	41.1	2043	113.3
Avangard - 264	2838	76.9	6.8	27.0	43.9	1617	103.7
Colorit - 409	2700	73.2	6.7	27.6	40.3	1905	105.0
Helius - 288	3619	98.1	7.1	26.7	42.7	2033	111.0
Natalia - 361	2455	66.5	6.6	27.5	40.9	1464	105.7
LSD 5%	47.0	21.9	0.9	2.6	2.0	19.8	24.5
LSD 1%	64.3	29.9	1.2	3.5	2.8	27.0	33.3

CONCLUDING REMARKS

All examined genotypes in the agro-ecological conditions of Strumica region belong to middle early-maturing varieties with a vegetation period of 123-130 days, while the lines belong to the group of early-matured varieties with a vegetative period of 119-122 days.

The highest yield of 5158 kg/ha average for two years was found for Veno variety. The lowest yield of 2853 kg/ha was obtained for Colorit-409.

The highest bolls weight was accounted in lines 5136 - 7.3g 5140 and 5141 - 7.2g, the smallest in Chirpan-539 - 6.5g. The others genotypes had boll weight of 6.7-7.1g. The highest lint percentage was found for varieties Chirpan-539 (45.4%) and Helius-288 (43.8%). Macedonian lines had lower lint percentage about 42-43%.

The longest fibre was found for the Bulgarian genotypes Colorit-409 (27.9mm) and Natalia-361 (27.8mm).

On average, in both years of research all genotypes showed lower lint percentage compared to the standard.

REFERENCES

Bourland, E., Beach, A., Kennedy, C., Martin, L., Rouse, A., &, Robertson B. (2015). Arkansas cotton variety test 2015. Arkansas agricultural Experiment station, University of Arkansas system division of agriculture.

Chen, Y., Liu, Z-H., Feng, L., Zheng, Y., Li, D-D, & Li, X-B (2013). Genome-wide functional analysis of cotton (Gossypium hirsutum). Hubei Key Laboratory of Genetic Regulation and Integrative Biology, College of Life Sciences, Central China Normal University, Wuhan, China.

Constable, G.A., & Bange, M.P. (2015). The yield potential of cotton (Gossypium hirsutum L). Department of agriculture and food (Pert Australia) Field crop research volume 182, 98-106.

Chaves, M.M. & Oliveira, M.M. (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of Experimental Botany, 55, 407, , 2365–2384.

Edwar, L. Lubbers, Peng, W., & Chee, (2009). The worldwide gene pool of G. hirsutum and its improvement. Genetics and genomics of cotton. University of Georgia.

Ganesan, S., Le Anne M.C., Lorraine P., Stipanovic, R.D. & Keerti S.R. (2006). Engineering cottonseed for use in human nutrition by tissue-specifi creduction of toxic gossypol. Institute for Plant Genomics and Biotechnology. National Academy of Sciences of the USA.

Jonathan F. Wendel & Richard C.C. (2003). Polyploidy and the evolutionary history of cotton. Department of Botany, Iowa State University, USA.

Rathore, KS., Campbell, LM., Sherwood, S., & Nunes, E. (2006). Cotton (Gossypium hirsutum L.). Methods of molecular biology. Institute for Plant Genomics & Biotechnology Texas A&M University College Station, USA.

Sunilkumar, G., Campbell, LM., Puckhaber, L., Stipanovic, R.D., & Rathore KS. (2006). Engineering cottonseed for use in human nutrition by tissue-specific reduction toxic gossypol. Institute for Plant Genomics and Biotechnology and Department of Soil and Crop Sciences, Texas.

Spasova, D., Spasov, D., Stoilova, A., Atanasova, B., Mihajlov, Lj., & Valkova, N. (2016). Application of cluster analysis for evaluation of new Bulgarian and Macedonian cotton varieties and lines. Bulgarian Journal of Agricultural Science, 22 (1). pp. 125-130.

Spasova, D., Mihajlov, L., Spasov, D., & Ilievski, M. (2010). Agroecological evaluation of new Bulgarian and Macedonian varieties of cotton. Goce Delcev University – Stip Faculty of Agriculture, Macedonia.

Spasova, D., Spasov, D., Kocevski, V., & Ilievski, M. (2001). Examination of some domestic and introduced varieties of cotton in the agroecological conditions of Strumica. Yearbook of Institute for Southern Crops, Vol. 1, 53-57.

Stoilova, A., Valkova, N., Hadzhiivanova, B., Koleva, M., & Nedyalkova S. (2014a). The cotton breeding in Bulgaria. Turkish Journal of Agricultural and Natural Sciences, Special Issue: 1, 992-999.

Stoilova, A., Valkova, N., Spasova, D., Spasov, D., & Mihaylov, L. (2014b). Agroecological assessment of new Bulgarian and Macedonian cotton varieties. Bulgarian Journal of Agricultural Science, 20 (2), 348-357.

КАРАКТЕРИЗАЦИЈА НА НЕКОИ ДОМАШНИ И ИНТРОДУИРАНИ СОРТИ ПАМУК ВО АГРОЕКОЛОШКИ УСЛОВИ НА СТРУМИЦА

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Резиме

Во периодот од 2015 до 2016 година во агроеколошките услови на Струмица беа изведени експерименти со 10 генотипови на памук (линиите *5136*, *5140*, *5141*, создадени во институтот во Струмица и бугарските сорти *чирпан*, *вено*, *перла 267*, *авангард 264*, *колорит 409*, *хелиус 288* и *наталија 361*), а целта беше да се проучат биолошките и стопанските карактеристики на памукот. Испитувањата се извршени во три повторувања по рандомизиран блок систем со големина на експерименталната парцела до 14 m^2. Сите испитувани сорти во агроеколошките услови во Струмица спаѓаат во средно раностасни сорти со вегетационен период од 125 до 130 дена, додека линиите спаѓаат во групата на ранозрели сорти со вегетационен период од 119 до 122 дена. Приносот на суров памук во годините на испитување се движи од 2853 kg/ha кај бугарската сорта *колорит*, до 5158 kg/ha кај сортата *вено*. Највисок рандман од домашите генотипови има линијата 5141 (42.6%), а од бугарските генотипови кај *чирпан-539* (45.4%) и *хелиус-288*, (43.8%).

Клучни зборови: *Gossypium hirsutum* L., *памук, принос, рандман, должина на влакно*

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EVALUATION OF TOTAL PHENOLS IN ALFALFA (*Medicago sativa* L.) COLLECTED FROM DIFFERENT LOCALITIES IN REPUBLIC OF MACEDONIA

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Abstract

Phenols are secondary biomolecules, which in their structure contain an aromatic ring attached to one or more substituents, such as flavonoids, which are one of the largest class plant phenols.

Phenolic compounds participate in biochemical processes that are important for the protection of plants against infections caused by fungi and viruses, mechanical damage, regulation of metabolism, and more. Phenolic compounds are oxidized by phenol oxidases to quinones, by releasing hydrogen and thus directly participating in the exchange of matter. The intensity of the biosynthesis of phenolic compounds increases after plant infection with pathogenic microorganisms. Therefore, the amount of total phenolic in plants can be used as biochemical parameter and indicator in the selection of plants for resistance to fungi, bacteria and viruses. Phenolic compounds are intensively synthesized in mechanical damage to plants, whereby they build protective film with active condensation in damaged areas. In this way they prevent cell wall degradation in infections caused by the necrotropic pathogens that destroy the cells in order to build their colonies on the dead tissues.

This paper presents the results of the research on the determination of the total phenols determined in three regions of the Republic of Macedonia in a total of 20 different locations. The content of the total phenols in the dry plant material of alfalfa in three mowings was determined by routine method in Folin-Ciocalteu (1927). The quantitative determination of total phenols in the plant ethanol extract was performed spectrophotometrically at a wavelength of 765nm.

Key words: *Lucern mowings, spectrophotometry, phenolic compounds, forage crop*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial fodder culture and is one of the many species of the *Medicago* genus. Of all species, the widespread production is common or blue alfalfa belonging to the family Fabaceae (Василиченко, 1949). It is one of the oldest cultivated species. It belongs to the oldest and most widely used forage crops in many parts of the world, but also in our country, but it is one of the most important forage crops.

Alfalfa, also called lucerne is characterized by high nutritional value (Julier et al., 2000), both in green mass, also in hay, silage, sage or alfalfa flour. Alfalfa is a fodder culture that ensures high yield and quality of protein foods (Dinic et al., 2005), which makes it one of the most important forage crops. The high yield of this crop comes from the ability of alfalfa to regenerate continuously through the year.

In addition to the nutritional components that make alfalfa useful as an animal feed or food supplement (Hatfield, 1992), the plants produce a variety of secondary metabolites showing biological activity. Many of these compounds help protect the plant against herbivores (Cambier et al., 2000; Awmack and Leather, 2002) and can influence the choise of food sources by insect herbivores (Shonle and Bergelson, 2000; Lankau, 2007; Mosleh et al., 2008).

In agricultural production, it has a huge influence and has a dominant role in the intensification of fodder production, due to the presence of vitally important vitamins, carbohydrates, mineral elements and other active components essential for the growth and development of plants that enable the ability for high productivity, quality and ability for strong regeneration (Hao et al., 2008).

Alfalfa has a powerful and developed root system, which improves the soil structure and deeper depths, but is also responsible for its resistance to drought on one side and very low temperatures on the other side (Markovic et al., 2007a). Through symbiotic nitrogenfixation with the bacterium *Rhisobium meliloti* var. *medicaginis* fixes large amounts of nitrogen from the air (Јакушкин, 1947); it reaches 300-400kg/ha per year of nitrogen, which corresponds to more than 60 tonnes of manure. In the soil significant amounts of phosphorus and potassium live. All this makes the alfalfa an excellent pre-culture for a range of important crops: cereal, industrial and horticultural.

In addition, alfalfa also contains phenolic compounds that have more significant functions, such as stimulating the growth and development of plants, protecting plants from pathogens, giving the color of flowers, and thus stimulate pollination, give the taste of green fruits and more.

Phenolic compounds are a group of chemical compounds that are widely distributed in nature. They are simple compounds present in most fresh fruits and vegetables, or complex compounds present in bark, roots and leaves of plants. A group of polyphenols, responsible for the color of many fruits, vegetables, and flowers, are known as anthocyanins. There are several important classes, of phenolic compounds. According to the basic skeleton, the structure of natural polyphenols varies from simple molecules, such as simple phenols (volatile phenols), to highly polymerized compounds, such as condensed tannins (Waterman and Mole, 1994).

Phenolic compounds as prevalent active secondary biomolecules that participate in various biochemical processes important for photosynthesis, regulation of metabolism and other, direct research mainly to confirming the quality of this culture as an excellent component in feeding stuffs.

MATERIALS AND METHODS

Plant material

The object of the examination was alfalfa (*Medicago sativa* L.), collected from three different regions on the territory of the Republic of Macedonia, Skopje region with coordinates (42.01^{o} N, 21.24^{o} E), Ovchepolski (41.45^{o} N, 22.11^{o} E) and Tetovo region (42.00^{o} N, 20.58^{o}E) from a total of 20 different locations, in three mowings. Table 1 describes the locations from the examined sites with their altitude (m), latitude (^{o}N) and longitude (^{o}E). The material is collected during the vegetative cycle of alfalfa. The experiments were carried out on dry plant material using modern quantitative methods.

Table 1. Description of the locations from the examined sites altitude (m) and latitude (˚N) and longitude (˚E).

Order number	Locations	Region	Altitude (m)	Latitude (°N)	Longitude (°E)
1.	Bogovinje	Tetovo	531.50	41.9236809	20.9168772
2.	Vrutok	Tetovo	682.41	41.7665300	20.8381550
3.	Dzepciste	Tetovo	474.48	42.0331690	21.0001650
4.	Galate	Tetovo	600.73	41.8381370	20.8813700
5.	Zelino	Tetovo	1605.94	41.9006530	21.1175770
6.	Pechkovo	Tetovo	991.87	41.7843700	20.8311530
7.	Jegunovce	Tetovo	658.34	42.1245655	21.0875064
8.	Avtokomanda	Skopje	246.68	42.0006868	21.4536642
9.	Sopiste	Skopje	1017.16	41.8638490	21.3083500
10.	Drachevo	Skopje	264.41	41.9352675	21.5098515
11.	Saraj	Skopje	424.88	42.0017493	21.2815977
12.	Radishani	Skopje	392.32	42.0732769	21.4479917
13.	Vlae	Skopje	256.07	42.0072938	21.3801924
14.	Glumovo	Skopje	274.74	41.9817742	21.3103747
15.	Dobroshane	Ovce Pole	302.06	42.1066200	21.7540130
16.	Cheshinovo	Ovce Pole	294.00	41.8735350	22.2905610
17.	Karbinci	Ovce Pole	342.98	41.7882100	22.2622460
18.	Obleshevo	Ovce Pole	297.63	41.8639320	22.2622460
19.	Lozovo	Ovce Pole	277.86	41.7806752	21.8995629
20.	Mustafino	Ovce Pole	289.18	41.8407190	22.0789350

The content of the total phenols in dry plant material from alfalfa was determined by the routine method in Folin-Ciocalteu (Singleton et al., 1965). The Folin-Ciocalteu reagent is most commonly used for the quantitative determination of the content of phenols and antioxidant compounds in plant extracts.

The quantitative determination of total phenols in the plant methanol extract was performed spectrophotometricaly at a wavelength of 765nm (Singleton et al., 1999), and the results are presented as mg GAE/g DW (mg Gallic acid equivalent/g dry weight).

Extraction method

The extraction of phenolic compounds from the plant material was carried out with 80% methanol (CH_3OH). Dry plant material (50mg) was macerated and then incubated. Then, the extracted plant extracts were centrifuged and the supernatant obtained was collected and used for quantitative analysis of the content of total phenolic compounds.

Method for determination of total phenols

For the quantitative determination of the content of the total phenolic compounds in the methanolic extracts, samples were taken, a series of standard solutions (in concentration rag from 0-0.4mg**.**ml^{-1}) against a blank test. The reaction mixture of the sample consists of 20μL of methanol extract, 80μL of 80% CH_3OH, 500μL diluted aqueous solution of Folin-Ciocalteu reagent (1:9) and 400μL of 0.7M Na_2CO_3. A series of standard solutions were prepared by diluting a gallic acid solution with a concentration of 0.4 mg**.** ml^{-1}. The blank was used for calibration of the spectrophotometer and contained 100μL of 80% CH_3OH, 500μL dilute aqueous solution of Folin-Ciocalteu reagent (1: 9) and 400μL of 0.7M Na_2CO_3. The prepared samples, the standard solutions and the blank were incubated on a temperature of 50^0C water bath for 5 minutes. Then, the samples were cooled to room temperature (15 minutes) and their absorbance was measured at a wavelength of 765nm. The results were expressed in mg GAE/g DW of plant material. The samples were prepared in triplicate for each analysis and the mean value of absorbance was calculated.

Statistical analysis

The data were statistically processed (XLSTAT 2014) with a one-way variance analysis (ANOVA), in order to determine the significant differences ($p < 0.05$) between the mean values of the samples. Subsequently, the results were Post hoc analyzed using Duncan's multiple ranking test. This test is used to determine statistically significant differences in the content of phenolic compounds within the three mowings.

RESULTS AND DISCUSSION

Content of total phenolic compounds at the first mowing

In this experiment, results of the studies for determining the total phenolic compounds have been presented. Phenolic compounds constitute a large class of plant secondary metabolites characterized by a high degree of structural heterogenity. Phenolic compounds such as 4-hydroxybenzoic, salicylic, r-coumarinic, gallic acid as well as some other phenolic compounds have a certain effect on the growth of plants. It has been found that the plant phenol-linullaric acid acts as a phytohormone and can replace the abscissic acid (Cvetanovska, 2016).

Form the results presented in Figure 1 and Table 2 can be seen that the significantly increased content of phenolic compounds was determined in the following regions and their localities: Tetovo (Dzepciste), Skopje (Glumovo), Ovchepole region (Lozovo) and the smallest content was measured in the Skopje region (Drachevo).

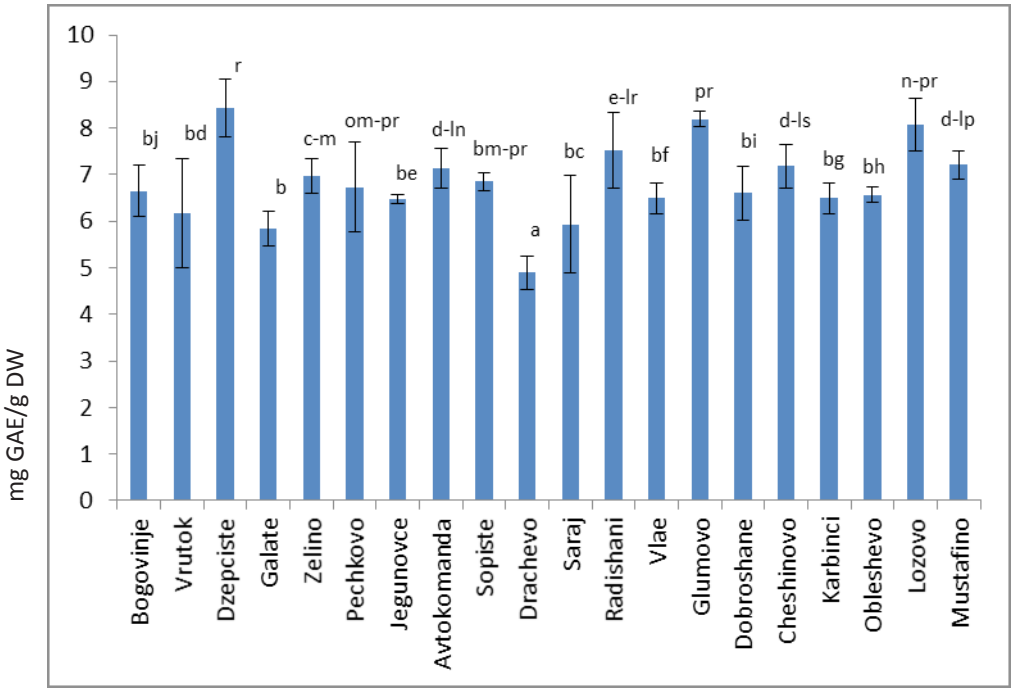

Figure 1. Content of total phenolic compounds in alfalfa (*Medicago sativa* L.), at certain localities on the territory of the Republic of Macedonia in the first mowing, expressed in mg GAE/g DW.

* If the small letters that are above of each column are the same there in no statistical difference, and if they are different there is a statistically significant differences.

Table 2. Content of total phenolic compounds in alfalfa (*Medicago sativa* L.), at certain localities on the territory of the Republic of Macedonia in the first mowing, expressed in mg GAE/g DW.

No.	Locations	First mowing mg GAE/ g DW	Standard deviation
1.	Bogovinje	6.65 bj	± 0.54
2.	Vrutok	6.17 bd	± 1.18
3.	Dzepciste	8.42 r	± 0.62
4.	Galate	5.84 b	± 0.36
5.	Zelino	6.96 c-m	± 0.37
6.	Pechkovo	6.73 om-pr	± 0.97
7.	Jegunovce	6.47 be	± 0.10
8.	Avtokomanda	7.14 d-ln	± 0.43
9.	Sopiste	6.85 bmpr	± 0.18
10.	Drachevo	4.89 a	± 0.37
11.	Saraj	5.93 bc	± 1.05
12.	Radishani	7.52 e-lr	± 0.81
13.	Vlae	6.49 b-p	± 0.33
14.	Glumovo	8.19 pr	± 0.17
15.	Dobroshane	6.6 bi	± 0.59
16.	Cheshinovo	7.18 d-ls	± 0.47
17.	Karbinci	6.49 bg	± 0.33
18.	Obleshevo	6.56 bh	± 0.17
19.	Lozovo	8.07 n-pr	± 0.57
20.	Mustafino	7.21 dlp	± 0.31

The intensity of the biosynthesis of phenolic compounds increases after infection of plants with pathogenic microorganisms. Therefore, the amount of certain phenolic compounds in the plant can be used as a biochemical parameter in the selection of plants resistant to fungi, bacteria and viruses.

Phenolic compounds are intensively synthesized and in mechanical damage to plants and damaged areas they build a protective film (with active condensation).

Content of total phenolic compounds at the second mowing

The content of the total phenolic compounds in alfalfa (*Medicago sativa* L.), collected from different localities of the territory of the Republic of Macedonia in the second mowing is presented in Figure 2 and Table 3.

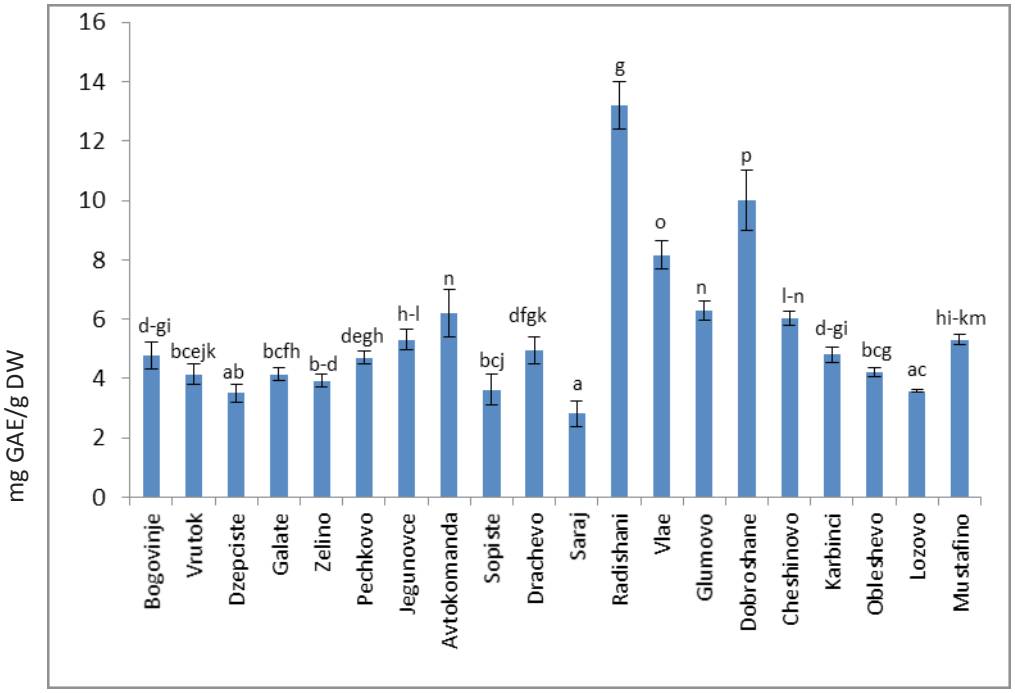

Figure 2. Content of total phenolic compounds in alfalfa (*Medicago sativa* L.), at certain localities on the territory of the Republic of Macedonia in the second mowing, expressed in mg GAE/g DW.
* If the small letters that are above of each column are the same there in no statistical difference, and if they are different there is a statistically significant differences.

Table 3. Content of total phenolic compounds in alfalfa (*Medicago sativa* L.), at certain localities on the territory of the Republic of Macedonia in the second mowing, expressed in mg GAE/g DW.

No.	Locations	Second mowing mg GAE/ g DW	Standard deviation
1.	Bogovinje	4.77 d-gi	± 0.46
2.	Vrutok	4.14 bcejk	± 0.34
3.	Dzepciste	3.51 ab	± 0.30
4.	Galate	4.14 bcfh	± 0.22
5.	Zelino	3.93 b-d	± 0.22
6.	Pechkovo	4.71 degh	± 0.21
7.	Jegunovce	5.31 h-l	± 0.34
8.	Avtokomanda	6.2 n	± 0.79
9.	Sopiste	3.63 bcj	± 0.53
10.	Drachevo	4.95 dfgk	± 0.46
11.	Saraj	2.82 a	± 0.43
12.	Radishani	13.21 q	± 0.80
13.	Vlae	8.16 o	± 0.46
14.	Glumovo	6.29 n	± 0.32
15.	Dobroshane	10.01 p	± 1.02
16.	Cheshinovo	6.04 l-n	± 0.23
17.	Karbinci	4.8 d-gi	± 0.25
18.	Obleshevo	4.21 bcg	± 0.16
19.	Lozovo	3.57 ac	± 0.04
20.	Mustafino	5.31 hi-km	± 0.16

The results show that the highest content of total phenolic compounds was found in the Skopje (Radishani) and the Ovche Pole region (Dobroshane), while the smallest quantity of measured phenolic compounds content was measured in the Skopje region (Saraj). The statistical analysis shows a significant difference between the amount of total phenols between the locations Radishani and Saraj ($p < 0.05$).

This data points to the fact that certain factors contributed to increased production of phenolic compounds in alfalfa grown in the Skopje region. As possible factors for increasing the total content of total phenols can be the following: altitude, external stress factors, climatic factors, etc.

The results of the authors, who analyzed the quantitative and qualitative phytochemical characteristics of plants, show different values. (Bystricka et al., 2010), reported that concentration and dynamics of the polyphenol synthesis in plant depends on the plant species, type of organs and growth stage. The results of some authors, who have comparatively analyzed the concentration of phenolic compounds in plant parts, support the fact that the highest concentration of phenolic compounds was found in leaves.

Content of total phenolic compounds at the third mowing

The obtained results, shown in Figure 3 and Table 4 are showing that there is a significant difference ($p<0.05$) between the Skopje region (Sopiste) and the Tetovo region (Vrutok) materials, taken in the third mowing.

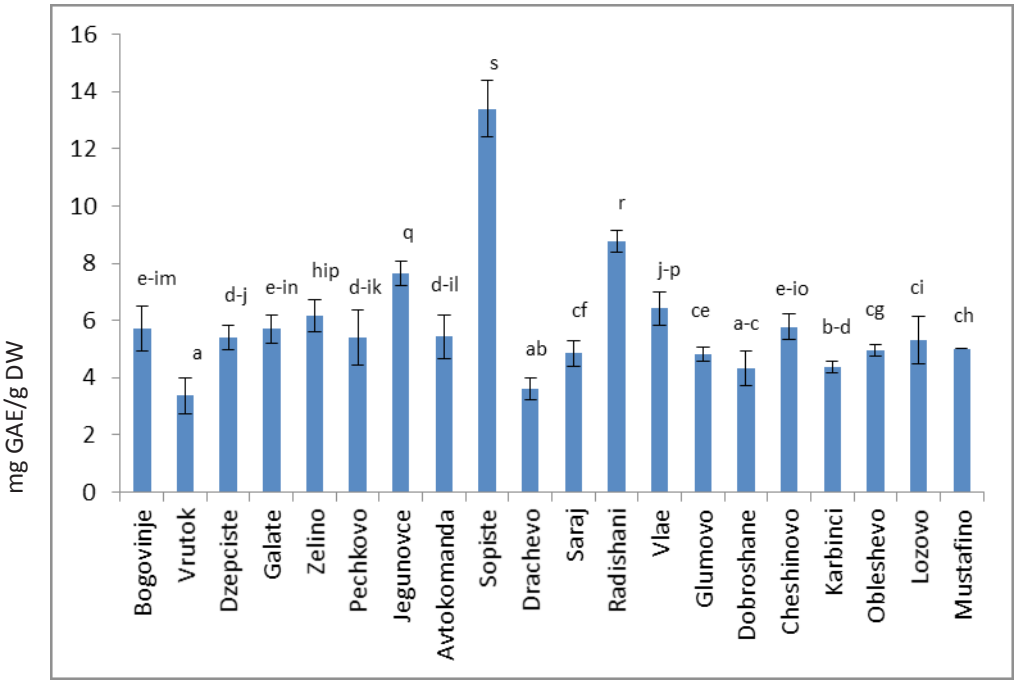

Figure 3. Content of total phenolic compounds in alfalfa (*Medicago sativa* L.), at certain localities on the territory of the Republic of Macedonia in the third mowing, expressed in mg GAE/g DW.

* IF the small letters that are above of each column are the same there in no statistical difference, and if there are different there is a statistically significant differences.

Table 4. Content of total phenolic compounds in alfalfa (*Medicago sativa* L.), at certain localities on the territory of the Republic of Macedonia in the second mowing, expressed in mg GAE/g DW.

No.	Locations	Third mowing mg GAE/ g DW	Standard deviation
1.	Bogovinje	5.7 e-im	± 0.78
2.	Vrutok	3.37 a	± 0.62
3.	Dzepciste	5.4 d-j	± 0.44
4.	Galate	5.71 e-in	± 0.49
5.	Zelino	6.15 hip	± 0.56
6.	Pechkovo	5.41 d-ik	± 0.97
7.	Jegunovce	7.64 q	± 0.43
8.	Avtokomanda	5.43 d-il	± 0.76
9.	Sopiste	13.4 s	± 0.99
10.	Drachevo	3.61 ab	± 0.38
11.	Saraj	4.85 cf	± 0.46
12.	Radishani	8.76 r	± 0.39
13.	Vlae	6.42 j-p	± 0.57
14.	Glumovo	4.8 ce	± 0.25
15.	Dobroshane	4.31 a-c	± 0.60
16.	Cheshinovo	5.78 e-io	± 0.45
17.	Karbinci	4.38 b-d	± 0.21
18.	Obleshevo	4.96 cg	± 0.20
19.	Lozovo	5.30 ci	± 0.83
20.	Mustafino	5.00 ch	± 0.01

The content of the phenolic compounds varies depending on the external conditions. Frequently there is a change in the content of the phenols, whose content is variable, depending on the eco-physiological conditions of the environment. The content may be increased even when it is necessary to absorb harmful ultraviolet radiation or when reducing the growth of surrounding competing plants (Koleva-Gudeva, 2010).

CONCLUDING REMERKS

Phenolic compounds are a large group of the secondary metabolites widespread in the plant kingdom. They are categorized into classes depending on their structure and subcategorized within each class according to the number and position of hydroxyl group and the presence of other substituents. The antioxidant properties of phenolics are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Commonly, the in vitro methods often do not correlate with the ability of phenolic compounds to inhibit oxidative deterioration of foods. Phenolic compounds are counted in such compounds that represent specificity in plant cells (because they contain enzymes responsible for their biosynthesis). Depending on their structure and degree of polymerization, they participate in various biochemical processes that are important for photosynthesis, plant protection against fungi and viruses, mechanical damage, regulation of metabolism, etc. Phenolic compounds are used in plant production in the selection of plants resistant to the action of oxygen radicals and pathogens. In the first mowing, significantly increased content of phenolic compounds in the three regions in certain sites, Skopje, Tetovo and Ovche Pole is confirmed.

In the second mowing, the phenomenon of significance was observed between location Radishani and Saraj ($p < 0.05$). Significant difference regarding the content of total phenolic compounds was observed between the Skopje region at the Sopishte location in

the third mowing and the Tetovo region at the location Vrutok ($p < 0.05$). Within the three mowings, a significant increase in the content of the phenolic compounds has been determined in the first mowing in certain location in all three regions, in the second and the third mowings we have a significant difference between certain locations in the given regions.

The alfalfa in agricultural production has a huge impact and today it is the most important multiannual fodder culture in the intensification of fodder production, which is due primarily to the high productivity, quality and ability for strong regeneration. The listed properties of alfalfa not only give economic but also very high ecological significance, and it has been increasingly applied in sustainable organic production, bio nutritionism and dietetics.

REFERENCES

Amorati R; & Valgimigli, L. (2012). Modulation of the antioxidant activity of phenols by noncovalent interactions. Org Biomol Chem. 10 (21), 4147-58

Awmack CS, and Leather Sr. (2002). Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology 47, 817–844

Bystricka J., Vollmannova A., Margitanova E., & Cicova I. (2010). Acta Agric. Slov., 95(3), 225-229

Cambier V, Hance T, & Hoffmann E. (2000). Variation of DIMBOA and related compounds content in relation to the age and plant organ in maize. Phytochemistry 53, 223–229.

Cvetanovska, L. (2016). Biohemija na rastenija. Univerzitet "Sv. Kiril i Metodij" – Skopje, Prirodno matematicki fakultet, Skopje.

Dinic B., Djordjevic N., Radovic J., & Ignjatovic S. (2005). Modern procedures in tehnology of conserving Lucerne in ensiling. Biotechnology in Animal Husbendry, 21(5-6), 297-303

Hao, C-c., Wang, L-j., Dong, L., Ozkan, N., Wang, D-c & Mao, Z-h. (2008). Influence of alfalfa powder concentration and granularity on rheological properties of alfalfa – wheat dough. Journal of Food Engineering, 89, 137-41.

Hatfield R.D. (1992). Carbohydrate composition of alfalfa cell walls isolated from stem sections differing in maturity. Journal of Agriculture and Food Chemistry. 40, 424-430.

Jakушкин И.В. 1947. Растениеводство. Огиз. Селхозгиз. Москва.

Julier, B., Huyghe, C.; Ecalle, C., 2000. Within- and among-cultivar genetic variation in alfalfa: forage quality, morphology and yield. Crop Science, 40 (2): 365-369

Khoddami A. (2013). Techniques for analysis of plant phenolic compounds. Molecules, 18(2): 2328-75

Koleva-Gudeva, L. (2010). Plant physiology. Faculty of agriculture, Goce Delcev University- Stip.

Lankau Ra. (2007). Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytologist, 175:176–184.

Markovic J., Radovic J., Lugic Z., & Sokolovic D. (2007). The effect of development stage on chemical composition of alfalfa leaf and steam. Biotechnology in Animal Husbendry, 23, 5-6, 383-388.

Mosleh Arany, A., De Jong, T.J., Van Dam, N.M., Choi, Y.H., Verpoorte, R., & Van Der Meijden, E. (2008). Glucosinolates and other metabolites in the leaves of Arabidopsis thaliana from natural populations and their effects on a generalist and a specialist herbivore. Chemoecology, 18, 65–71.

Robbins, Rebecca J. (2003). Phenolic acids in foods: An overview of analytical methodology. Journal of Agricultural and Food Chemistry, 51(10), 2866-2887.

Singleton, V.L. & Rossi, J.A. Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic, 16,144-158

Singleton, V.L., Orthofer, R., & Lamuela – Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299, 152-178

Shonle, I., & Bergelson, J. (2000). Evolutionary ecology of the tropane alkaloids of Datura stramonium L. (Solanaceae). Evolution, 54, 778–788

Василченко И.Т. (1949). Љуцерка. Лучшее кормовое растение. Труди Ботанического Института. СССР.

Waterman, P.G. & Mole, S. (1994). Analysis of Phenolic Plant Metabolites. Blackwell Scienctific Publications, Oxford.

ОДРЕДУВАЊЕ НА ВКУПНИ ФЕНОЛИ ВО ЛУЦЕРКА (*Medicagosativa* L.) КОЛЕКЦИОНИРАНА ОД РАЗЛИЧНИ ЛОКАЛИТЕТИ ВО РЕПУБЛИКА МАКЕДОНИЈА

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Резиме

Фенолните се секундарни биомолекули кои во својата структура содржат ароматичен прстен со еден или повеќе супституенти, а растенијата во својот секундарен метаболизам ги синтетизираат овие соединенија кои содржат една или повеќе фенолнни групи.

Фенолните соединенија учествуваат во биохемиски процеси кои се значајни за заштитата на растенијата од инфекции предизвикани од габи и вируси, механички оштетувања, регулација на метаболизмот и друго. Фенолните соединенија се оксидираат со фенол-оксидази до кинони, при што ослободуваат водород и така директно учествуваат во размената на материјата. Интензитетот на биосинтезата на фенолните соединенија се зголемува по инфекција на растенијата со патогени микроорганизми. Заради тоа и количината на вкупните фенолни во растенијата може да се користи како биохемиски параметар и индикатор во селекцијата на растенијата за отпорност на габи, бактерии и вируси. Фенолните соединенија интензивно се синтетизираат и при механичките повреди кај растенијата, при што на оштетените места градат заштитен филм со активна кондензација. На тој начин ја спречуваат деградацијата на клеточните ѕидови при инфекции предизвикани од некротрофните патогени, кои ги уништуваат клетките за да на изумрените ткива изградат свои колонии.

Во овој труд се презентирани резултати од истражувањата за одредувањето на вкупните феноли одредувани на три региони на Република Македонија на вкупно 20 различни локации. Содржината на вкупните феноли во сув растителен материјал од луцерка во три откоси е одредувана со рутинска метода по Folin-Chioclateau (1927). Квантитативното одредување на вкупните феноли во растителниот етанолен екстракт е вршено спектрофотометриски на бранова должина од 765 nm.

Клучни зборови: *секундарни биомолеули, спектрофотометрија, фенолни соедининија*

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PECULIARITIES OF THE HORSE MEAT AGING

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Abstract

Over the last decade the horse meat has gone deeper into the field of vision of both consumers and scientists. The objective of this study is to identify the specific features during aging of the horse meat. The changes in microstructure, morphology, protein autolysis, soluble proteins, pH, WHC, drip loss and colour were studied in horse m. Longissimus dorsi during 12 days of wet aging at 0 - 4°C. At 3 d post mortem the A- and I-zones were more difficult to distinguish. Some shortening of the sarcomere was observed. The rigor mortis period in the horse meat occurs between day 3 and day 5. Within this period the muscle fibres were contracted, the red colour component was decreased by 2 - 3 units, the pH and the WHC were minimal - 5.80 and 13 - 14%, respectively, and the drip losses were maximum about 20%. In intra-cellular spaces released free water was found. After 5 d post mortem single cracks and strains were observed - an indicator of the ongoing autolytic processes. The solubility of the proteins is stabilized at about 1.750 mg/ml. An increased share of protein fractions with a molecular weight of 28 - 23 KDa, considered as an indicator of increased meat fragility, was found after 5 days. From 5 to 12 day, higher levels of α-actinin, desmin and light meromyosin were found. After 7 d of post mortem the destructive changes were deepening. Z-lines were very much torn. A- and I-discs were difficult to distinguish. Myofibrils were highly fragmented and I-zones were not distinguishable.

Key words: *m. Longissimus dorsi,* microstructure, morphology, protein autolysis, soluble proteins, drip loss, color

INTRODUCTION

Post mortem changes, leading to the transformation of muscle tissue into meat, have been in the spotlight of scientists since the middle of the twentieth century (Fujimaki & Arakawa, 1958; Hultin, 1984). Longo et al. (2015) confirm the hypothesis that at the centre of this transformation is the process of apoptosis. Post-mortem chemical changes in muscle are associated with the aging of the meat (Davey, 1983) and its tenderness (Lian et al., 2013). Proteolysis processes are referred to as primarily responsible for the aging and maturation of meat (Geay et al., 2001).

In the literature publications discussing the problems of aging meat from 3 to 10 year old horses (Litwinczuk et al., 2008) and foals (Ruiz et al., 2018) and accelerated aging of horse meat by marinating with solutions of calcium chloride can be found (Lourdes Perez et al., 1998). There were no studies on aging of two years old horses bred in the Balkans. This is why we have set ourselves the aim of this study being to identify the specific features in the process of the horse meat aging.

MATERIALS AND METHODS

Horse meat

The objective of the study was horse m. Longissimus dorsi. The meat was supplied by Unitemp Ltd., village of Voyvodinovo, district of Plovdiv. An average sample was taken from 21 horses. The 42 muscles were obtained, packed in plastic containers in air and wet-aged for a period of 12 days at 0 - 4°C. The samples of m. Longissimus dorsi (from the area between 13th and 18th thoracic vertebra about 500 g for each sample) were taken for analysis.

Part of those muscle particles was used for a morphological, microstructural and colour analysis, determination of water holding capacity (WHC) and pH. The meat samples for protein autolysis and pH were mixed and minced in a grinder with a hole diameter 4 mm. The mince meat was homogenized in a homogenizer Knife Mill GRINDOMIX GM 300 (AZoNetwork UK Ltd., Manchester, UK). The homogenized samples were vacuum-packed in table-top vacuum packers LYNX 32 (INTRAMA Group, Dobrich, Bulgaria) and stored for 8h at 0-4°C up to the moment of analysis.

The changes in microstructure, morphology, protein autolysis, soluble proteins, pH, WHC, drip loss and colour were studied.

Light microscopy

For morphological analysis samples of 2 x 1 x 1 cm were used. The fixing and the contrast of the samples were carried out according to the method described by Barbut et al. (2005).

Transmission electron microscopy

The preparation of the samples and the transmission electron microscopy were conducted in accordance with the method of Lawrence et al. (2002).

SDS-PAGE electrophoresis of myofibrillar proteins

SDS-PAGE electrophoresis of the myofibrillar extracted proteins was carried out under the Laemmli method (1970).

Free amino nitrogen

The amount of α-free amino groups in the myofibrillar extracted proteins was established as described in Analalytica - EBC (Welten, 2013).

Soluble proteins extracted from myofibrils

Extraction of myofibrillar proteins was performed with PBS buffer (ionic strength 0.55) using Khan describtion (1962). The protein concentration of the extract was determined by Lowry et al. (1951) method.

Water holding capacity of meat

The water holding capacity of meat was determined by the Grau & Hamm method (Modzelewska-Kapitula and Cierach, 2009).

pH value

The pH of the samples was measured electropotentiometrically by the Korceala et al. (1986).

The meat colour characteristics

The colour characteristics of the horse meat samples were determined spectrophotometrically with the CIELab system (Brewer and Wu, 1993).

Statistical analysis

The statistical analysis was made using the method of Draper and Smith (1998). Differences between values below $p \leq 0.05$ were considered statistically significant. All statistical procedures were performed using software Microsoft Excel 5.0.

RESULTS AND DISCUSSION

Morphological changes

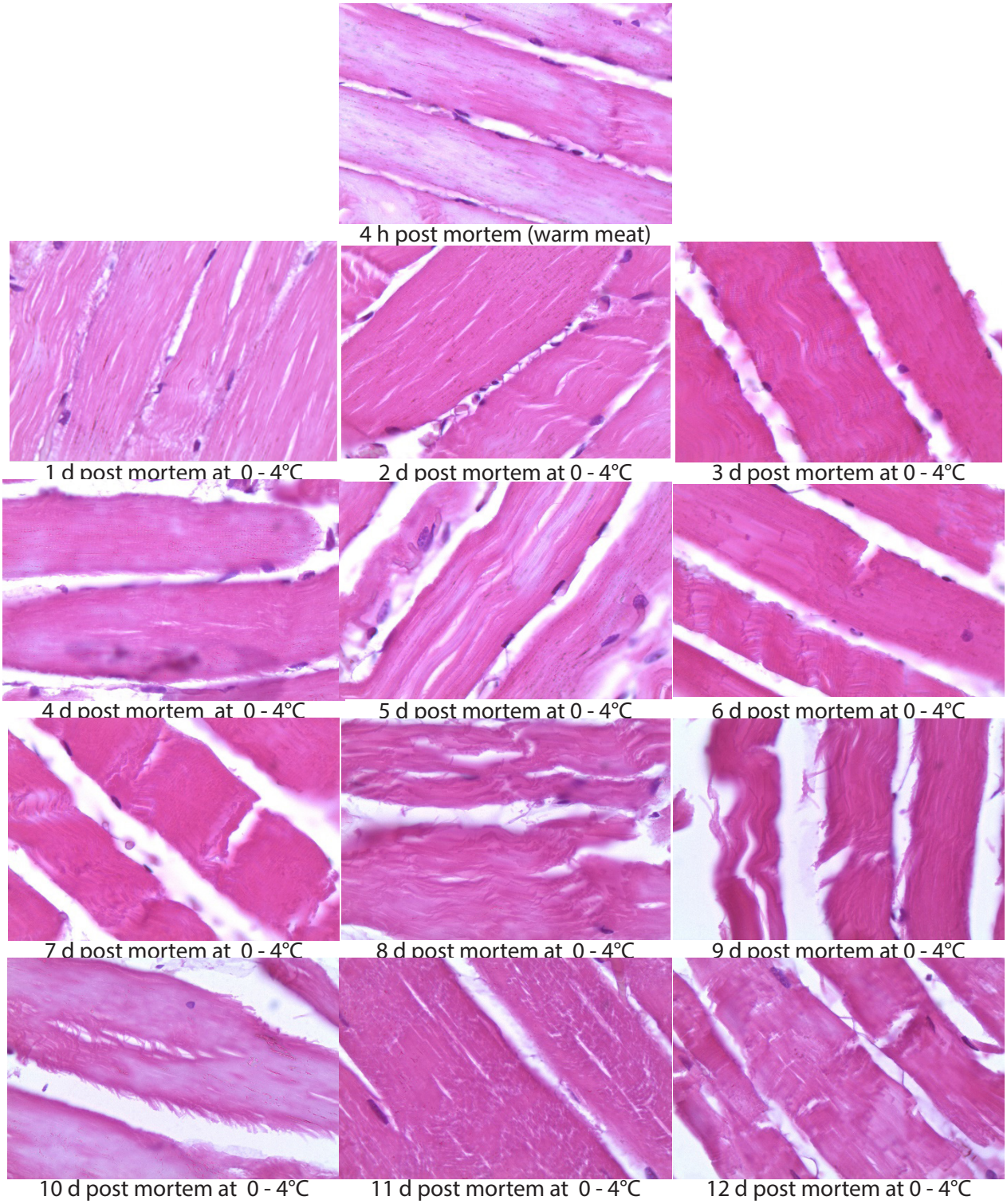

Figure1. Morphological changes of horse *m. Longissimus dorsi* during a period of 12 days of storage at 0 - 4°C. Horse fillets longitudinal cut contrasted with hematoxylin, 1000x.

In Figure 1 are presented changes of the morphology in horse muscle tissue samples during a period of 12 days of storage at 0 - 4°C. Up to the 3 days of cold storage in muscle and connective tissues there were not detected any significant destructive changes. Muscle fibres had a loose structure and preserved their integrity. They were tightly attached to one another. With the onset of rigor mortis the muscle fibres were contrasted. Exudate was excreted in the intercellular spaces. In the initial stages of autolysis (4 days) the muscle fibres recover their loose structure. Some unique cracks and strains appear. This is an indication of the ongoing autolysis changes up to 5 days of refrigeration. Some single cracks and feathering were found. These changes are an indication of ongoing autolytic changes till the 5 days of cold storage. From 6 days to 12 days of the horse meat refrigeration at 0 - 4°C the destructive changes in muscle tissue were getting worse. Larger cross cracks were observed. There was also observed a partial decomposition of protofibrils (Fig. 1).

Changes of electron microscopic determined muscle structure

Figure 2 shows the changes in the microstructure of horse meat samples (m., *Longissimus dorsi*) for a period of 12 days of refrigerated storage at 0 - 4°C.

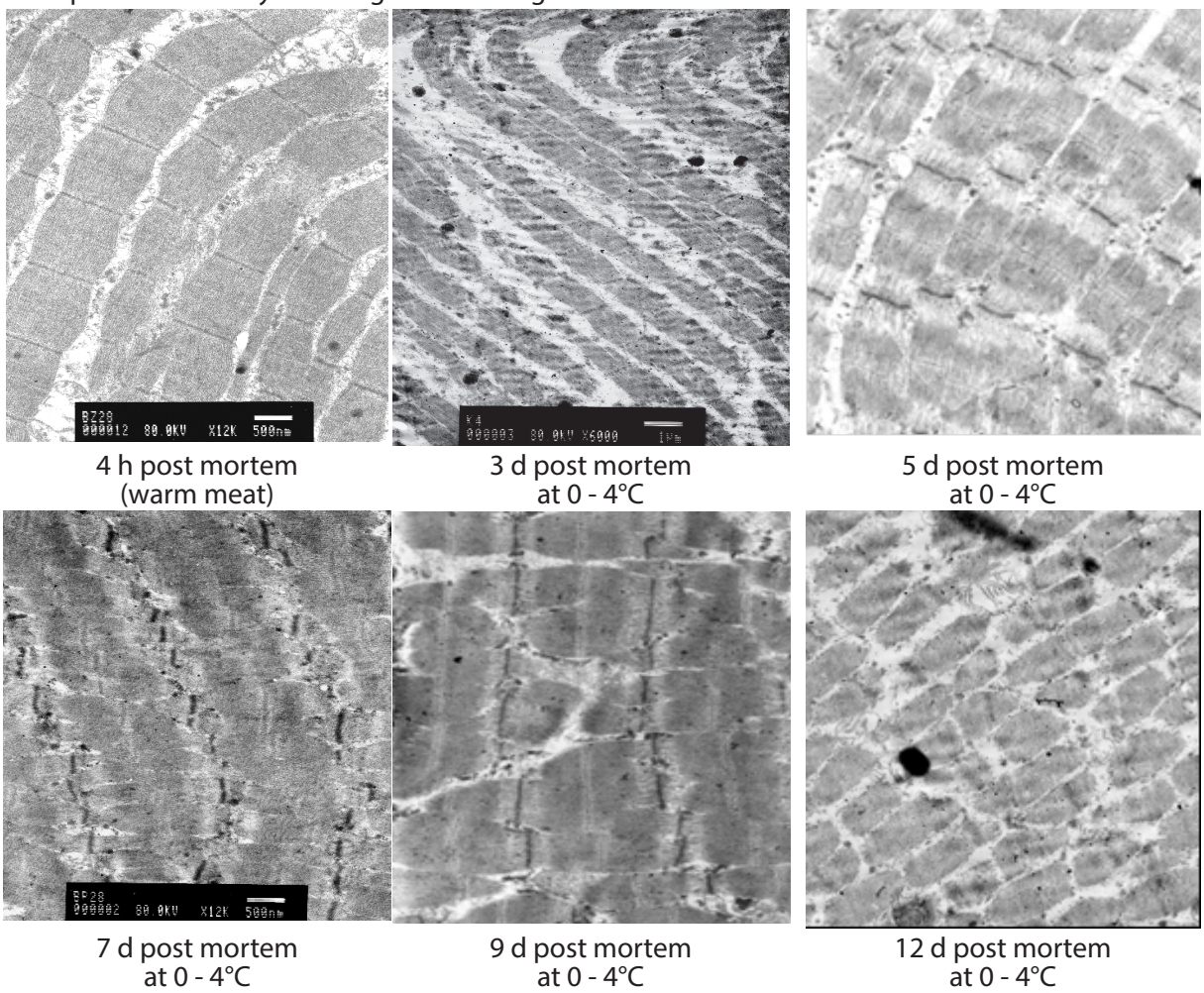

Figure 2. Electron microscopic preparations from longitudinal cuts of horse *m. Longissimus dorsi*, 12000x.

After 4 h post mortem individual sarcomers were clearly identifiable and could retain integrity. A- and I-zones were distinguishable. H-zones and M-lines were clearly visible, no Z-lines were observed. After 3 days post mortem storage A- and I-zones were distinguished with more difficulty. There was established some shortening of the sarcometers. After rigor mortis (6 days) a loosening of myofibrils and a partial recovery of their natural structure was observed. The A- and I-disks were still clearly visible, the Z-lines were preserved. Some changes typical of the meat maturation were noticed. Cross-cracks in the Z-lines and the myofibrils were spotted. After 9 days post mortem the destructive changes in the myofibrils were getting worse. The Z-lines were heavily torn; the A- and I-discs were very hard to distinguish. The myofibrils were highly fragmented, and the I-zones were virtually indistinguishable.

Results from SDS-Page gel electrophoresis

SDS-PAGE electrophoresis during aging of horse meat (0-4 °C) showed that the heavy meromymisin chains(200 KDa) were identified up to 48 hours post mortem. At the 4-day of storage (0-4 °C), the α-actinin content (95 KDa) was minimal. With increasing the aging time (after 5 days of storage at 0-4 °C) due to ongoing proteolytic changes, the protein fractions with a molecular weight of 28-23 KDa, considered as an indicator of tenderness (Huff-Lonergan, 1999) increase. After the 5 day of refrigerated storage at 0 - 4 °C to the end of studied period (12 days) an increase in the α-actinin fraction, desmine and light meromyosin was observed.'

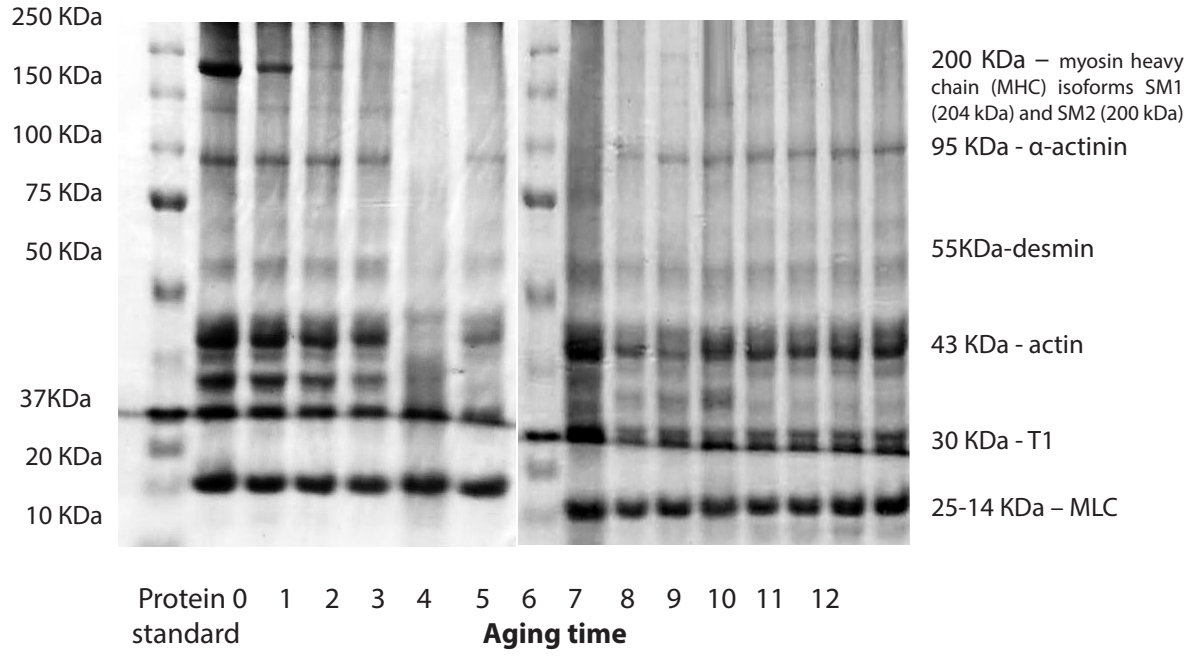

Figure 3. SDS-Page gel electrophoresis of horse *m. Longissimus dorsi* during 12 days of aging at 0 – 4°C

Free amino nitrogen

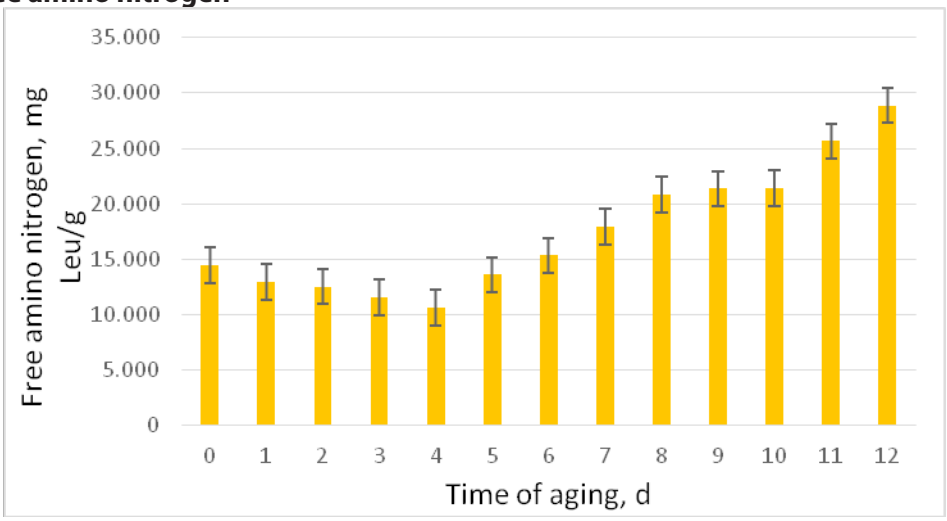

Figure 4. Changes of free amino nitrogen in horse *m. Longissimus dorsi* during 12 days of aging at 0 – 4°C.

During the first 5 days of the horse meat storage at 0 - 4°C (Fig. 4) there were no statistically significant ($p > 0.05$) changes in the content of free amine nitrogen (FAN). From 6 day to 8 day of the experiment due to proteolysis processes (Geay et al., 2001) the amount of free amine nitrogen in the meat increased by 46.7% ($p \leq 0.05$).

From 8 day to the end of the study period - 12 days (0 - 4°C) a reverse trend was found, namely: the FAN content of the horse meat was reduced by 35% (Fig. 4, $p < 0.05$). The decrease in FAN in the final stages of sample storage (0 - 4°C) corresponds well with the reduction of the solubility of proteins during the same study period (Fig. 4).

Soluble proteins

Immediately after obtaining (4 h post mortem) horse meat was characterized by a relatively high pH (pH = 6.75, Tab. 1) and water holding capacity (WHC) (Fig. 6). For the next 3 d (0 - 4°C) the solubility of the protein fraction was decreased by 5.7% ($p \leq 0.05$, Fig. 5). The results obtained correspond to the pH and WHC data (Tab. 1 and Fig. 6) and are indirect evidence of a rigor mortis. After 3 days autolysis was detected under the effect of endogenous tissue enzymes and a number of biochemical transformations (Hultin, 1984). As a result, after 6 days the solubility of the meat proteins increased significantly ($p \leq 0.05$). From 6 day to the end of the study period (12 d, 0 - 4°C) a reverse trend was established.

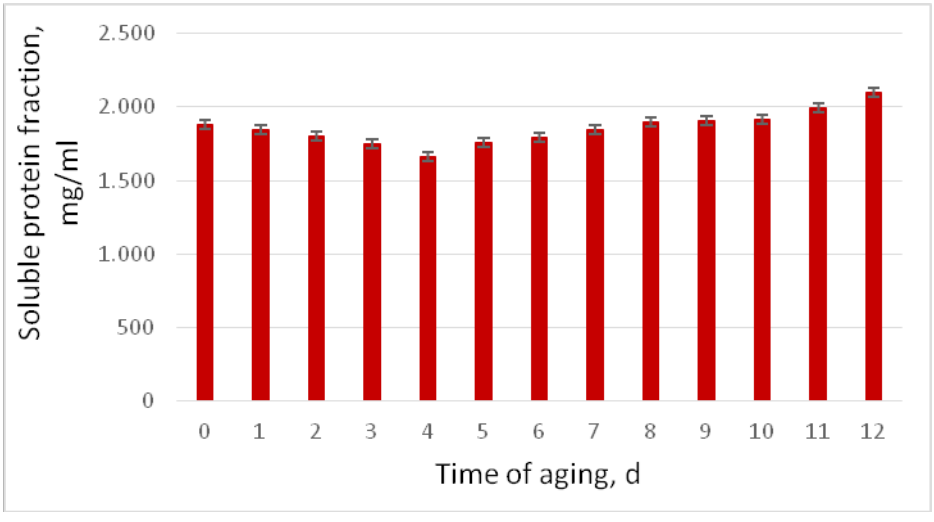

Figure 5. Changes of the soluble protein fraction extracted from horse *m. Longissimus dorsi* for a period of 12 days of aging at 0 – 4°C.

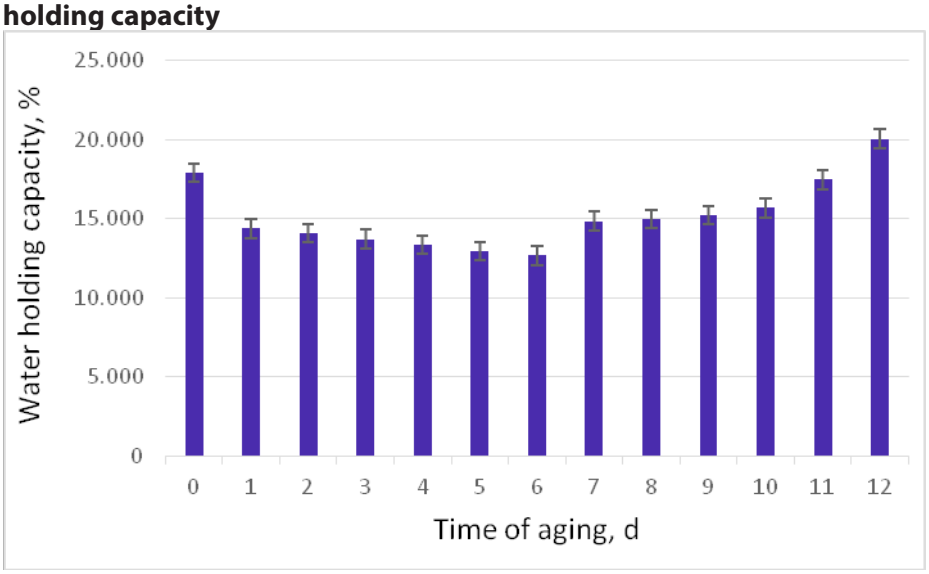

Figure 6. Changes of water holding capacity of horse *m. Longissimus dorsi* during 12 days of aging at 0 – 4°C.

The identified changes in water holding capacity (WHC) of the horsemeat samples (Fig. 6) correspond to the data obtained for the pH (Tab. 1). From 1 day to 6 day of the experiment, the WHC decreased by 45.8% (Fig. 6, $p \leq 0.05$). From 6 day to 12 day of the storage of samples of horse meat (0 - 4°C) the WHC increased by 7.5% ($p < 0.05$) (Fig. 6).

pH value

Immediately after slaughter (4h post mortem) the horse meat was characterized by a normal pH of the order of 6.75 (Tab. 1). A statistically significant decrease of pH of 14.07% ($p \leq 0.05$) was found on the 4 day of the horse meat storage at 0 - 4°C. These results are indirect evidence of a rigor mortis stage. Similar results for the pH at 45 min, 24 and 48 h post mortem in the lumbar segment of Longissimus dorsi muscle (Longissimus dorsi) and m. Semitendinosus are found by Litwinczuk et al. (2008). Unlike our study Lourdes Perez et al. (1998) and Litwinczuk et al. (2008) found a 8.39% lower pH at 24 h post mortem. This is probably due to the fact that the samples in the studies were taken from horses considerably older and probably in purer health.

From 4 days to 8 days of the refrigeration storage the rigor mortis progressively passed and the pH of the horse meat increased by 8.62% ($p \leq 0.05$) (Tab. 1).

From 8 days to 12 days at the end of the cold storage period of the horse meat at 0 - 4°C the pH again decreases and reaches acidic values commensurate with those in the post-mortem state (Tab. 1). The resulting decrease in pH at the end of meat storage (0 - 4°C) is probably due to the development of lactic acid microflora. Contrary to our data Seong et al. (2014) found a constant increase in the pH of vacuum packed and 30 days aged at 4°C m. Longissimus dorsi. Similar as our results were found in thawed horse meat after 30 days storage at -20°C.

Colour determination

Table 1. Changes of pH and colour characteristics of horse m. *Longissimus dorsi* for a period of 12 days of aging at 0 - 4°C.

Time for post mortem aging d	pH	The color brightness L*	The color redness a*	The color yellowness b*
0	$6.75^{e} \pm 0.05$	$30.01^{a} \pm 0.26$	$16.91^{e} \pm 0.10$	$3.45^{b} \pm 0.38$
1	$6.20^{b,c} \pm 0.05$	$30.35^{a} \pm 0.14$	$17.01^{e} \pm 0.37$	$3.58^{b} \pm 0.17$
2	$6.15^{b,c} \pm 0.05$	$30.82^{a} \pm 0.79$	$17.86^{f} \pm 0.21$	$3.61^{b} \pm 0.35$
3	$6.10^{b} \pm 0.05$	$31.71^{b} \pm 0.13$	$19.93^{g} \pm 0.43$	$4.89^{d} \pm 0.28$
4	$5.80^{a} \pm 0.08$	$31.34^{b} \pm 0.52$	$19.67^{g} \pm 0.32$	$4.62^{d} \pm 0.17$
5	$6.16^{b,c} \pm 0.05$	$30.42^{a} \pm 0.34$	$15.55^{d} \pm 0.36$	$4.21^{c} \pm 0.23$
6	$6.20^{b,c} \pm 0.05$	$30.29^{a} \pm 0.72$	$14.62^{c} \pm 0.73$	$3.78^{b} \pm 0.27$
7	$6.25^{c} \pm 0.05$	$30.30^{a} \pm 0.56$	$14.42^{c} \pm 0.41$	$3.75^{b} \pm 0.21$
8	$6.30^{c,d} \pm 0.02$	$30.36^{a} \pm 0.44$	$12.62^{b} \pm 0.20$	$3.21^{a,b} \pm 0.07$
9	$6.12^{c} \pm 0.07$	$30.52^{a} \pm 0.27$	$12.17^{a} \pm 0.23$	$3.08^{a,b} \pm 0.23$
10	$6.00^{b} \pm 0.06$	$30.42^{a} \pm 0.68$	$12.14^{a} \pm 0.28$	$2.99^{a,b} \pm 0.23$
11	$5.85^{a} \pm 0.08$	$30.50^{a} \pm 0.45$	$11.98^{a} \pm 0.58$	$2.71^{a} \pm 0.20$
12	$5.80^{a} \pm 0.10$	$30.78^{a} \pm 0.56$	$11.84^{a} \pm 0.15$	$2.70^{a} \pm 0.23$

Means within each column having different letters were significantly different according to Duncan's test at $p < 0,05$.

No statistically significant differences ($p > 0.05$, Tab. 1) were observed when the colour brightness (L*) measured at the beginning (4 h, 0 - 4°C) and at the end of the experiment (12 days, 0 - 4°C) of horse m. Longissimus dorsi were compared. Statistically significant increase of the colour brightness (L*) by about 5.66% ($p \leq 0.05$) was found on the 3 and 4 day of the storage of the horse meat at 0 - 4°C (Tab. 1). From the 4 d (Tab. 1) until the end of the study period the colour brightness decreases with no statistically significant difference ($p > 0.05$) of the originally determined value on the 12days. These conclusions are consistent with the results reported by Seong et al. (2014) for vacuum-packed and 30 days aged at 4°C d horse m. Longissimus dorsi but differ significantly from the results reported by Ruiz et al. (2018) for 9 d aged at 4°C foals m. Longissimus dorsi.

After the 4 days of horse meat storage (Tab. 1) the color redness (a*) increases by approximately 17.86% ($p \leq 0.05$). After the 4 days of the meat refrigeration a reverse trend was established and by the end of the study the colour redness was reduced by 7.83 ($p < 0.05$).

A statistically significant increase in the colour, with an approximately 41.74% ($p \leq 0.05$) increase in the yellowness (b*), was found on the 3 day of the horse meat storage at 0 - 4°C (Tab. 1). From the 4 day to the 12 day of the storage at 0 - 4°C a reverse trend was established as well.

On the 12 day of the experiment the colour yellowness (b*) decreased with 1.92 units ($p \leq 0.05$) from the baseline. These results are not in agreement with those reported by Seong et al. (2014) who found that the yellowness of the vacuum packaged and 30 days aged at 4°C d horse m. Longissimus dorsi significantly increases and those of Ruiz et al. (2018) for 9 days aged at 4°C d foals m. Longissimus dorsi.

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REFERENCES

Barbut, S., Zhang, L., & Marcone, M. (2005). Effects of pale, normal, and dark chicken breast meat on microstructure, extracted proteins, and cooking of marinated fillets. Poultry Science, 84(5), 797-802.

Brewer, M. S., & Wu, S.Y. (1993). Display, packaging, and meat block location effects on color and lipid oxidation of frozen lean ground beef. Journal of Food Science, 58(6), 1219 -1236.

Davey, C. L. (1983). Post mortem chemical changes in muscle - meat aging. 36th Reciprocal Meat Conference, American Meat Science Assosiation, North Dakota State University, Fargo, North Dakota, USA. Proceedings: 108-115.

De Palo, P., Maggiolino, A., Centoducati, P., & Tateo, A. (2012). Colour changes in meat of foals as affected by slaughtering age and post-thawing time. Asian-Australasian Journal of Animal Science, 25(12), 1775-1779.

Draper, N. R., & Smith, H. (1998). Applied Regression Analysis. Somerset, NJ, USA: John Wiley & Sons, Inc.

Fujimaki, M., & Arakawa, N. (1958). Chemical studies on the autolysis of meats. Part VII. On the chemical chanches of the myosin B during aging of meats. Bulletin of Agriculture Chemical Society of Japan, 22(4), 249-255.

Geay, Y., Bauchart, D., Hocquette, J.-F., & Culioli, J. (2001). Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. RND Reproduction, Nutrition, Development, 41(1), 1-26.

Huff-Lonergan, E., & Lonergan, S. M. (1999). Postmortem mechanisms of meat tenderization: The roles of the structural proteins and the calpain system: Quality attributes muscle foods. New York, NY: Kluwer Academic/Plenum Publishers.

Hultin, O. H. (1984). Post mortem biochemistry of meat and fish. Journal of Chemical Education, 61(4), 289-298.

Khan, A. W. (1962). Extraction and fractionation of proteins in fresh chicken muscle. Journal of Food Science, 27(5), 430-434.

Korkeala, H., Mäki-Petäys, O., Alanko, T., & Sorvettula, O. (1986). Determination of pH in meat. Meat Science, 18(2), 121-132.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227(5259), 680-685.

Lawrence, T. E., Waylan, A. T., & Kastner, C. L. (2002). Myofibrillar structural changes caused by marination with calcium phosphate or calcium chloride and sodium pyrophosphate. Conference of Cattlemen's Day, M. C. Hunt, Ed., Kansas State University. Agricultural Experiment Station and Cooperative Extension Service, Manhattan, Kan, USA. Proceedings: 102-105.

Lian, T., Wang, L., & Liu, Y. (2013). A new insight into the role of calpains in post mortem meat tenderization in domestic animals. A review. The Asian-Australian Journal of Animal Science, 26(3), 443-454.

Litwinczuk, A., Florek, M., Skałecki, P., & Litwinczuk, Z. (2008). Chemical composition and physicochemical properties of horse meat from the Longissimus lomborum and Semitendinosus muscle. Journal of Muscle Foods, 19(3), 223-236.

Longo, V., Lana, A., Bottero, M. T., & Zolla, L. (2015). Apoptosis in muscle-to-meat aging process. The omic witness. Journal of Proteomics, 125(1), 29-40.

Lourdes Perez, M., Escalona, H., & Guerrero, I. (1998). Effect of calcium chloride marination on calpain and quality characteristics of meat from chicken, horse, cattle and rabbite. Meat Science, 48(1/2), 125-134.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry, 193(1), 265-275. 1951.

Modzelewska-Kapitula, M., & Cierach, M. (2009). Effect of pressure and sample weight on free water content in beef estimated according to Grau-Hamm method using computer image analysis. Nauka PrzyrodaTechnologie, 3(4), 1-6.

Ruiz, M., Beriain, M. J., Insausti, K., Lorenzo, J. M., Cantalejo, M. J., & Saries, M. V. (2018). Aging effect on a foal meat preservation. ITEA, 114(1), 45-60. [In Spanish]

Seong, P.N., KyoungMi, P., SooHyun, C., GeunHo, K., SunMun, K., BeomYoung, P., & HoaVan, B. (2014). Effect of postmortem ageing time on quality characteristics of horse meat. Proceedings of 60th International Congress of Meat Science and Technology, Maldonado, Uruguay, 17-22 August 2014. Archivos Latinoamericanos de Producción Animal, 22(5), 69-71.

Welten, E. (2013). Free Amino Nitrogen in Beer by Spectrophotometry (IM): Analalytica – EBC. Nuremberg, Germany: Fachverlag Hans Carl.

ОСОБЕНОСТИ НА СТАРЕЕЊЕТО НА КОЊСКОТО МЕСО

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Резиме

Во текот на последната деценија коњското месо отиде подлабоко во видното поле на потрошувачите и научниците. Целта на оваа студија е да се идентификуваат специфичните карактеристики за време на стареењето на коњското месо. Промените во микроструктурата, морфологијата, протеинската автолиза, растворливите протеини, pH, капацитет за задржување на вода (КЗВ), загубата на вода и бојата беа испитувани кај m. Longissimusdorsi од коњ за време на 12 дена на на температура од 0 до 4°C. На 3 ден postmortem А и I-зони потешко се разликуваа. Беше забележано скратување на саркомерата. Периодот на постморталната вкочанетост (rigormortis) во коњското месо се јавува помеѓу 3 и 5 ден. Во овој период мускулните влакна се контрахирани, компонентата на црвената боја е намалена за 2-3 единици, pH и КЗВ се минимални - 5,80 и 13-14%, соодветно, и загубите на вода беа најмногу околу 20%. По 5 ден postmortem беа забележани поединечни пукнатини - показател за тековните автолитички процеси. Растворливоста на протеините се стабилизира на околу 1750 mg/ml. Зголемен дел од фракциите на протеините со молекуларна тежина од 28 до 23 KDa, сметано како индикатор за зголемена кршливост на месото беше пронајдена по 5 дена. Од 5 до 12 ден се откриени повисоки нивоа на α-актинин, дезмин и лесен меромиозин. По 7 ден postmortem деструктивните промени се продлабочуваат. Z-линии беа многу искинати. А- и I-дисковите тешко се разликуваа. Миофибрилите, исто така, беа многу фрагментирани и I-зоните не можеа лесно да се разликуваат.

Клучни зборови: *m. Longissimus dorsi, микроструктура, морфологија, протеинска автолиза, растворливи протеини, загуба на вода, боја.*

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BIOASSAY IN SAFETY ASSESSMENT OF NEW GRAIN PRODUCTS

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Abstract

The article is devoted to the issue of food safety as an important indicator of consumer properties and a decisive criterion of their quality. It describes that various alternative toxicological methods of research using the biological test-objects, the so-called bioassay methods are used along with the traditional toxicological control methods for safety assessment of food products. Safety of new grain crisp bread on the basis of spelt using bioassay methods were evaluated in experimental studies. The first method is based on determining the toxicity of crisp bread using test-object of *Colpoda steinii infusoria*, the second – on testing for a mortality rate of Daphnia Magna Straus crustaceans, the third – by method of determining the toxicity of objects using bioassay methods on the test-object Drosophila melanogaster fruit flies. On the basis of the performed bioassay the safety of new grain crisp bread was determined based on spelt. Ecotoxicological studies allowed assessing the safety of products being developed, as well as the prospect of introduction of new grain crisp bread into the market as a safe food product will be described in future.

Key words: *grain crisp bread, product safety, toxicity, ashberry, brier*

INTRODUCTION

Over the last decade the number of toxicants that affect the safety of raw materials and foodstuffs has increased, moreover, methods of their determination are quite complicated. In 2016 the Act On the Basic Principles and Requirements for Food Safety and Quality was adopted in Ukraine. One of the main priority lines is to prevent losses, preserve quality and guarantee food safety at all stages of production and storage [3]. Pursuant to Act a safe foodstuff is a foodstuff which has no harmful effects on human health and is fit for use.

Contaminants may occur in our food from various sources. They typically pose a health concern, resulting in strict regulations of their levels by national governments and internationally by the Codex Alimentarius Commission. Therefore, analysis of relevant chemical contaminants is an essential part of food safety testing programmes to ensure consumer safety and compliance with regulatory limits. Modern analytical techniques can determine known chemical contaminants in complex food matrices at very low concentration levels. Moreover, they can also help discover and identify new or unexpected chemical contaminants [4].

Many strategies to detect biological and chemical contaminants in foodstuffs have been developed to solve food safety problems [5]. Bioassay is one of the methods of study which is used to determine the degree of negative impact of chemicals being potentially dangerous to living organisms through recording of changes of biologically significant parameters (test functions) of the experimental test-objects with a subsequent assessment of their condition in accordance with the chosen toxicity criterion [6].

Foodstuff bioassay means toxicity studies of aqueous extracts from the product using live test-objects. Test-objects (test organisms) are experimental biological objects (organisms) that are used in determining toxicity. The discovered toxic effect is registered and evaluated in the experiment. Test-objects allow replacing complex chemical analyses and quickly identify the toxicity of the product [6, 7]. The method of determining the toxicity with the test-objects is fast enough; it does not require the use of experimental animals or expensive equipment and has prospects for accelerating safety control of raw materials and foodstuffs [4]. Infusoria, hydras, planarians, leeches, molluscs, crustaceans, representatives of different groups of plants and algae, insects etc. are used as test objects [13]. The choice of the test object for each case is determined by standards, sensitivity level to toxic substances and environment you want to study [6]. In the study we used test objects from different systematic groups: *Colpoda steinii* infusoria, *Daphnia Magna Straus* crustaceans and *Drosophila melanogaster* fruit-flies. The specific properties of these life forms allow obtaining detailed information on the likely negative impact or food stuff safety.

MATERIAL AND METHODS

Bioassay in evaluation of grain crisp bread safety

The objective of the study was to determine the safety of consumption of new grain crisp bread with ashberry and brier using bioassay methods on aquatic organisms and insects.

New types of grain crisp bread made with spelt including enriching additives. Spelt is specie of soft wheat, which is in contrast to the traditional wheat has a high content of proteins, nutrient rich fibres, mineral substances and vitamins [15]. Dry powders of ashberry and brier were used as nutrient additives. Their inclusion into the composition of the crisp bread contributes to giving curative and preventive effect, improving the organoleptic properties to the finished products.

The following samples were the test objects:

- sample 1 – grain crisp bread without additives (control);
- sample 2 – grain crisp bread with ashberry;
- sample 3 – grain crisp bread with brier.

When conducting ecotoxicological control of grain crisp bread bioassay methods with test organisms from different taxonomic groups were used. The first stage of the study was to determine the toxicity of grain crisp bread by bioassay method on the death of Daphnia Magna Straus crustaceans. These test-organisms are widely used in determining the toxicity of soils, waste water, feed and food products [12]. Studies were conducted on a synchronized Daphnia culture. A synchronized culture is one age culture obtained from one female by using acyclic parthenogenesis in the third generation. Such a culture is genetically homogeneous [8]. The technique is based on identifying the differences between the number of dead Daphniae in the test sample (experience) and that which is cultivated in water. Bioassay is the criterion of the acute lethal toxicity.

10 cm^3 of dechlorinated drinking water was poured into each test tube where Daphniae are cultured, 1 cm^3 of water extract of the test sample and 10 Daphniae into each tube. The bioassay was performed in diffused light at a water temperature of (20±2) °C for 96 hours.

According to GOST 32536 – 2013 [9] live Daphniae are the one which move freely in the water column or emerge from the bottom of the vessel after its slight shaking. The rest of Daphniae are considered dead.

RESULTS AND DISCUSSION

Daphniae were not fed during bioassay of samples, at the end of the experience the number of live test-objects was counted at the end of the experiment (Fig. 1).

The toxicity level of the experimental product during testing of aqueous solution of test samples was determined by % of maxillopods that survived in accordance with table 1.

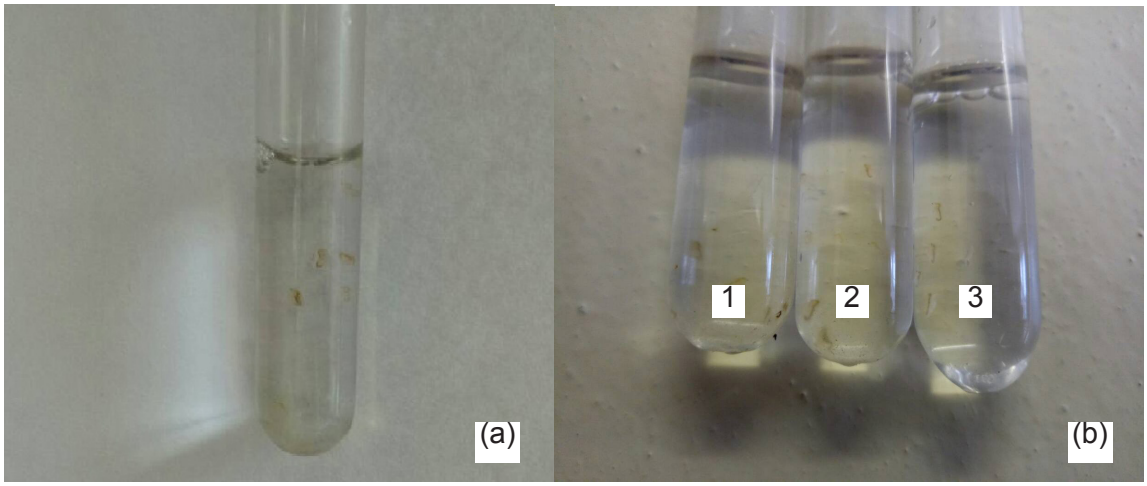

Figure 1. (a) Daphnia magna Straus culture in water; (b) Daphnia magna Straus culture in medium of the tested samples: 1 – grain crisp bread without additives; 2 – grain crisp bread with ashberry; 3 – grain crisp bread with brier

Table 1. The scale of toxicity level of grain crisp bread when testing an aqueous solution

Toxicity level of the experimental product	Survival rate of Daphnia magna Straus maxillopods, %
Non-toxic	93–100
With low toxicity	62–92
Toxic	0–61

The results of study were as follows: in sample № 1 – grain crisp bread without additives (control) the number of dead Daphniae was 1 %, sample № 2 and № 3 – grain bread with ashberry and brier – 3 % on each, that the evidence of new product safety.

The second method is based on determining the toxicity of grain crisp bread using the test-object *Colpoda steinii* infusoria (Fig. 2).

Figure 2. *Colpoda steinii culture*

The method is based on extraction of different fractions from the experimental products – polar and non-polar toxic substances respectively with water and with hexane and the subsequent impact of these extracts (hexane was previously evaporated) on Colpoda steinii culture. Dry culture *Colpoda stenii* represents o colpoda cysts and *Bac. Subtilis* spores attached to the wall of the vial and visible on increase of 80 – 150 min. Developed standardized rating scale of toxicity is given in table 2 [10]. The specimen used in study is manufactured in a

certified laboratory (Vidrodzhennya m LLC, Odessa), in accordance with TU U 46.15.243-97 requirements and [14]. Colpoda culture is harmless to humans and animals; it was stored dry up to 6 months at a temperature of 10 – 25 °C. Vials with Colpoda culture and nutrient medium were opened for studies not earlier than 12 – 24 hours before use. 2 cm^3 of nutrient medium were poured into each vial with Colpoda culture. One made certain of relevance of Colpoda culture immediately before the use. This culture was studied by pendent drop method under the microscope. Colpodae in maximum number of 6 cells per high power field should actively move. Weighing of 20 g was added to a flask with a capacity of 250 cm^3 and poured 100 cm^3 of distilled water. The flasks with the content have been shaken for 20 min, and then the mixture was filtered. 2 cm^3 of extract were added to the tube containing the active culture of Colpoda and stirred. 2 cm^3 of nutrient medium were brought to the control sample with the active culture of Colpoda. In 10 min and then in 3 hours one drop of the mixture was selected from the experimental and control test tubes and reviewed them under the microscope using pendent drop method. Available live and dead infusoriae were considered in the test samples. The criterion of toxicity is the time from beginning of exposure of the experimental extract till death of the majority (90 %) of Colpodae. The fact of death of Colpodae was stated on the basis of a complete termination of their movement and the presence of decay. In the control sample all Colpodae should remain movable in a control sample [10].

The product under study is considered toxic, if death of Coolpodae occurs within 10 min after introduction of extract in a live culture of Colpodae. The product under study is slight toxic, if death of Colpodae occurs within the interval up to 3 hours of studies. The product under study is non-toxic, if all Colpodae remain movable in 3 hours of studies.

Table 2. Toxicity assessment scale of finished product [10]

Toxicity	Criteria
Very toxic	Death of the most Colpodae occurs within 3 minutes
Toxic	Death of the most Colpodae occurs within 10 minutes
Slight toxic	Death of the most Colpodae occurs in 3 hours
Non-toxic	Most Colpodae remain movable within 3 hours

Fig. 3 shows the obtained results of determination of ecological and toxicological parameters of grain crisp bread safety by bioassay method using Colpoda Steinii.

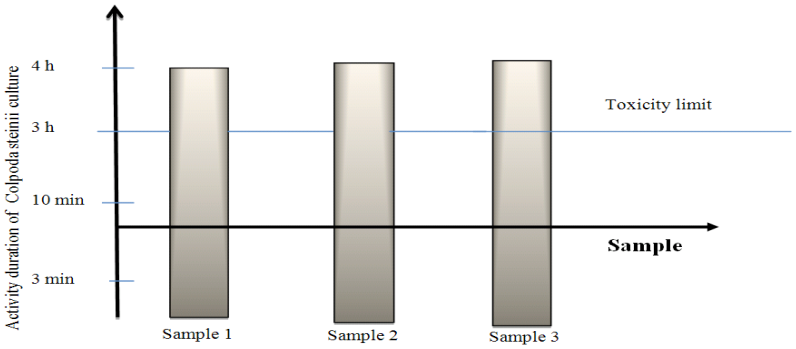

Figure 3. Grain crisp bread toxicity by bioassay method using Colpoda Steinii

On the basis of the performed studies it is established that the sample № 1 – grain crisp bread without additives (control) and samples № 2 and № 3, namely grain crisp bread with ashberry and brier do not contain toxic substances, because most Colpodae remained alive in all the experimental samples within three hours. The results correlate with the studies performed by bioassay method using *Daphnia magna Straus*.

The third method is based on bioassay of grain crisp bread samples by determining the availability or absence of acute toxic action on the test-objects. Chronic toxicity was determined in toxicological analysis of grain crisp bread quality. Biological tests on *Drosophila melanogaster Meig* flies were used during bioassay [11] shown in Fig. 4.

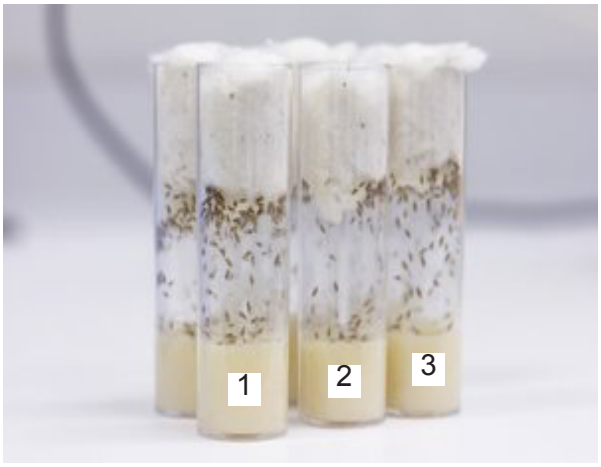

Figure 4. *Drosophila melanogaster Meig* in medium of the experimental samples; 1 – grain crisp bread without additives; 2 – grain crisp bread with ashberry; 3 – grain crisp bread with brier

The toxicity criterion in bioassay method on fruit flies is a probable deviation from frequency control of occurrence of dominant lethal mutations

Determination of toxicity of objects using bioassay method on Drosophila melanogaster fruit flies has as compared to other test organisms (bacteria, plants, tissue culture) a number of advantages due to the fact that it is possible to find all types of mutations in Drosophila. It has a small number of chromosomes, short life cycle and great fertility; metabolic activation of substances proceed the organism which is the same as in humans. Data obtained using this test organism can be extrapolated to highly organized animals including mammals and used as forecast of risk to human health [11].

Grain crisp bread was tested with the aim of establishing the use of bioassay method on *D. melanogaster* to determine the genotoxicity. The presence or absence of genotoxic and mutagenic actions to *D. melanogaster* during bioassay was determined in samples. According to the results of testing of samples of grain crisp bread genotoxic and mutagenic actions were not found in either of the samples that the evidence of the safety of the product.

CONCLUDING REMARKS

Based on ecotoxicological control of grain crisp bread with the test-organisms from different taxonomic groups, namely *Colpoda steinii* infusoria, *Daphnia Magna Straus* crustaceans, *Drosophila melanogaster* fruit flies it was established that the consumption of new cereal products is safe.

Biological analysis of all test objects showed that the samples under study of the crisp bread do not have a negative impact on living form and can be recommended for consumption by a human. The conducted studies are the evidence of expediency of the further work and industrial production of new types of grain crisp bread

based on spelt with ashberry and brier that will allow expanding the assortment and fills the market with safe food stuffs.

The lack of significant material costs, high sensitivity of methods and the obtained results with a high degree of reproduction demonstrate the feasibility to recommend the conduction of bioassay for all new types of food stuffs, particularly on introduction of non-traditional raw materials and additives.

REFERENCES

Biotesting as a method of assessing the quality of drinking water. (2006). Bulletin of the National Academy of Sciences of Ukraine. 10. 55-57. (in Ukrainian).

Bochkov, N., & Chebotarev, A. (1989). Human heredity and mutagens of the environment. Moscow, Russia, Medicine. (in Russian).

Braginsky, L. (2000). Methodological aspects of toxicological biotesting at Daphnia magna Str. and other branching crustaceans (critical review). Hydrobiological Journal. 36(5), 50-70. (in Russian).

Eremeeva, A. S., Donchenko, M. I., Buchelnikov, V. S., Peregudina, E. V., & Azarova, S. V. (2015). Review of bioindication and biotesting methods for environmental assessment. Young Scientist. 11, 537-540. (in Russian).

Food safety. Fact sheet (October 2017). Retrieved from http://www.who.int/mediacentre/factsheets/fs399/en/ (in Russian).

GOST 13496.7-97 Feed grain, grain by-products, compound feeds. Methods for determination of toxicity (with Change No. 1). (in Russian).

GOST 32536-2013 Test methods for chemical products that present a danger to the environment. Determination of acute toxicity for daphnia. (in Russian).

Kohajdová, Z., & Karovičová, J. (2008). Nutritional value and baking applications of spelt wheat. Acta Scientiarium Polonorum, Technologia Alimentaria. 7 (3), 5-14.

Law of Ukraine "On basic principles and requirements for the safety and quality of food products" Retrieved from http://zakon3.rada.gov.ua/laws/show/771/97-%D0%B2%D1% 80 (in Ukrainian).

Lyashenko, O. (2012). Bioindication and biotesting in environmental protection. St. Petersburg, Russia, SPb GTURP. (in Russian).

Man, Lv, Liu, Y., Geng, J., Kou, X., Xin, Z., & Yang, D. (2018). Engineering nanomaterials-based biosensors for food safety detection. Biosensors and Bioelectronics, 106, 122-128. Retrieved from https://www.sciencedirect.com-/science/article/pii/S0956566318300605.

Omarov, R., Agarkov, A., Rastovarov, E., & Shlykov, S. (2017) Modern methods for food safety. Engineering for rural development, Jelgava, Latvia Proceedings: 960-963.

Shaw, J. R., Pfrender, M. E., Eads, B. D., Klaper, R., Callaghan, A., Sibly, R. M., Colson, I., Jansen, B., Gilbert, D., & Colbourne, J. K. (2008). Daphnia as an emerging model for toxicological genomics. Advances in Experimental Biology. 2, 165-219, 327-328.

Vinokhodov, D. (2007). Scientific fundamentals of bit-testing using infusorians. The dissertation of the Doctor of Biological Sciences. St. Petersburg, Russia, St. Petersburg Institute of Technology. (in Russian).

Yevlash, V., Samoilenko, S., Otroshko, N., & Buryak, I. (2016). Express-methods of studying the safety and quality of food products. Kharkiv, Ukraine, KhDUHT. (in Ukrainian).

БИОТЕСТОВИ ЗА АНАЛИЗА НА БЕЗБЕДНОСТ НА НОВИ ЗРНЕСТИ ПРОИЗВОДИ

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Резиме

Трудот е посветен на прашањето за безбедноста на храната како важен показател за потрошувачкит есвојства и одлучувачки критериум за нивниот квалитет. Во трудот се опишува дека се користат различни алтернативни токсиколошки методи за истражување со користење на биолошки тестови, таканаречени биолошки методи, заедно со традиционалните токсиколошки методи за контрола за проценка на безбедноста на прехранбените производи. Безбедноста на новиот двојнопечен леб базиран на житната култура спелта со методи за биоанализа беа евалуирани во експериментални студии. Првиот метод се базира на утврдување на токсичноста на свеж леб, користејќи биолошки тест од *Colpoda steinii infusoria*, вториот – за тестирање на стапката на смртност на рак од *Daphnia magna* Straus, третата – со метод на одредување на токсичноста на предметот на проучување, кои користат методи за биоанализа на тест-објектот *Drosophila melanogaster*. Врз основа на извршениот биотест, безбедноста на новиот двојнопечен леб беше определена врз основа на спелата. Екотоксиколошките студии овозможија проценка на безбедноста на производите што се развиваат, како и можноста за воведување на нов двојнопечен леб на пазарот како производ за безбедна храна, кои ќе бидат опишани во иднина.

Клучни зборови: *двојнопечен леб, безбездност на производ, токсичност, природни конзерванси*

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THE INFLUENCE OF THE PERLITE AS A SUBSTRATE FOR IMPROVING ON SOME WATER PROPERTIES ON THE FLUVIAL SOIL WITH AN APLICATION OF RETENTIONAL CURVES

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Abstract

This work is focused on the determination of potential of moisture retaining in perlite and fluvial soil. For assessing this parameter, the method of bar extractors and Porous plate extractors has been explored. The method is applied on 7 different regimes of pressure, (0.1; 0.33; 1; 3; 6.25; 11; 15 bars) in samples composed of perlite and fluvial soil present in different ratios of 20/80, 30/70, 50/50. The major goal of this study is to explore the experimental results of moisture content and to show the effectiveness of the water retention properties of perlite. Water retention curve is the relationship between the water content, and the soil and substrate water potential. This curve is characteristic for different types of soil and substrate, and is also called the soil moisture characteristic. The retention curve reflects the moisture content during different tension. The data determined in this work are useful to assess the effective zone of the root system. We show that perlite exhibits specific features in respect to the water retention in several types of soils. Because of the good physical properties and the high porosity, the expanded perlite has a significant role in maintaining and improving the water-air regime in the fluvial soils. In addition, it gives better accessibility of air and moisture for the plants, having a very positive influence on the soil features.

Key words: *Water retention, root system, physical properties.*

INTRODUCTION

Perlite is a glass volcanic rock of rhyolitic composition containing 2-5 % of combined water (Roulia et al. 2006). Technically, the term perlite is used for glassy volcanic rock which can be thermally expanded (Koukouzaset al.1994). The commercial product, commonly designated as expanded perlite, is produced by heating the material at 760–1100 °C, there by converting its indigenous water to vapour and causing the material to expand to 4 to 20 times its original volume while forming lightweight high-porosity aggregates, snow-white granules are composed of many tiny closed air cells or bubbles. They are very light in density, they have excellent thermal insulation properties, and they are fully inert and neutral (Dogan and Alkan, 2004; Harben, 1990).

Figure 1. a) Expanded perlite b) Raw perlite
Photo: (Markoska, 2018)

Expanded perlite has several attractive physical properties for commercial applications including low bulk density, low thermal conductivity, high heat resistance, low sound transmission, high surface area, and chemical inertness Ennis (2011). Precisely, because of the expansion process and the series of positive features, the perlite is applied in many branches, (agriculture, construction, industry, technology, etc). As far as applications of perlite are concerned, it is mainly consumed as fillers, filter aids, in producing building construction materials (Morsy et al., 2008; Aglan et al., 2009), adsorptive materials, precursor for geo-polymer formation (Vance et al., 2009), removal of heavy metal ions and other pollutants from atmosphere (Mostaedi et al., 2010), in thermal insulation (Vaou et al., 2010), removal of dyes (Vijaykumar et al., 2009), Dogan and Alkan (2003) in horticulture sorption of oil etc. Perlite is a naturally occurring waste, estimated with about 700 million tons worldwide reserves. We use it in our research in expanded form as a substrate. The application of substrates to improve the properties of the soils requires knowledge of their physical and chemical properties that are responsible for providing adequate support and a reservoir for air, water and nutrients. The use of various organic and inorganic substrates such as perlite allows for better nutrient intake, sufficient growth and development due to optimization of water and oxygen (Verdonck and Demeyer, 2004). These media should guarantee better rooting conditions and provide anchorage for the root system, supply water and nutrients to plants and suitable aeration environment to roots (Gruda et al. 2013, 2006). One of the most important properties of substrates is by adding such improvers and substrates in the soil itself; they improve the soil regardless of what type of soil it is. The substrates are used to solve problematic soils such as sandy soils that do not retain enough water or too clayey soil which, on the contrary, retains too much moisture and less oxygen. But also, when soil conditions change, substrates serve as part of preventive care, even in the absence of the familiar problems. The reason why the substrates are to be added to the soil is to provide a better environment for the root system and plant growth. This includes improving the soil structure and water storage capacity, the availability of nutrients and living conditions for soil organisms that are important for plant growth and development.

MATERIAL AND METHODS

The experimental part served to determine the retention of moisture of perlite and fluvial soil at different pressures. The experimental part was divided into two parts: field part and laboratory part. The field part consisted of taking soil samples from Strumica and from the locality of the exploitation of the raw material perlite "Cera Poliana" in Mariovo, Gradesnica, Republic of Macedonia. The raw material perlite in this research will be used in its expanded form as a substrate. These soils are prevalent in all valleys of the area. Most of them are in Gevgelija and Strumica valleys. The hydrological maps of individual sheets on the area give a precise idea of their prevalence. During the pedological mapping of the valleys, it was established

that the total area of alluvial soils is 8955 ha. Markoski (2015). The laboratory part consisted of preparation of the soil / substrate for analyses and conducting quantitative laboratory analysis. The soil and perlite were analysed in all three of their different ratios: 20/80, 30/70, 50/50 with the ultimate goal to determine the ability to retain water in the soil or substrate, and the role of perlite in improving the aquatic regime in examined soil. Soil samples were taken from the mentioned sites, perlite was used in expanded form as an expanded perlite or as a substrate, which further served us as a material for analysis. The soil samples from Strumica were taken at depth of 0-30cm. In laboratory conditions, soil samples were brought to an airy dry state. Then the soil was finely milled and sifted through a sieve with 2mm openings, and an average analytical sample was prepared in which further soil analysis was carried out. In laboratory conditions there was determined retention of perlite moisture and soil at higher pressures with application of a pressure limiter with Bar extractor for determination of moisture retention at 0.1 bar (pF - 2); 0.33 bar (pF - 2.54); 1 bar (pF - 3); To determine soil moisture retention in higher pressures, the Richard Porous plate extractor method was applied, 2.00 bar (pF - 3.3); 6.25 bar (pF - 3.90); 11 bar (pF - 4.04) and 15 bar (pF - 4.2), described by (Resulović, 1971; Belić et al., 2014). The obtained results for moisture retention in mass percent are presented in a tabular manner. Keeping water in the soil or perlite is marked as retention. The characteristics of moisture retention include the relations between the matrix potential and the moisture content and can be represented by a retention curve. It shows the moisture content at different tensions. Water retention is the result of two forces: adhesion (attraction of water molecules by the particles) and cohesion (attraction of water molecules to each other). Adhesion is much stronger than cohesion.

Figure 2. Preparing soil/substrate and placing samples on Bar extractor and Porous plate extractor

The force with which the water is retained in the soil, that is, the force it needs to squeeze out of the soil is denoted as capillary potential and is closely related to the water content. The free water in the soil has a capillary potential equal to zero, a condition in the soil when all pores, capillary and non-capillary, are filled with water. Markoski (2013) To obtain a clearer representation of the intensity of moisture retention, especially for soil and perlite, the fluvial soil along with perlite, the humidity values in mass percent tabular and graphic with pF values are displayed, the height of the water column in cm (1 bar = 1063 cm / cm^2).

RESULTS AND DISCUSSION

All examined samples of perlite and fluvial soil and their respective ratios were placed on 7 different pressure modes (0.33; 1; 3; 6.25; 11; 15 bar) using Bar extractor and Porous plate extractor, and the obtained results for moisture retention in mass percent's are presented in a tabular manner.

Table 1. Moisture retention in weight % at different tension in substrate perlite and fluvial soil na 0.1 bar; 0.33 bar; and 1bar

Substrate Perlit(P) Fluvial soil(Aa) and Corelations		0.1bar		0.33 bar		1bar	
	n	x	SD	x	SD	x	SD
Aa50/P50	3	27.52	0.02	24.53	0.03	20.51	0.01
Aa70/P30	3	25.05	0.17	23.14	0.15	19.07	0.12
Aa80/P20	3	22.15	0.12	20.72	0.22	15.08	0.23
P-perlite	3	67.88	1.88	58.35	1.59	47.70	1.57
Aa-soil	3	9.28	0.02	7.83	0.01	7.03	0.01

P- Perlite;Aa- Fluvialsoil; Corelations: P20/Aa80, P30/A70, P50/Aa50

Table 2. Moisture retention in weight % at different tension in substrate perlite and fluvial soil na 3 bar; 6.25 bar; 11bar and 15 bar

Substrate Perlite(P) Fluvial soil(Aa) and Corelations		3bar		6.25 bar		11bar		15bar	
	n	x	SD	x	SD	x	SD	x	SD
Aa50/P50	3	17.06	0.03	15.78	0.02	12.10	0.10	11.92	0.02
Aa70/P30	3	16.10	0.22	13.21	0.27	11.10	0.18	10.07	0.18
Aa80/P20	3	13.84	0.78	10.68	0.80	9.34	0.67	7.92	0.55
P-perlite	3	39.78	2.58	34.84	2.66	30.10	2.40	26.65	2.75
Aa-soil	3	5.24	0.01	4.39	0.01	3.99	0.01	3.02	0.01

P-Perlite ;Aa- Fluvial soil; Corelations: P20/Aa80, P30/A70, P50/Aa50

To understand more clearly the intensity of moisture retention in fluvial soil with perlite, the mean moisture values in mass percentage are shown. In the data given in Table 1 and 2 is noted that the P-perlite substrate has the largest retention capacity in all variants and at all points of pressure tension such as: At a pressure of 0.1 bar with an obtained result of an average value of 67.85% at pressure of 0.33 bar with an average value of 58.35%, at a pressure of 1 bar 47.70% of 3 bar 39.78% of 6.25 bar 34.84 at a pressure of 11 bar 30.10%, of 15 bar with average value of 26.65. The retention capacity of the fluvial soil is lower than the perlite in all pressures of different tension: from 0.1 bar = 9.28%, from 0.33 bar = 7.83%, from 1 bar = 7.03% , from 3 bars = 5.24%, from 6.25 bars = 3.99%, from 11 bars = 3.99%, from 15 bars = 3.02%.In other analysed ratios, where the perlite is represented by 20%, 30% and 50% in soil, the soil retention capacity is increased dramatically, for example in the ratio P20/80Aa at a pressure starting at 0.1 bar with an average value of 22.15%, at a pressure of 0.33 bar, the retention pressure equals an average value of 20.72%, at a pressure of 1 bar, the retention pressure equals the mean value of 15.08%, of 3 bar the retention pressure equals the mean value from 13.84%, from 6.25 bars with an average value of 10.68% at a pressure of 11 bars with an average value of 9.34%, a pressure of 15 bar retention pressure amounts to an average value of 7.92%. The retention pressure of other relations, such as P30/Aa70, and P50 /Aa50, is presented in table 1, with the addition of a larger percentage of perlite to soil, the retention pressure will increase. Figure 3 and 4 represent the retention curves of the substrate perlite and the fluvial soil. From the curves it can be noted that the percentage of moisture in the substrate perlite is higher, compared to

the retention curve in the fluvial soil. The ability of the substrate to retain and maintain moisture is crucial for improving the efficiency of water use for growing crops in closed (greenhouses, greenhouses, etc.) and open conditions. According to the author Richards (1955) Retention curves have great practical and theoretical importance, because they show all data about the water in the soil and substrates. Retention curves or moisture retention curves (MRCs) were first described in Bunt (1961) and were obtained in a similar manner as in soils. The equivalent pores ratio can be estimated according to the retention curve. The tension occurring in the meniscus of water in one cylindrical pore depends on the pore diameter. Therefore, it can be concluded that the amount of water that soil releases in a certain dimension corresponds to this interval of tension. Moisture retention curves provide us data about soil and substrate capacity for available moisture, with the upper limit of field water capacity and the lower limit of the coefficient of the set. For estimating soil moisture, using capillary potential quantified Vućić (1987)pF values were determined, whereby the water force in the soil was expressed through the height of the water column in cm (1 bar = 1063 cm/cm2). pF values affect the change in the mechanical composition of the soil. The higher fraction of small fractions results in higher pF values, especially at a pressure of 0.33 bar. Markoski (2013) Apart from the mechanical composition of the soil, water - physical relations affect the mineral composition, the content of organic matter and others. This influence was studied Hillel (1980) and (Maclean and Yager, 1972) in many soils in America, Europe and Asia. In their research the soil moisture retention in West Midland mostly depends on organic matter, mechanical composition and mineral composition of soil. Filipovski (1996) also explains that retention of moisture in various tensions is closely related to the content of humus, clay, dust and mineral clay composition. According to the author Kutilek M. and Novak V., 1998 the hydrological characteristics of soils, such as water retention and the rate of water movement, depend on a large degree on the total porosity and pore-size distribution of the material while the moisture content in the perlite substrate depends on a higher percentage of the porous material. For our exploration of fluvial soil, the samples were taken at a depth of 0-30 cm. Fluvial soils are young roots of river that contain regulates and soil material from the obtained areas. These soils have good physical properties: they have good water resistance, and are well aerated. (Markoski et al., 2015) With the appearance of a sandy layer or gravel on the surface of the soil, the physiologically active layer of low humus content decreases. These types of soils are characterized by high water tolerance. Sometimes such negative examples of deterioration of the physical properties of the soil, using substrates or enhancers as perlite in our research can positively affect the improvement of these properties.

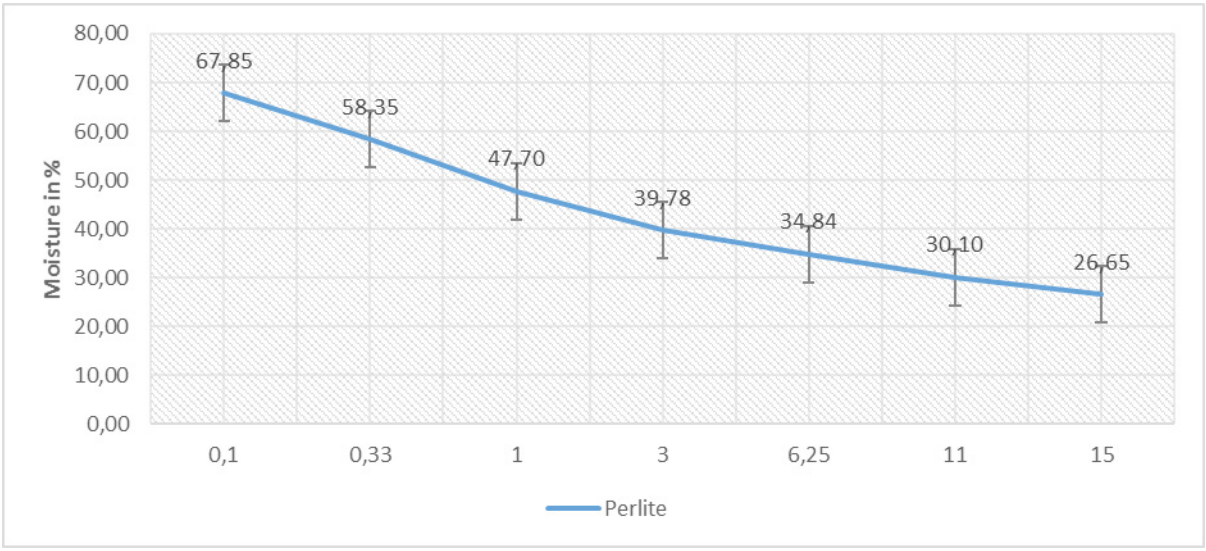

Figure 3. Moisture retention in substrate perlite

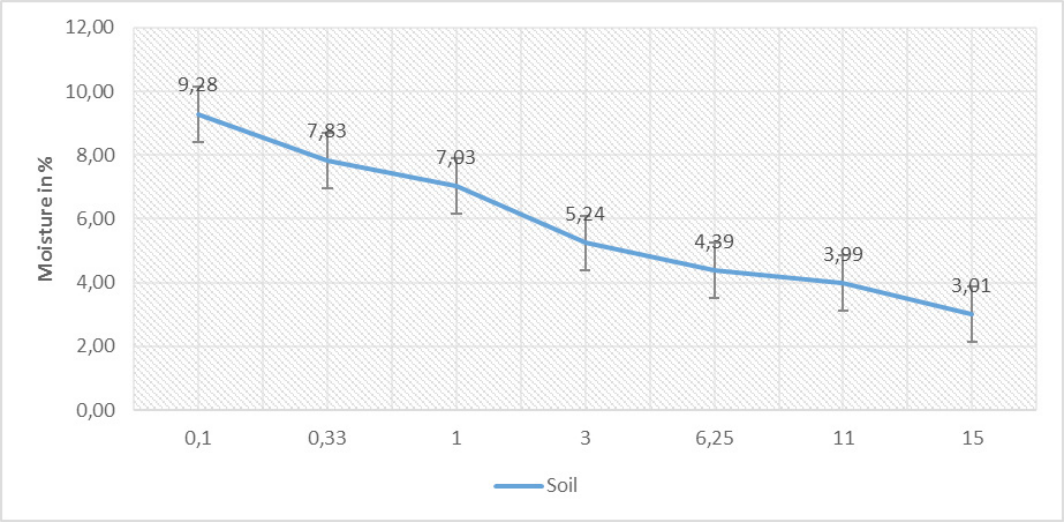

Figure 4. Moisture retention in fluvial soil type

Figure 5 shows the values of the retention curves of relations Aa80 / P20, also fluvial soil and pure perlite substrate. It can be noticed that the highest curve shows pure perlite, and the lowest shows fluvial soil. In the Aa80/P20 ratio, moisture retention increases by 10 to 22%, where retention curves increases in parallel. Because of the addition of 20% perlite to 80% of soil, where perlite with high porosity capacity can retain water with its particles, the percentage of moisture increases. Figure 6 shows the retention curves of three ratios, perlite as a pure substrate, fluvial soil and perlite ratio of 30/70 to fluvial soil. The largest retention ratio is the ratio of pure perlite substrate. It can also be noted on the curve that higher retention moisture occurs at 0.1 bar and 0.33 bar where all the pores are filled with water. The lowest retention occurs in the fluvial type of soil. In the ratio of Aa30/P70 where 30% of perlite and 70% of soil is fluid, the retention curve is growing from 10% to 28% of available moisture.

Figure 7 presents the retention curves of the relations between perlite and fluvial soil, and the ratio between perlite and fluvial soil is 50/50. Moisture retention in pure substrate perlite is represented by the highest retention curve due to its effective porous space to retain and maintain water in its pores, to create a relatively high content of available moisture. The retention curve of the soil shows lower retention of the perlite substrate; a significant difference between the soil type and the perlite substrate can be observed here. Whereas in the proportion of the mixture of 50% perlite and 50% fluvial soil, we have higher retention compared to the retention of pure fluvial soil, or a moderate increase in retention, the perlite as a substrate keeps the water in its pores, which can increase the retention of soil moisture

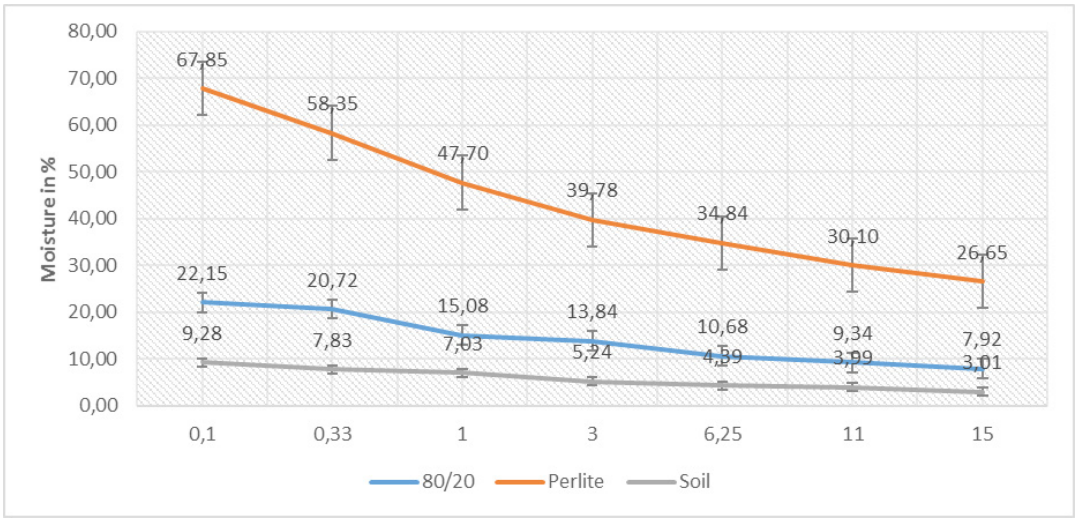

Figure 5. Moisture retention in perlite P20/80 Aa-fluvial

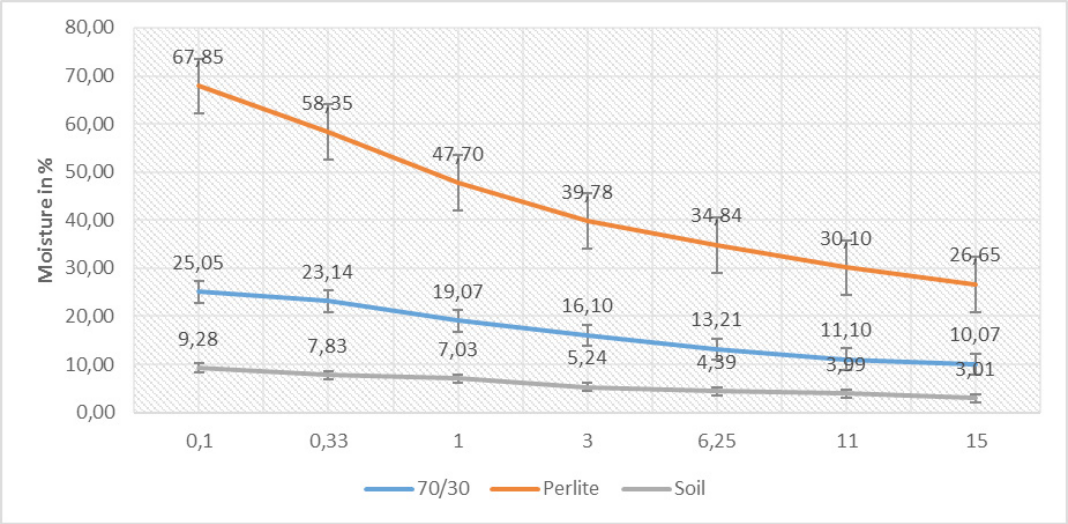

Figure 6. Moisture retention in perlite P30/70 Aa-fluvial

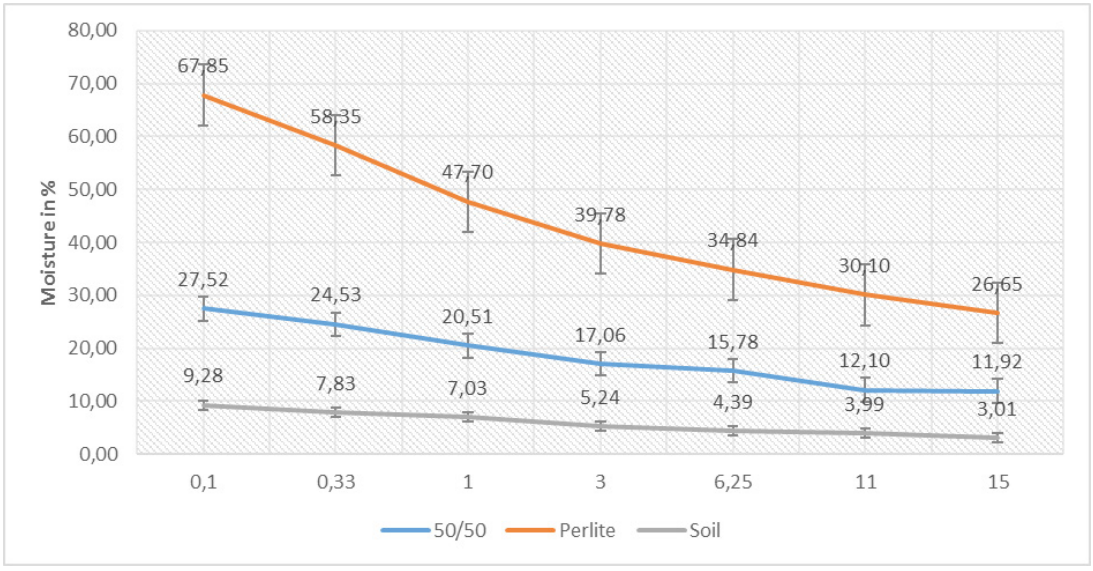

Figure 7. Moisture retention in perlite P50/50 Aa-fluvial

Table 3. Correlation coefficients between the tension points of 0.33; 1; 3; 6.25; 11; 15 bars

Correlation coefficients	0,1bar	0,33 bar	1bar	3bar	6.25bar	11bar	15bar
0,1 bar	1	0.998**	0.982**	0.972**	0.978**	0.958**	0.951**
0,33 bar		1	0.985**	0.977**	0.982**	0.962**	0.957**
1 bar			1	0.987**	0.991**	0.991**	0.979**
3 bar				1	.993**	0.978**	0.989**
6.25 bar					1	0.975**	0.991**
11 bar						1	0.972**
15 bar							1

In table 3 the coefficients of correlation of the researched properties in different ratios of the perlite substrate and the fluvial soil are given. On the basis of the correlation analysis, it can be noted that there is a high positive correlation between all points of tension within the mutual relations. The authors of the paper Jeb S, Fields, William C, Fonteno, and Brian E, Jackson, (2014), have examined the physical properties of the perlite and tested the moisture retention by methods from the manual (Fonteno and Harden, 2010), with Volumetric Pressure Plate Extractors with (-Kra), which yielded similar results with ours, the percentage of moisture in the perlite substrate was 66% per 0.1 bar, 43% per 1 bar and 31% of moisture per 10 bar.

CONCLUDING REMARKS

Based on the results obtained in the laboratory analysis of the perlite as a substrate and the fluvial soil, we can conclude the following: The analysis of moisture retention in the perlite shows high moisture retention at all points of tension (0.1; 0.33; 1; 3; 6.25; 11; 15 bar). The analysis of the retention capacity of the analysed fluvial soil shows a lower retention capacity compared to the perlite's retention capacity at all points of tension. In other proportions of Aa80/P20 or the addition of 20% perlite in river soil, the value increases several times, which again confirms that the addition of perlite in the fluvial soil samples causes an increase in retention capacity at all points of tension such as 0.1; 0.33; 1; 3; 6.25; 11; 15 bar, starting from 0.1 bar with an average value of 22.15% to 15 bar of retention soil, which amounts to 7.92% moisture. The Aa70/P30 ratio also shows a slight increase in retention capacity of fluvial soil at all points of tension such as 0.1; 0.33; 1; 3; 6.25; 11; 15 bar. With a further 50% perlite increase in soil at the Aa50/P50 ratio, retention capacity increases with an average value of 27.52%, starting from 0.1 bar to 15 bar with a moisture content of 11.92%.A high retention curve which reflects the characteristics of moisture retention in pure perlite substrate shows that this results in the fact that it's effective porous space keeps and maintains water in its pores. It is noted that the addition of substrate perlite in the soil also increases the values for retention capacity. Perlite due to its high porosity, which keeps water in its pores, influence the improvement of the water regime of the examined fluvial soil by retaining and maintaining moisture and providing adequate support and a water reservoir that is needed for proper growth of the plants and more healthier soil. Retention curves have great practical and theoretical significance, because through them almost all data about water in the soil and substrate can be obtained, these curves give the opportunity to determinate when and what content of water the plant's needs. In this way we can see the relations among the water, soil, and plants. The strength of water persistence in the soil or substrate can be determined for all content of water. Humidity retention curves provide us with data about the capacity of the humidity available within the soil and substrates, whose upper limit defines the water capacity of the ground and lower limit the coefficient of fading. These data, particularly for the effective area of the root system, become applicable when water is exploited in the soil.

REFERENCES

Aglan, H., Morsy, M., Allie, A., & Fouad, F. (2009). Evaluation of fiber reinforced nanostuctured perlite-cementitious surface compounds for building skin applications, Construction and Building Materials, Vol.23 (1), pp.138-45.

Bunt, A.C. (1961). Some physical properties of pot-plant composts and their effect on plant growth. Plant Soil, 13: 322-332.

Vance, E.R., Perera, D.S., Imperia, P., Cassidy, D.J., Davis, J., & Gourley, J.T., (2009). Perlite Waste as a Precursor for Geopolymer Formation. Journal of the Australian Ceramic Society, Vol. 45 (1), 44-49.

Vaou, V. & Panias, D. (2010). Thermal insulating foamy geopolymers from perlite. Miner. Eng., Vol. 23 (14), 1146-51.

Vijaykumar, G., Dharmendirakumar, M., Renganathan, S., Sivanesan, S., Baskar, G., Elango, K.P. (2009). Removal of Congo Red from Aqueous Solutions by Perlite. Clean-Soil, Air, Water, Vol.37 (4-5), pp.355-64.

Verdonck & Demeyer (2004). The influence of the particle size on physical properties of growing media. ActaHort, 644: 99-101.

Vućić, (1987). Vodni vozdusni I toplotni rezim zemjista. VANU, Matica srpska, Novi Sad

Gruda, N., Prasad, M, & Maher, M.J. (2006). Soilless Culture, In: R. Lal (ed.) Encyclopaedia of soil sciences. Taylor & Francis, Boca Raton, FL, USA.

Doganm, A. (2004). Some physicochemical properties of perlite as an adsorbent. Fresenius Environmental Bulletin,13, 252–257

Dogan, M., & Alkan, M. (2003). Removal of methyl violet from aqueous solution by perlite. J. Colloid Interface Sci., Vol. 267 (1), 32-41.

De Boodt, M., & Verdonck, O. (1972). The physical properties of the substrates inhorticulture. ActaHort., 26: 37–44.

Ennis D.J. (2011). Perlite mining and reclamation in the no aqua peaks, Taos County, New Mexico. New Mexico Geological Society Guidebook, 62nd Field Conference, Geology of the Tusas Mountains – Ojo Caliente, 409–18.

Jeb S., Fields William, C., Fonteno B.E., Jackson Joshua L., Heitman, James, S, & Owen, Jr. (2014). Hydrophysical properties, moisture retention, and drainage profiles of wood and traditional components for greenhouse substrates Giancarlo fascella, growing substrates alternative to peat for ornamental plants.

Koukouzas N, & Dunham A. (1994). Genesis of a Volcanic Industrial Rock. Trachilas perlite deposit, Milos island, Greece

Kutilek, M. & Novak, V., (1998). Exchange of water in the soil-plant-atmosphere system. Int. Agrophysics, 12,28-33.

Richards, L.Á. (1955). Retention and transnêission of water in soil. Yearbook of Agriculture, 144-15.

Maclean, A.H., & Yager, T.V. (1972). Available water capacities of Zambian soils in relation to pressure plat measurements and particle size analysis. Soil Scinces, 113, 23-29.

Markoski, M., & Mitkova, T. (2012). Soil moisture retention changes in terms of mineralogical composition of clays phase. Clay Minerals in Nature – Their characterization, modification and application. Publisher, In Tech. p.p. 101 – 119.

Markoski, M. (2013). Genesis and properties of soil formed uponlime stones and dolomites in the Republic of Macedonia. Doctoral disetation. Faculty of Agricultural Sciences and Food, Skopje. pp. 1-163.

Миле Маркоски, Јосиф Митрикески, Татјана Миткова. (2015). Почвите распространети на подрачјето опфатено со листовите Струмица 1,2,3,4 и Демир Капија 2 и 4 на топографска основа 1:50 000 (Источно од гринич). Толковник. Издавач: Универзитет Св. „Кирил и Методиј"-Скопје. Земјоделски институт-Скопје.

Morsy, M.S., Shebl, S.S., & Abd El Gawad Saif, M., (2008). Development of perlite-gypsum-slag-Lime sludge-composite system for building application. Building Research Journal, Vol.56, pp.49-58.

Mostaedi, M.T., Ghassabzadeh, H., Maragheh, M.G., Ahmadi, S.J., & Taheri, H. (2010). Removal of cadmium and nickel from aqueous solution using expanded perlite. Brazilian Journal of Chemical Engineering, 27 (2), 299-308.

Roulia M. (2006). Influence of thermal treatment on the water release and the glassy structure of perlite. Journal of Materials Science, 5870–5881.

Resulović, H. (1971). Metode istraživanja fizičkih svojstva zemljišta, kni. V. JDZPZ, Belgrade.

Roulia, M., Chassapis, K., Kapoutsis, J., Kamitsos, E., & Savvidis, T. (2006). Influence of Thermal Treatment on the Water Release and the Glassy Structure of Perlite. Journal Materials Science, 41, 5870-5881.

Filipovski, G. (1996). Soil of Republic of Macedonia. Macedonian Academy of Sciences and Arts. Skopje. Vol II. p.p.11-55.

Harben, P.W. (1990). The determination of quartz in perlite by x-ray diffraction. Advances in X-ray Analysis. Volume 23. Serial publication. New York: Plenum Press.

Hillel, D. (1980). Application of soil physics. Department of Plant and Soil Sciences. Massachusetts, Academic press.

Fonteno, W.C., & Harden, C.T. (2010). North Carolina State University Horticultural substrates lab manual. North Carolina State University, Raleigh, NC.

ВЛИЈАНИЕ НА ПЕРЛИТОТ КАКО СУПСТРАТ ЗА ПОДОБРУВАЊЕ НА НЕКОИ ВОДНИ СВОЈСТВА НА ФЛУВИЈАТИЛНА ПОЧВА СО ПРИМЕНА НА РЕТЕНЦИОНИ КРИВИ

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Резиме

Во овој труд ќе бидат прикажани податоците за потенцијалот на задржување на влагата кај перлитот и флувијатилната почва со методот на Barextractor и Porous plate extractor на 7 различни режими на притисок, при различна тензија од (0,1; 0,33; 1; 3; 6,25; 11; 15 бари), кај перлитот и флувијатилна почва со нивни различни соодноси од 20/80, 30/70, 50/50. Резултатите од кривите на ретенција ќе бидат прикажани графички. Исто така, прикажани ќе бидат и резултати од регресискиот мултиваријантен статистички модел за влијанието на различните варијанти, различниот сооднос во варијантите и нивната интеракција врз различни режими на притисок. Целта на оваа студија е да се презентираат и да се дискутираат експерименталните резултати на содржината на влага и својствата на ефективно задржување на вода кај почвата и супстратот перлит. Под карактеристики на ретенцијата на влага се подразбира односот помеѓу содржина на вода и потенцијалот на вода во почвата /супстратот и може да се претстави со ретенциона крива, таа ја покажува содржината на влага при различна тензија. Овие податоци се корисни особено за ефективната зона на кореновиот систем и наоѓа примена во употреба на вода во почвата или супстратите. Поради добрите физички својства и висок порозитет, експандираниот перлит има улога да го одржува и подобрува водно-воздушниот режим во флувијатилната почва, при што влагата и воздухот ги прави подостапни за растенијата и со тоа влијае позитивно врз почвата.

Клучни зборови: *ретенција на влага, коренов систем, физички карактеристики*

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VARIATION OF MORPHOLOGICAL PROPERTIES IN VIRGINIA TOBACCO TYPES

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Abstract

The properties that identify the type of tobacco, or the variety in one type, are divided into two groups: qualitative and quantitative. Morphological properties belong to the group of quantitative (metric) and are conditioned by a large number of genes with additive effect (minor genes or polygenes**).** These properties are to a largely dependent on the conditions of the external environment and applied agrotechnology during tobacco screening. Tests were performed in 2016 at the Scientific Tobacco Institute - Prilep, on a field experiment in four repetitions with the following varieties: Virginia MV-1 CMS - Control, Virginia McNair-944, Virginia K-394 and a newly prospective line MV-1 / 14 CMS.

The results of the test are processed variationally-statistically through the parameters: mean value ($\bar{x}$), mean error ($S\bar{x}$), standard deviation (S), variation coefficient (CV%), and variation width (WV). The aim of this paper is to present the variation of the more important morphological properties: the height of the plant with the inflorescence, the number of leaves per plant, and the length and width of the largest plant leaf in the varieties concerned. From the studies, we found that the tested varieties are very stable, and they have very little scattering of the morphological properties, since the variation coefficient everywhere showed a value less than 10%. We note that the newly created line MV-1/14 CMS has the slightest variation, while at the same time it has the largest leaves, which is a positive feature in large-scale tobacco of the Virginia type.

Key words: *tobacco, Virginia type, morphological properties, variability*

INTRODUCTION

In the agricultural economy of the Republic of Macedonia, tobacco belongs to the group of strategic cultures. Tobacco cultivation ranges between 12,000 and 15,000 hectares with an annual output of 20 to 25 million kilograms of quality tobacco raw material. From the aspect of the type of representation, over 95% of these areas belong to the oriental types of Prilep and Jaka (type Prilep is most present in our country), while the large (Virginia and Burley), in recent years, are almost encountered in the fields. By the end of the nineties of the last century, although to a lesser extent, they were growing in our country (an average of 1500 tons per year by the Virginia type only), which reduced the import of these tobacco raw materials - the main components in the harmanes for making the more popular "American bland" cigarettes. The type of Virginia in the composition of these cigarettes accounts for 45 to 65% (Mickovski, 2004). According to the author, the largest producers of tobacco of this type in the world are: China, USA, Brazil, Argentina, Italy, Spain, Zimbabwe and Oceania. It is important to point out that in the Republic of Macedonia there are regions with excellent conditions for growing quality tobacco of the type Virginia which is a challenge for its return to production. Having in mind the aforementioned, recent scientists from the Scientific Tobacco Institute - Prilep, create and select varieties that will meet the requirements and needs of the cigarette factories. Therefore, the object and purpose of this study is the variation of the most important

morphological properties of Virgina tobacco type varieties that have good combination skills, which is a condition for creating new, more productive and better quality than existing ones. This would bring back the interest of the producers for this large type of tobacco, which is constantly requested in the world market, after which the financial effects would be positive and guaranteed to the satisfaction of everyone in the tobacco industry of the Republic of Macedonia.

MATERIAL AND METHODS

The tests were conducted on three varieties and a one newly created Virginia-type line: MV-1 CMS as a control variety (Ø), McNair-944, K-394 and the newly created MV-1/14 CMS line. The control variety MV-1 CMS (male-sterile) was created at the Tobacco Institute - Prilep. The Federal Classification Committee of the former Yugoslavia was recognized in 1987. Since then, by the end of the nineties of the last century, it was the only variety of Virginia-type tobacco that was produced in Macedonia and in certain regions in Serbia and Montenegro. The McNair-944 and K-394 varieties are fertile and originate in the United States. In the past, they were fairly represented in Virginia production around the world, and to a lesser extent they are now produced in the home country and some countries in South America. These varieties as well as the control MV-1 CMS are still relevant due to their quality and other positive properties, and are used in the selection for the outward processing of tobacco of this type. The new line MV-1-14 CMS was created at the Scientific Tobacco Institute - Prilep by crossing and selecting Virginia tobacco varieties. The experiment was set in the experimental field of the Scientific Tobacco Institute- Prilep in 2016 on diluvial-colluvial soil in four repetitions. The tobacco is seeded manually at 80x50 cm. For basic fertilization, NPK fertilizer with combination 8:22:20 is used in quantity of 350 kg/ha. During the vegetation, the necessary agrotechnical operations are performed for ensuring normal growth and development of the plants (feeding with nitrogen fertilizer, trapping and treating tobacco according to the program of the Scientific Tobacco Institute - Prilep for protection from diseases, pests and weeds). The tobacco in the experiment is sprinkled 3 times with an average level of 400 m3 / ha water. We note that the 2016 production was assessed as a good year for tobacco production. The studies on morphological properties (height of the plant with inflorescence, number of leaves and length and width of the largest leaf from the middle belt of the plant) were carried out in the field in the "full blossoming" phase of the tobacco by standard methods in the selection, is the mean value ($\bar{x}$), for each property is determined based on 15 randomly selected plants of each variety in the experiment. The obtained data from the measurements are statistically processed through parameters of property variability (Najcevska, 2002), and the results are shown in tables.

RESULTS AND DISCUSSION

The examined morphological features of the Virginia-type tobacco varieties belong to the group of quantitative properties. They are of great importance in the genetics and selection of tobacco because they determine (identify), the type and the varieties they belong to. In addition, the number and size of the leaves determine the yield and quality of tobacco. They are dictated by their own genotype but are also dependent on soil-climatic conditions in the region where it is grown, as well as from applied agrotechnical operation during vegetation. We have mentioned above that we present the results of the researches in tables, especially for each property for better visibility, comparison between the investigated varieties and the new line and drawing appropriate conclusions.

Height of the plant with its inflorescence

The height of tobacco plants is a great feature. Uzunoski (1985), according to this characteristic, divides the tobacco varieties into three groups: 1. Varieties with low growth, the height of which is up to 70 cm (Prilep, low spot); 2. Medium growth varieties with height

of plants with inflorescence, which varies from 70 to 130 cm (Jaka, Dzebel); 3. Varieties of high growth, with a height above 130 cm (Virginia and Burley). Risteski and Kochoska (2004), in their research on 6 varieties of tobacco of the Virginia type, point out that the height of the plant with the inflorescence ranged from 159 cm in the variety V-27/01 to 192 cm in the MV-1 CMS, which was and the highest. The results of our trials for this morphological feature are shown in Table 1.

Table 1. Height of the plant with its inflorescence (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV%	WV
MV-1CMS Ø	15	190	1.76	6.81	3.59	180 - 200
McNair-944	15	158	1.18	4.55	2.88	150 - 165
K-394	15	147	1.27	4.93	3.35	140 - 155
MV-1/14 CMS	15	180	1.24	4.81	2.66	170 - 185

The table shows that the average height of 190 cm is the highest control class MV-1 CMS, and with 147 cm the lowest is the K-394. In terms of variation, it can be noted that the varieties tested are stable in this capacity, since the value of the variation coefficient (CV%) is low (everywhere is below 10%) and ranges from 2.66% to the new line MV-1/14 CMS up to 3.59% in control.

Number of leaves on the plant

The number of leaves of the plant depends on the genetic structure of the variety and the conditions of cultivation. It is thought that the number of tobacco leaves is one of the most stable quantitative properties. The number of leaves is a variegated feature and represents a high-yielding quantitative property (Atanasov, 1972). In their research on the number of leaves of 7 Virginia tobacco varieties Risteski and Kochoska (2014), found that the highest number of leaves of one plant is distinguished by the variety V-88/09 CMS, which on average for the two years of examination had 33.3 leaves, while the least leaves has the K-326 variety (28.8 leaves). Since the multi-year research of 5 Virginia tobacco varieties in the Republic of Croatia, it has been determined that the average number of leaves in the three newly created varieties is as follows: Kutjevo (H 30), 23 leaves, Drava (H 31), 22 leaves and Bilogora (H 32), has 22 leaves (Devcic and Triplat, 1982). In our research with the highest number of leaves (Table 2), is the variety MV-1 CMS, where on average we counted 28 leaves of the plant and with at least McNair-944 with 24 leaves.

Table 2. Number of leaves

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV%	WV
MV-1CMS Ø	15	28	0.32	1.25	4.48	26 - 30
McNair-944	15	24	0.37	1.44	5.97	22 - 27
K-394	15	27	0.30	1.16	4.30	25 - 29
MV-1/14 CMS	15	27	0.23	0.88	3.26	25 - 28

Regarding the variability of this feature, the statistical parameters have shown that it is very small. The coefficient of variation is from 3.26% on the line MV-1/14 CMS to 5.97% in the McNair-944 variety. The standard deviation (S) is also small and ranges from 0.88 to 1.44 leaves.

Length of the biggest leaf of the plant

The length of the leaves in all types of tobacco is an important feature because it is closely related to the quality of the tobacco raw material. In the type Virginia, the larger the leaves the higher the yield and the better the quality is. Boceski (2003), points out that the length and width, and therefore the surface of the leaves during curing, are reduced by 20 to 30%, which is very important in the technology of processing and processing of tobacco. Risteski and Kochoska (2014), examining the length of the leaves in 7 domestic and foreign varieties of the Virginia type, state that with the largest 10 leaves (the 10th leaf is the largest of the plant) is the variety V-79/09 CMS, with the length of

the leaf of 62.4 cm, and with the smallest leaves, with a length of 44.3 cm is the Delcrist variety. The K-394 variety is on the fifth place with an average length of the 10th leaf of 52.5 cm. The results of our measurements and the variability of this property are shown in Table 3.

Table 3. Length of the biggest leaf (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV%	WV
MV-1CMS Ø	15	60	0.32	1.22	2.04	58 - 62
McNair-944	15	57	0.34	1.33	2.34	55 - 59
K-394	15	56	0.34	1.31	2.34	54 - 58
MV-1/14 CMS	15	62	0.25	0.96	1.55	60 - 63

The subject varieties have long leaves which are characteristic for large-scale tobacco, including the type Virginia. The longest leaves of the plant have the new line MV-1/14 CMS ($\bar{x}$=62 cm), with the smallest is K-394 ($\bar{x}$=56 cm). The standard deviation is from 0.96 in the new line to 1.33 in McNair-944, with a CV% within 1.55 to 2.34%, so it can be said that the variability is meaningless.

Width of the largest leaf of the plant

The width of the leaves and the length depends on the soil and climate conditions and technical measures during the cultivation of tobacco. Drazic et al. (2012), examining the morphological properties and yield of 12 newly created genotypes (7 in the Republic of Serbia and 5 in Republic of Macedonia), concluded that with the widest leaves the variety Hevesi 9 ($\bar{x}$=36 cm) was standard in the experiment. Of the new genotypes, with an average width of the largest leaf of 34 cm, the V-814 took the second place, while with a width of 24 cm, the genotype V-30/09 was in the last place. From our examinations we obtained the results we present in Table 4.

Table 4. Width of the biggest leaf (cm)

Varieties	n	$\bar{x}$	$S\bar{x}$	S	CV%	WV
MV-1CMS Ø	15	31	0.31	1.19	3.81	30 - 34
McNair-944	15	27	0.30	1.16	4.30	25 - 29
K-394	15	29	0.30	1.16	4.00	27 - 31
MV-1/14 CMS	15	32	0.33	1.28	3.99	30 - 34

The table shows that on the average with the widest leaves is the line MV-1/14 CMS ($\bar{x}$=32 cm) and it is followed by the control with 31 cm, K-394 with 29 cm, while with the narrowest leaves is McNair-944 with width of the largest leaf of 27 cm. The standard deviation ranges from 1.16 to 1.28, while CV% of 3.81 in control to 4.30 at McNair-944.

CONCLUDING REMARKS

The highest altitude is the control type MV-1 CMS ($\bar{x}$=190 cm), and the lowest is K-394 ($\bar{x}$=147 cm). The variation coefficient showed that with the slightest variation in this property is the line MV-1/14 CMS (CV=2.66%).

At least leaves has McNair-944 (an average of 24), while with 28 leaves the control is in the first place. The newly created line and variety K-394 have on average 27 leaves each. The variation coefficient yielded values that show the stability of this feature in the tobacco varieties concerned.

Variation of the length of the largest plant leaf is the lowest in the line MV-1/14 CMS (CV=1.55%).

From the measurements we found that with the widest leaves is the new line ($\bar{x}$=32 cm), and with the narrowest is McNair-944 ($\bar{x}$=27 cm).

The tested Virginia type tobacco types are genetically stable, and the variation of the morphological properties is insignificant (CV is everywhere below 10%), which means they are a good material for the improvement and creation of new varieties. The new line MV-1/14 CMS is stable, with more positive properties, so it is expected to be included in the National Variety List of the Republic of Macedonia.

REFERENCES

Atanasov, D. (1972). *Tobacco producing*. ITTI Plovdiv.

Boceski, D. (2003). *Knowledge and processing of tobacco*. Tobacco Institute-Prilep, II additional edition.

Devcic, K., & Triplat, J. (1982). Some properties of the new flue-cured varieties created in Tobacco institute in Zagreb. *Tutun/Tobacco*, 32(11-12), 5-10.

Drazic, S., Risteski, I., Filiposki, K., & Kocoska, K. (2012). Results of studies on recently developed Virginia tobacco genotypes under different growing conditions in Serbia. *Tutun/Tobacco,* 62(1-6), 3-8.

Mickovski, J. (2004). *Tobacco in Republic of Macedonia*. Society for Science and Art-Prilep.

Najcevska, C. (2002). *Experimental statistics applied in agricultural and biological research*: *Variability of the properties of living organisms – research and statistical analysis*. Bona–Skopje.

Risteski, I., & Kocoska, K. (2005). *Results from a comparative experiment on Virginia tobacco*. Report on the project tasks of the scientific and research work in 2004, University "St. Kliment Ohridski" - Bitola, Tobacco Institute – Prilep, 26-31.

Risteski, I., & Kocoska, K. (2014). *Results from a comparative experiment with Virginia tobacco varieties and lines*. Report on the work of the Scientific Tobacco Institute-Prilep for 2012, University "St. Kliment Ohridski" - Bitola, Scientific Tobacco Institute-Prilep, 25-30.

Risteski, I., & Kocoska, K. (2014). Results of the investigations of some bio-morphological traits of Virginia tobacco varieties and lines in the producing region of Prilep in 2010 and 2011. *Tutun/Tobacco*, 64(1-6), 28-36.

ВАРИРАЊЕ НА МОРФОЛОШКИТЕ СВОЈСТВА КАЈ СОРТИ ТУТУН ОД ТИПОТ ВИРЏИНИЈА

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Резиме

Својствата што го идентификуваат типот на тутунот, односно сортата во еден тип, се поделени во две групи: квалитативни и квантитативни. Морфолошките својства спаѓаат во групата на квантитативните (метрички) и се условени од поголем број гени со адитивен ефект (минор гени или полигени). Овие особини во голема мерка се зависни од условите на надворешната средина и применетата агротехника за време на одгледувањето на тутунот. Испитувањата се извршени во 2016 година во Научниот институт за тутун – Прилеп, на полски опит во четири повторувања, со следниве сорти: *вирџинија MB-1 (ЦМС)* – контрола, *Virginia McNair-944, Virginia K-394* и една новосоздадена перспективна линија *MB-1/14 – ЦМС*. Резултатите од испитувањето се обработени варијационо-статистички преку параметрите: средна вредност ($\bar{x}$), грешка на средната вредност (S$\bar{x}$), стандардна девијација (S), варијационен коефициент (CV - %)и варијациона ширина (WV).

Целта на овој труд е да го прикажеме варирањето на поважните морфолошки својства: височина на растението со соцветие, број на листови на едно растение и должина и широчина на најголемиот лист на растението кај предметните сорти.

Од истражувањата потврдивме дека испитуваните сорти се многу стабилни, односно варирањето на морфолошките својства им е многу мало, бидејќи варијациониот коефициент секаде покажа вредност помала од 10%. Истакнуваме дека новосоздадената линија MB-1/14 – ЦМС има најмало варирање, истовремено има и најголеми листови, што е позитивно својство кај крупнолисниот тутун од типот вирџинија.

Клучни зборови: *тутун, тип вирџинија, морфолошки својства, варијабилност*

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THE EFFECT OF PRUNING ON FRIUTING CAPACITY OF MICHELE PALIERI TABLE GRAPE VARIETY GROWING IN TIKVES VINEYARD

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Abstract

The subject of research was the fruiting capacity measured by the table grape variety Michel Palieri, grown in the Tikves vineyards. The aim of this paper was to determine the effect of various types of pruning on fruiting capacity of Michel Palieri variety, and no research have been done for this variety so far. The research lasted three years (2014, 2015 and 2016), and three pruning variants were used (16, 20 and 24 buds/vine).

Different values were obtained for the examined elements primarily as a result of the varietal specificity and the extent of the load of the vines with the native buds. In the years of examination, the percentage of fruiting buds in all variants is quite stable, with insignificant variations.

The coefficient of variation ranges from 2.74 (variant II) to10.24 (variant III). The average number of bunches per fruiting canes (absolute coefficient) is in the range of 1.3 to 1.4 and with high stability both in variants and in the years of examination.

The average mass of bunches ranges from 401 g (variant II) to 492 g (variant III). After years, significant variation in variants I (28.84) and III (20.25) was observed in the mass of bunches.

Key words: *table grape, pruning, fertility, mass of bunches*

INTRODUCTION

The grapevine is a perennial plant, which remains on the projected vineyard plantation or in one place for almost 25-30 years depending on the correct application and utilization of numerous agrotechnical, ampelotechnical, agrobiological and agro-ecological measures. The grape is one of the most important agricultural crops in the world and special attention is paid to improving the yield and quality of the grapes (Bruhn et al., 1991). In the recent years, the Tikvesh wine-growing region in Macedonia has been introducing a large number of newly created varieties with different agrochemical and technological features. So far, these varieties have not been studied from the aspect of their adaptation to the conditions in this region in Macedonia. Therefore, the subject of this paper was to study in details the effect of the pruning and fruiting capacities of Palieri, one of the large number of newly introduced table grape varieties in the Republic of Macedonia.

The Pallieri table grape variety was discovered by Michel Palieri in Velette, a famous area around Rome, Italy, obtained by crossing Alfonso Labale and Red Malaga. In the grape, there are an average of 2 seeds.

The yield moves in the interval between 15-20 tons / ha. The period of awakening is the third decade of March, and the period of maturation is early September, which classifies it as a late grape variety. The vine is very lush with big, large leaves. The grapes are large, cylindrical - pyramidal, winged, very loose with a mean weight of 450-500 grams. The grain is quite large with a weight of 7 - 8 grams, oval or slice. It is medium resistant variety with large ash powder crop with dark - purple colour. Mesothelium is juicy, sweet in taste, neutral, with sugar content of 14 - 15%. Up to - 15°C the buds do not freeze. Recommended grafting pads for Palieri are: Kober 5 bb, SO4, 5C, Telescope - 8B, Shasla x Berlandieri 41 b. Palieri is a variety with a high-transportability (Z.Bozhinovic 2010).

MATERIAL AND METHODS

Field studies were carried out in the locality "Gornicki" - CA Marena for the table variety Palieri with: Spalding, construction and placement of the rows in the east-west direction, with length of the rows 120 m.

In the studied vineyard plant, the experiment was aimed at determining the influence of the two-fold Gyiv system of pruning with different lengths of the fruiting canes and the impact of the load on the native buds on the yield and the fertility of the buds (V. Dimovska 2011). The vineyard was planted in 2007, with a distance between the vines of 2.30 m and vines in the row of 1 m, with the use of the two-fold Gyiv system of pruning. Palieri was grafted on the Kober 5 BB vineyard, the soil type is a degraded deluvium. In the vineyard there is drip irrigation system, regular feeding and regular use of these agro-technical measures: pruning of mature or cutting out of the variety, download and export of the cut out, fruiting canes, tying, protection from diseases and pests, fertilization, irrigation, etc.

In terms of the experimental location, this variety is in intensive production with regular application of agricultural practices. The necessary green ampelotechnical operations that are carried out during the vegetation are: green pruning, defoliation, punching, barking, and secretion of the canes. Defoliation of this variety is very characteristic by the fact that moderate removal of the leaves at the base of the fruiting canes is carried out, better ventilation and creating a favourable microclimate around the bunches, which enables improvement of the nutritious organic assimilates of the grain and better maturing of the grapes.

In the vineyard, the experiment was set by the selection case method, and the health status and uniformity of the vegetative potential of the vine was good. The three variants of the pruning during the trials were studied on M. Palieri table grape variety (16, 20 and 24 buds per vine (Table 1)). The experiment was set to 30 vines in three iterations for each variant (10 iteration vines), or a total of 90 vines for all variants.

Table 1. The experiment covers the following variants of pruning:

Variants	Pruning	Number of short canes	Number of long canes	Number of buds per vine
Variant I	Short	2x2	2x6	16
Variant II	Control	2x2	2x8	20
Variant III	Long	2x2	2x10	24

The fruiting capacity of the tested varieties was determined and expressed per vine, variant and repetition,

- Untreated buds (%);
- Buds developed in canes (%);
- Native buds (%);
- Fruiting Capacity Coefficient: Potential, Relative and Absolute;
- Productivity of buds (grape weight per vine (g) and
- Average mass of a bunch (g).

The obtained results were statistically processed and represented by a coefficient of variation and standard deviation.

RESULTS AND DISCUSSION

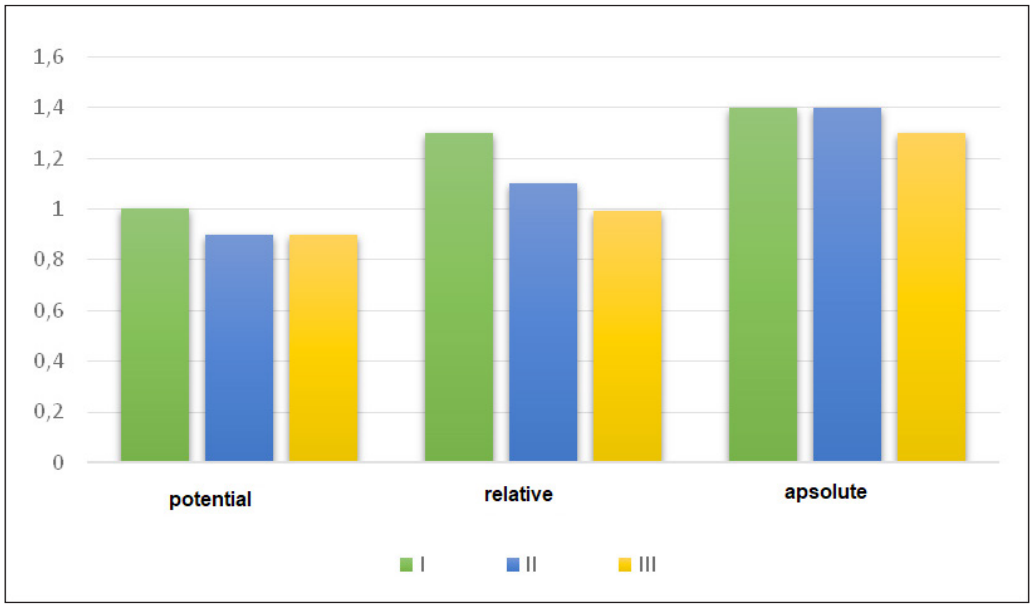
The fruiting capacity of the varieties is an important economic feature and is determined by the fruiting capacity of the buds and the yield. In a large number of table grape varieties, the first 2 to 3 buds per fruiting cane are very small or not treated at all. Therefore, the fruiting capacity of the buds or canes in vegetation begins to manifest itself in the second or third and even in some varieties the fourth bud, so it is the basic and only reason why mixed or long

pruning is applied to table grape varieties (M. Delic at. al 2017). The obtained results for the fruiting capacity elements of the M.Palieri variety are given in Table 2. Per variants, for all elements of fruiting capacity (untreated, non-native and native eyes), have statistically significant variation in variant III (2x10 + 2x2). Individually, in all variants, statistically significant variation is found in % of untreated buds ranging from 20.6% (variant I) to 25% (variant II) with a variation coefficient of 10.20 (variant II) to 28.12 (variant III). This is the result of the variety and variant, i.e., the number of buds per vine. While in % of native buds, as per variants and so for years, high stability with insignificant variation has been established.

The results obtained for the fruiting capacity coefficients: the potential (number of canes per bud), the relative (the number of bunches per developed bud in the cane) and the absolute coefficient (number of bunches by the native bud) are presented graphically (Graph 1). For a better comparison, the graph gives the average values of the coefficients of fruiting capacity per varieties. The values for all three fruiting capacity coefficients, by variants and years of testing are quite stable, and the differences are statistically insignificant, i.e. with a small coefficient of variation. This suggests that the variety and the way of pruning (the different load on the vine with the native buds), have no effect on the fruiting capacity coefficients of the Palieri variety.

Table 2. Elements of buds fruiting capacity per variants

Variant	Year	Untreated buds (%)	Non-native buds (%)	Native buds (%)
I (2 x 6+2 x 2)	2014	25.0	13.8	60.0
	2015	22.5	16.3	61.3
	2016	14.4	16.3	69.4
	2015/2016	*20.6*	*15.5*	*63.6*
	CV%	26.8	9.33	8.01
	SD	5.54	1.44	5.09
II (2 x 8+2 x 2)	2014	24.5	21.5	54.0
	2015	23.0	20.0	57.0
	2016	28.0	16.0	56.0
	2015/2016	*25.2*	*19.2*	*55.7*
	CV%	10.20	14.83	2.74
	SD	2.57	2.84	1.53
III (2 x 10+2 x 2)	2014	28.3	12.5	59.2
	2015	25.0	21.7	53.3
	2016	15.8	18.8	65.4
	2015/2016	*23.0*	*17.7*	*59.3*
	CV%	28.12	26.62	10.24
	SD	6.48	4.70	6.05



Graph 1. Fruiting capacity coefficient

Table 3. Productivity of the buds in kg: Variant 1 (2x6 + 2x2)

Year	Bud					
	I	II	III	IV	V	VI
2014	0.574	0.738	0.902	0.779	0.902	0.328
2015	0.354	0.561	0.590	0.561	0.502	0.266
2016	0.746	0.533	1.013	0.853	1.013	0.959
2015/2016	0.558	0.610	0.835	0.731	0.810	0.520
CV%	35.21	18.20	26.27	20.77	33.36	74.07
SD	0.20	0.11	0.22	0.15	0.27	0.38

Table 4. Productivity of the buds in kg: Variant 2 (2x8 + 2x2)

Year	Bud							
	I	II	III	IV	V	VI	VII	VIII
2014	0.830	0.568	0.656	0.612	0.787	0.656	0.743	0.524
2015	0.356	0.570	0.570	0.570	0.392	0.712	0.427	0.178
2016	0.410	0.656	0.697	0.533	0.574	0.615	0.738	0.492
2014/2016	0.532	0.598	0.641	0.572	0.584	0.661	0.636	0.398
CV%	48.78	8.40	10.11	6.91	33.83	7.37	28.46	48.04
SD	0.26	0.05	0.06	0.04	0.20	0.05	0.18	0.19

Table 5. Productivity of the bud in kg: Variant 3 (2x10 + 2x2)

Year	Bud									
	I	II	III	IV	V	VI	VII	VIII	IX	X
2014	0.561	0.673	1.009	0.842	0.785	0.449	1.066	0.842	1.122	0.617
2015	1.130	0.699	0.861	0.699	0.807	0.699	0.753	0.807	0.646	0.484
2016	0.680	0.643	0.529	0.454	0.529	0.605	0.756	0.567	0.794	0.529
2014/ 2016	0.790	0.672	0.780	0.665	0.707	0.584	0.858	0.739	0.854	0.543
CV%	37.97	4.17	30.74	29.51	21.86	21.61	20.95	20.27	28.53	12.45
SD	0.30	0.03	0.25	0.20	0.15	0.13	0.18	0.15	0.24	0.07

Fruiting capacity of the buds represents a mass of grapes per bud along the length of the fruiting cane. An important element on the basis of which we can determine the length of the fruiting cane during the pruning and the number of the native eyes (Tadijanović, Đ., 1993).The obtained results for the productivity of the buds are given by variants:

- Table 3 – Variant I (2x6 + 2x2),
- Table 4 – Variant II (2x8 + 2x2),
- Table 5 – Variant III (2x10 + 2x2).

Based on the results obtained in Table 3, in variant I (2x6 + 2x2) we concluded that the productivity of the buds increases from the first (0.558 kg) to the fifth eye (0.810 kg). Per years, statistically significant variation occurs in the first and sixth bud, where the coefficient of variation ranges from 35.21 (first eye) to 74.04 (sixth eye). This is due to the varietal characteristic i.e. the first eye with the lowest fruiting capacity (number and mass of bunches).

The results for the productivity of the bud in variant II (2x8 + 2x2) are given in Table 4. In this variant, the productivity of the eyelids along the length of the fruiting cane is quite stable from first to the seventh bud, and significantly decreases to the last, the eighth bud. It ranges from 0.532 kg (first bud) to 0.636 kg (seventh bud) average for the study period (2014/2016). In terms of years of testing, the largest variation is observed in the first bud where the coefficient of variation is 48.78. This is due to the varietal characteristic i.e. the first bud with the lowest fruiting capacity (number and mass of bunches).

In Table 5, the results for the productivity of the bud in variant 3 (2x10 + 2x2) are presented. The productivity of the buds along the length of the fruiting cane to the ninth bud is quite stable, and it is significantly decreasing in the tenth bud. After years of testing, in this variant as well, the largest variation was found at the first bud, where the coefficient of variation is 37.97.

The average mass of the bunch ranges from 413 g (variant I) to 492 g (variant III), and after years of testing, a minimum mass of 295 g was obtained in variant I in 2015 and a maximum of 561 g in variant III. The results obtained for the mass of the bunches in our research are significantly higher than the results of Palieri grown in Podgorica-Montenegro (PejovicLj., Vesna Maras, 1998), where the mass of the bunch ranges from 226 g to 374 g. According to the average mass, bunches of all varieties fall into the group of varieties with very large bunches (> 400 g, Bozhinovic, 2010).

Table 6. Mass of bunches (g) by variants and sub-variants

Variant	Year	Mass of a bunch (g)
I (2 x 6+2 x 2)	2014	410
	2015	295
	2016	533
	2014/2016	413
	CV%	28.84
II (2 x 8+2 x 2)	2014	437
	2015	356
	2016	410
	2014/2016	401
	CV%	10.29
III (2 x 10+2 x 2)	2014	561
	2015	538
	2016	378
	2014/2016	492
	CV%	20.25
	min	295
	max	561

CONCLUSION

The biological potential, that is, the varietal characteristics and the degree of the load of the vines with native buds (16, 20 and 24 buds per vine), affect the fruiting capacity of the buds in the variety Michel Palieri.

From the examined elements of fruiting capacity, % of native buds, both by variants and after years of examination, there was a high stability, i.e. no statistically significant difference was noted.

The fruiting capacity coefficients: the potential (number of bunches per bud), the relative (the number of bunches per developed bud in the cane) and the absolute coefficient (number of bunches per native bud), by variants and years of examination are quite stable. The variety and method of pruning (the different load of the vine with the native buds) have no effect on the coefficients of fruiting capacity of the variety Palieri.

The variety has an impact on the first bud productivity of all variants after years of investigation where high variability or high values for the coefficient of variation are noted. Bunches of all varieties, according to the average mass, fall into the group of varieties with very large bunches (> 400 g) which meets the quality criteria for table grapes of this group.

REFERENCES

Dimovska, V., Ivanova V., Ilieva F., Sofijanova E., & Kletnikoski P. (2011). The State of Table Grape Varieties in R. Macedonia. International scientific on-line journal "Science & Technologies", I (6). pp. 30-34. ISSN 1314-4111

Bruhn, C.M., Feldman, N., Garlitz, C., Harwood, J., Ivans, E., Marshall, M., Riley, A., & Williamson, M. (1991). Consumer perceptions of quality: Apricots, peaches, pears, strawberries, and tomatoes. J. Food Qual., 14:187–195.

Maletić, E., Karoglan Kontić, J., & Pejić, I. (2008). Vinova loza - ampelografija, ekologija, oplemenjivanje. Školska knjiga, Zagreb.

Nicolaescu, Gh., Derendovskaia A., Perstniov, N., Stirbu, A., Tcaciuc. O., Nicolaescu. A., Ciobanu, T. & Josan, S. (2009). Gibberellin – as a determinant factor of grapes quality of Codreanca (Black Magic) variety. Lucrărişti inţifice USAMUB, Seria B, LIII, 573 – 576.

Bozinovc, Z. (2010). Ampelografija. Agrinet. Skopje.

Tadijanović, Đ. (1993). Oblici čokota i rezidba sa planiranjem prinosa vinove loze. Nolit – Beograd.

Mersija Delić, F., Behmen, S., Sefo, Drkenda, P., Dimovska, V. & Sunulahpašić, A. (2017). The effect of pruning on fruiting capacity of Black Magic table grape variety. Works of the Faculty of Agriculture and Food Sciences, University of Sarajevo, Vol. LXII, N0 67/2, 146-153.

Piña, S., & Bautista, D. (2006). Evaluation of vegetative growth on several tablegrape cultivars under semiarid tropic conditions in Venezuela. Rev. Fac. Agron. (LUZ), 23: 402-413.

Pejovic, Lj., & Maras, V. (1998). Rezults of three-year studying of table vine varieties Muskatplevenski, Pleven and Palieri 5 in agro-ecological conditions of Podgorica (Montenegro). Poljoprivreda i sumarstvo, 44(1-2):1-153.

Piras, F., Lovicu, G. & Zurru, R. (2014): Observation on some agronomic traits of cultivar of table grapes. ActaHort., 1032, 243-251.

ВЛИЈАНИЕ НА РЕЗИДБАТА ВРЗ РОДНОСТА НА ТРПЕЗНАТА СОРТА МИШЕЛ ПАЛИЕРИ, ОДГЛЕДУВАНА ВО ТИКВЕШКОТО ВИНОГОРЈЕ

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Резиме

Предмет на истражувањето беше сортата на трпезно грозје *мишел палиери*, одгледувана во тиквешкото виногорје.

Целта беше да се утврди влијанието на начинот на резидба врз елементите на родност кај сортата *мишел палиери*, со оглед на тоа што до сега не се направени истражувања за оваа сорта кај нас. Истражувањето траеше три години (2014, 2015 и 2016 година), а беа користени три варијанти на резидба (16, 20 и 24 окца/лоза).

Добиени се различни вредности за испитуваните елементи, пред сè, како резултат на сортната специфичност и степенот на оптоварување на лозите со родни окца.

Во годините на испитување процентот на родни окца кај сите варијанти е достастабилен и со незначителни варирања. Коефициентот на варирање се движи од 2.74 (варијанта 2) до 10.24 (варијанта 3). Просечниот број на гроздови по роден ластар (апсолутен коефициент) е во границите од 1.3 до 1.4 и со висока стабилност, како по варијанти така и во годините на испитување. Просечната маса на гроздовите се движи од 401 g (варијанта II) до 492 g (варијанта III). По години, кај масата на гроздови е констатирано значително варирање кај варијантите 1 (28.84) и 3 (20.25).

Клучни зборови: *трпезно грозје, резидба, родност, маса на грозд*

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GENETIC AND ENVIRONMENTAL EFFECT ON THE GRAIN YIELD OF SPRING BARLEY VARIETIES CULTIVATED IN THE REPUBLIC OF MACEDONIA

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Abstract

The aim of this paper was to evaluate the genetic and environmental effect on the grain yield of spring barley varieties cultivated in the Republic of Macedonia.

Five spring barley varieties (Makedo, Xanadu, Josefin, Gladys and Scarlet) were used as an experimental material. Makedo is Macedonian variety and the other barley cultivars are imported. The field experiments were carried out during 2013 and 2014 on the field areas in Probistip, Republic of Macedonia. The experiment design was randomized complete block with three replications. The results were analyzed to synthesize the relative proportion of the influence factor (η %) such as variety or year, as well as the interaction between variety and year on the grain yield. Besides the year conditions, the impact of variety contributed significantly to the overall variability of yield (over 98 %), compared to the influence of year and the interaction between variety and year. Makedo variety showed the highest value for grain yield (6 844 kg/ha), followed by Xanadu (6 638 kg/ha). The average grain yield for all tested varieties for both years of examination was 6 544 kg/ha. Also, Makedo variety has the highest values for number of spikes per m^2 and the number of grain per spike.

Key words: *grain yield*, *barley*, *variety*, *yield components*

INTRODUCTION

After wheat, rice and maize, barley is the fourth most significant crop (Abebe, 2010). Barley is used as a livestock feed, for malt and for preparing foods. Among the cereal crops, barley is a species with the greatest adaptability to a wide range of environments. Barley is cultivated from arctic latitudes to tropical areas, grown at the highest altitudes and adapted to specific sets of agro-ecological areas (Alemayehu and Parlevliet, 1997).

In Republic of Macedonia barley and wheat are the principal cereal crops. In 2016, barley production was 144 832 t with average grain yield of 3 507 kg/ha (FAOSTAT, 2016). On the National variety list in the Republic of Macedonia, besides autumn forms of barley, only one variety (Makedo) is registered as a spring domestic variety (MAFWE, 2008).

Successful growing of spring barley depends on many factors. Productivity is the final result of the effect and interactions of several yield-related traits, which are basically polygenic, (Madić et al., 2014). The grain yield and quality traits of barley are determined by its genetic makeup and environment conditions during growth, harvest and storage. Variation of yield and major chemical components of barley grain is genetically controlled (Eagles et al., 1995), but it is also influenced by environmental factors (Helm, 1992; Paynter and Young, 2004).

Also, numerous tests have been performed which confirmed that proper and balanced cereals diet is essential to improve the yield and quality and can directly or indirectly affect the efficiency of the other agro-technical measures (Popescu et al., 1997). Basic nutrients, such as

nitrogen, potassium, sulphur and magnesium are crucial elements in many processes in the development of the plant and the formation of the yield (Randahwa and Arora, 2000), but besides this elements, microelements play a large role in the quality of final product as well. Awasti and Brahm (1994) reported that barley yield was increased by increasing the dose of nitrogen.

The yield formation can be defined as the interaction effect of soil and climatic conditions, genotype, fertilization and growing technology (Barczak and Majcherczak, 2008). Grain yield is made up of three main different yield components, the number of spikes, the number of grains per spike and the thousand-kernel weight. According to Kavitha et al., (2009), Sukram et al., (2010) productive tiller per plant, number of grains per spike and 1 000 grains weight would be more useful criteria for selecting high yield barley varieties.

The objective of this study was to determine the genetic potential and the influence of variety, environment and their interaction on grain yield in spring barley varieties, grown in Macedonia.

MATERIAL AND METHODS

Plant material and experimental design

The experiment was carried out on the field areas in Probistip, Republic of Macedonia. Probistip is an urban municipality in eastern part of the Republic of Macedonia, located at 42°00'11" N and 22°10'42" E, with an elevation above sea level of 589 m. The annual mean temperature of this city is 10°C with relative humidity around 70 % and average annual rainfall of 450 - 500 mm.

Field trials were conducted during two years (2013 and 2014). Five spring barley varieties (*Makedo*, *Xanadu*, *Josefin Variety*, *Gladys* and *Scarlet*) were used as an experimental material for this study. Only *Makedo* variety is Macedonian and the other barley cultivars are imported. The experimental material was placed by using randomized block design in three replications. The size of experimental plots was 5m^2.

In 2013 the sowing was done on 19 March and in second testing year on 17 March. The sowing was made by hand with 20 cm space within rows. The standard growing measures were applied during the vegetation. The harvest was done by hand also.

Data collection

To quantify the yield, we measured its three most important components, respectively the number of spike per m^2, the number of grain per spike and 1 000 grain weight.

Ten randomly selected plants from each repetition have been analyzed for the plant height (cm) and number of grains per spike. The number of spike per m^2 was determined by counting the plants from m^2 of each repetition. 1 000 grains weight, has been determined to measure 1 000 grains of each repetition. Grain yield obtained from the 5 m^2 was calculated in kg/ha.

Statistical analysis

For the analysis of variance (ANOVA) the statistical package SPSS (2010) was used. Least significant difference (LSD) was calculated using Statistical analysis system software JMP (2002).

RESULTS AND DISCUSSION

Due to short vegetation period extending for about 100 days and poorly developed root system, spring barley is very sensitive to drought stresses, even if they are temporary (Pecio and Wach, 2015). Increasing temperature, even without significant changes in precipitation may cause the deepening of the already negative water balance (Kozyra et al., 2009).

In our study, meteorological data do not differ for both years of testing. The average air temperature during the first year of study was 29,6° C, while for the second testing year was 29,2° C. This means that the temperature values were favourable for optimal plant development and high yield.

Figure 1 illustrates the average, lowest and the highest values of grain yield of the tested barley varieties in the period of study. Furthermore, Figure 1 presents that *Makedo* variety has the highest average value for grain yield and in the same time had the lowest rang of variation, followed by *Xanadu* and *Gladys*. The lowest grain yield was record by *Scarlet* variety.

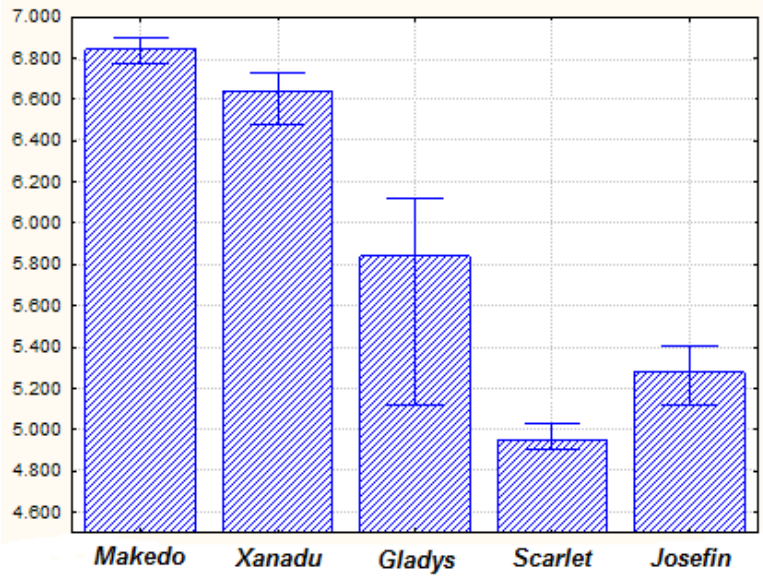

Figure 1. Average values and rang of variation for grain yield (kg/ha) at barley varieties during the period of study

Barley varieties are known to have different yield potentials which depend on many factors (Fettel, 1999; Mackenzie, 2005).

In Table 1 are given the average values for grain yield and some yield-related traits of barley varieties during the study period. LSD test showed significant difference between tested varieties and traits.

Table 1. Average values for grain yield and some yield-related traits during the period of study

Variety	Plant height (cm)	Number of spike per m^2	Number of grain per spike	1 000 grain weight (g)	Grain yield (kg/ha)
Makedo	64,8c	672a	22a	49,4a	6 844a
Xanadu	64,4c	617c	20b	43,5c	6 638a
Gladys	68,8b	576d	20b	50,1a	5 841a
Scarlet	69,8b	638b	20b	46,3b	4 949b
Josefin	76,3a	517e	20b	45,8b	5 279ab
Mean	**68,8**	**604**	**21**	**47,1**	**6 544**
$LSD_{0.05}$	**1,32**	**20,32**	**1,05**	**1,81**	**1 922,3**
CV (%)	**1,05**	**1,85**	**2,80**	**2,12**	**18,72**

Our research shows that Josefin variety has the highest value for plant height (76,3 cm), followed by Scarlet variety (69,8 cm). No significant differences were found between Makedo and Xanadu variety. Plant height has the lowest coefficient of variation (1.05 %).

Number of spike per m^2 plays important role in yield formation. LSD test show that all tested varieties significantly differ and cultivars belong to different group (Table 1). Makedo variety had the largest number of spike per m^2 (672) and the lowest was notes by Josefin cultivar. The mean value for this trait during the period of study was 604.

Number of grain per spike is one of the main yield-related components. According to LSD, there were not significant differences between tested barley varieties except for Makedo variety. This cultivar has the largest number of grain per spike (22) and the mean value for this property was 21 grains per spike for all analyzed varieties.

1 000 grain weight is a quality trait and it is indicator for the size and grain thickness. This property depends on variety genetic, but also of environmental conditions. On the other hand, 1 000 grain weight is less sensitive to climatic factors compared to grain yield. In this research

1 000 grain weight ranges from 43,5 g to 50,1 g, with average value of 47,1 g. *Gladys* variety had the highest average value (50,1 g), followed by Makedo (49,4 g). Bleidere (2008) also reported similar values for this trait.

From all tested barley varieties, the genetic potential of grain yield, mostly come to expression at Makedo variety. Macedonian variety showed 6 844 kg/ha average grain yield, followed by *Xanadu* (6 638 kg/ha). The average grain yield for all tested varieties for both years of examination was 6 544 kg/ha. According to Andrejčíková et al., (2016) the average value for grain yield during period of study (2011-2012) was 7 178 kg/ha.

In order to see how changes of environmental conditions and variety influence to the grain yield, it is necessary to assess the interaction between variety and the environmental conditions. Today, there are many papers in which the influence of variety, year conditions and their interactions on grain yield is explained (Markova Ruzdik et al., 2015). According to the results of ANOVA (Table 2), grain yield was significantly affected by the variety (98.79 %). The effect of year conditions and the interaction between variety and year had lower impact on grain yield (0.08 % and 1,13 %, successively).

Table 2. The influence of variety, year and their interaction on grain yield

Factor	Sum of Squares	df	Mean Squere	F	η
Total	17,204	30			
Factor (A) - variety	16,363	4	4,091	127,734	**98,79***
Factor (B) - year	0,013	1	0,013	0,399	0,08
A x B	0,187	4	0,047	1,462	1,13
Error	0,641	20	0,032		

CONCLUDING REMARKS

From the performed research it can be concluded that Makedo variety had the highest value for grain yield, followed by Xanadu variety. Also, Makedo cultivar had the highest number of spike per m^2 and the number of grain per spike. These results showed that Makedo variety is suitable for cultivation and should be more present in barley production. The study had proved that the variety has the strongest impact over grain yield during the period of research.

Except Makedo variety, also Xanadu and Gladys cultivars can be introduced in barley production or to be chosen as the most suitable varieties for new parents in any future breeding process, in order to get the new high yielding varieties suitable for cultivation in Republic of Macedonia.

REFERENCES

Abebe, T.D. (2010). *Genetic diversity and population differentiation analysis of Ethiopian barley (Hordeum vulgare L.) landraces using morphological traits and SSR markers*. Wellega, Ethiopia.

Alemayehu, F., & Parlevliet, J.E. (1997). Variation between and within Ethiopian barley landraces. *Euphytica*, 94, 183-189.

Andrejčíkova, M., Macàk, M., & Habàn, M. (2016). Yield potential of spring malting barley (*Hordeum vulgare* L.) varieties in the growing conditions of south-western Slovakia. *Journal of Central European Agriculture*, 17(4), 932-940.

Awasti, U.D., & Brahm, S. (1994). Physiological response of barley genotipes to nitrogen levels under moisture scarce conditions on light extrude soils of Central Utter Pardash. *Indian Journal of Plant Physiology*, 37, 32-34.

Barczak, B., & Majcherczak, E. (2008). Effect of varied fertilization with sulfur on selected spring barley yield structure components. *Journal of Central European Agriculture*, 9 (4), 777-784.

Bleidere, M. (2008). Genetic and environmental effects on the grain quality of spring barley. *Latvian Journal of Agronomy*, 11,

33-38.

Eagles, H.A., Bedggod, A.G., Panozzo, J.F., & Martin, P.J. (1995). Cultivar and environmental effects on malting quality in barley. *Australian Journal of Agricultural Research*, 46, 831-844.

FAOSTAT (2016). World crop production. Published online ahttp://www.faostat.fao.org.

Fettell, N.A., Moody, D.B., Long, N., & Flood. (1999). Determinants of grain size in malting barley. In ''9th Australian Barley Technical Symposium". Melbourne, Victoria. (Eds. D. B. Moody, R. G. Flood) (The Regional Institute Ltd).

Helm, J.H. (1992). Cultivar and growing location effects on composition of barley grains, Barley for food and malt. Proceedings of International symposium, September 7-10, Sweden, 308-312.

JMP (2002). Version 5.0 1a, A Business Unit of SAS 1989 - 2002 SAS Institute Inc.

Kavitha, G., Dhindsa, G.S., Singh, S., & Singh, J. (2009). Stability analysis of yield and its components in malt barley (*Hordeum vulgare* L.) genotypes. *Journal of Crop Improvement,* 36(1), 29 – 34.

Kozyra, J., Doroszewski, A., & Nieróbca, A. (2009). Climate change and its expected impact on agriculture in Poland. *Studia i Raporty IUNG-PIB*, 14, 243-257.

Madić, M.R., Djurović, D.S., Knezević, D.S., Paunović, A.S., & Tanasković, S.T. (2014). Combining abilities for spike traits in a diallel cross of barley. *Journal of Central European Agriculture*, 15 (1), 108-116.

MAFWE (2008). *National variety list of Republic of Macedonia*. RI Grafica, Skopje, Republic of Macedonia.

Markova Ruzdik, N., Vulcheva, D., Mihajlov, Lj., Mitrev, S., Karov, I., & Ilieva, V. (2015). The influence of environment on yield and yield components in two row winter barley varieties. *Bulgarian Journal of Agricultural Science*, 21, (4), 863-871.

Mckenzie, R., Middleton, H.A.B., & Brener, E. (2005). Fertilization, seeding rate and seeding date of malting barley yield and quality in southern Alberta. *Canadian Journal of Plant Science*, 85, (3), 603-614.

Paynter, B.H., & Young, K.J. (2004). Grain and malting quality in two-row spring barley are influenced by grain filling moisture. *Australian Journal Agricultural Research*, 55, 539-550.

Pecio, A., & Wach, D. (2015). Grain yield and yield components of spring barley genotypes as the indicators of their tolerance to temporal drought stress. *Polish Journal of Agronomy*, 21, 19-27.

Popescu, S., Idriceanu, A., Stan, S., Mihãilã, V., Negrilã, M., Povarnã, F.I., & Ionescu, F. (1997). Implicaþii ale dezechilibrelor nutritive in sintenza proteinei la grau. An. I.C.C.P.T Fundulea.

Randahwa, P.S., & Arora, C.L. (2000). P and S interaction effect on dry matter yield and nutrient uptake by wheat. *Journal of Indian society of soil science*, 48, (3), 536-540.

SPSS Statistics 19 (2010). SPSS Inc., an IBM Company.

Sukram, P., Tejveer, S., & Ramesh, B. (2010). Estimation of genetic parameters in barley (*Hordeum vulgare* L.). *Journal of Crop Improvement* (Abs.) 37, (1), 52 – 56.

ГЕНЕТСКИОТ ПОТЕНЦИЈАЛ И ВЛИЈАНИЕТО НА НАДВОРЕШНАТА СРЕДИНА ВРЗ ПРИНОСОТ КАЈ ПРОЛЕТНИ ФОРМИ НА ЈАЧМЕН ОДГЛЕДУВАНИ ВО РЕПУБЛИКА МАКЕДОНИЈА

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Резиме

Целта на овој труд е да се одредат генетскиот потенцијал и влијанието на надворешната средина врз приносот кај пролетни форми на јачмен одгледувани во Република Македонија. Како експериментален материјал се користени пет пролетни форми на јачмен (*македо*, *ханаду*, *јозефин, гладус* и *скарлет*). *Македо* е македонска сорта, а останатите сорти на јачмен имаат странско потекло. Полските експерименти беа реализирани во текот на 2013 и 2014 година на површините во Пробиштип, Република Македонија. Опитот беше поставен во рандомизиран блок-систем во три повторувања. Истражувањата беа направени со цел да се утврди дали сортата, надворешните услови или нивната интеракција е главниот фактор на влијание (η) врз приносот на зрно. И покрај значајноста на климатските фактори врз приносот на зрно, во овие истражувања се покажа дека главен фактор кој има силно влијание врз приносот е сортата. Влијанието на сортата врз формирањето на приносот е над 98%. Највисок принос беше добиен од сортата *македо* (6844 kg/ha), а веднаш по неа следуваше ханаду со 6638 kg/ha. Просечниот принос на зрно од двете години на испитување беше 6544 kg/ha. Сортата *македо,* исто така, имаше и најголем број на класови на m^2 и најголем број на зрна во клас.

Клучни зборови: *принос на зрно*, *јачмен*, *сорта*, *компоненти на принос*

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CHANGES IN FATTY ACID COMPOSITION OF POULTRY MEAT AFTER HEAT TREATMENT

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Abstract

The purpose of the paper is to determine the changes in the fatty acid composition of fat in poultry meat after the heat treatment, after frying in different types and combinations of fats. Lard, sunflower oil, palm oil and a combinations of three parts of lard and one part of sunflower oil, three parts of lard and one part of palm oil and three parts of palm oil and one part of sunflower oil were used for the examination. After frying with lard in the meat, there is a greater change in the fatty acid composition, where the fatty acid C18:1n9cof 45.84%, which it had it before frying, increased to 49.05%, C18:O decreased from 10.18% to 7.45% and decreased C16:O from 29.55 % to 27.45%. After frying in sunflower oil and palm oil, there has a formation of new fatty acids in insignificant amounts. In combination of three parts of lard and one part of sunflower oil, minimal changes in the fatty acid composition of less than 1% have been observed. In frying with three parts of lard and one part of palm oil fatty acid C14:O from 0.88% to 23.81%, fatty acids C16:O decreased from 27.24% to1.72%, C18:1n9c from 48.05 % to 15.16% and C18:2n6c from 12.44% to 0.20%. After the heat treatment of the meat in three parts of palm oil and one part of sunflower oil, there is an increase in the content of fatty acid from C18:2n6cfrom 8.42% to 11.62 %.

Key words: *quality, frying, lard, palm oil, sunflower oil*

INTRODUCTION

Thermal processing of meat is regular action in cuisine and in the meat industry. The objective of thermal processing of meat is to prepare the meat for consuming and to increase its sustainability. One of the most applied and fastest ways of thermal processing of meat for consuming in households is frying. Soft, boneless pieces of meat from the shoulder part, the thigh, the loin and the neck, and from which the visible fat tissue was removed, are usually used for frying. Sunflower oil and lard are used in the households to fry meat, and lately the palm oil became very popular. During frying with less or more oil, sensory characteristics which are very alike to those formed during grilling or roasting, are formed in the meat. During frying of the meat with less or more oil, attractive chestnut brown colour is being formed on its surface, while golden yellow to chestnut colour is formed on the surface of the fat tissue. The chestnut brown colour of the meat surface is consequence of Maillard reaction, which occurs during thermal processing.

During thermal processing – frying of meat, the temperature on the meat surface reaches 180-150°C (Rahelič et al., 1980) and numerous physical and chemical reaction happen within the meat. As a result of the heat, the water evaporates from the meat, the fats are extruded and they leak into the frying pan.

Changes which occur in the oils in which the meat is fried are primarily result of heat denaturation of fats, as well as a result of reaction between fats in the meat and soluble proteins.

MATERIAL AND METHODS

Material samples

The poultry that were used for examination are purchased from trade network usually from freshly slaughtered broilers (meat from the chest). The meat was cut down to size and shape as for frying, 80-100 g.

Fats used for the heat treatment of the meat (frying) and for examining their chemical changes after the heat treatment are lard, sunflower oil, palm oil and mixture, lard and sunflower oil in a ratio of 3:1, palm oil and sunflower oil in a ratio of 3:1, lard and palm oil in a ratio of 3:1. Lard used for the heat treatment of meat and for the examination of chemical changes in fat after the heat treatment of the meat is obtained from the trade network. The sunflower oil used to test the chemical changes in fats after the heat treatment of the meat is commercial oil produced in the oil factory ''Blagoj Gorev'' from Veles, R. Macedonia.

Thermal processing of the meat

Thermal processing – frying of the meat is performed in laboratory. The meat samples are frying after the melting and reaching the boiling point. The meat is fried in a large amount of fat, that is, the level of fat in the plate in which the meat was fried was enough to cover them. The determination of fried meat was subjective, that is, the conclusion that the meat was sufficiently thermally treated was the formation of the attractive colour on its surface. After the heat treatment, the meat is taken out of the plate in which it is thermally processed and it is transferred to another plate in which a kitchen paper for absorption of fat is placed on the bottom. The changes in meat weight were monitored by measuring meat before and after thermal processing with electronic weighing scale accurate to 0.1 g. Prior to the heat treatment of the meat, one sample of the fat in the amount of 25 cm^3 was taken or a total of 12 samples of fat were taken for testing the fatty acid composition before the treatment. Fat-sampling before heat treatment is done after fat fading. The fats are placed in glass bottles with a volume of 30 cm. After the heat treatment of the meat and the cooling of the fats, four more samples are taken to examine the changes that occur in the fatty acid composition of the fats.

Determination of fatty acids content

Examination of changes in fatty acid composition of the fats is conducted before and after thermal processing .The fats from the analyzed samples of the poultry meat were extracted by the Rose-Gottlieb, (AOAC, 1990) method (modified method of Secchiari and Coll. (2003). Butylated hydroxytoluene (BHT) (50 µg BHT/ml in methanol) was added in order to avoid oxidative degradation of the fatty acids. Methylation of the fatty acids was done by the AOAC Official Method 996.06 (2005).

The produced metal esters of the fatty acids (FAMES) were analyzed with gas chromatograph Hewlett Packard 5890 series II with flame ionization detector and capillary column HP 88 (60m x 0.250mm x 0.20 µm). Helium was the gas carrier, and the total duration of one cycle was 38.50 minutes. The FAMES produced from the analyzed samples of the fermented sausages were identified on the basis of the comparison of their retention time with the retention time of the standards of FAMES consisted in 37 component mixture (Supelco 37 component FAME mix). The content of each of the analyzed fatty acids is shown in percent (%).

Analyses of changes in fatty acid composition of the fats are examined in the laboratory of the Food Institute at the Faculty of Veterinary Medicine in Skopje.

Statistical analysis

All data were presented as mean value with their standard deviation indicated (mean ± SD).

RESULTS AND DISCUSSION

Significant physical changes, which contribute to change of basic characteristics of meat and forming new sensory characteristics important to its quality, occur during thermal processing of meat.

One of the basic changes which occur during thermal processing is weight loss – meat shrinkage. The weight loss of the meat which occurs after thermal processing could be seen best from data given in Table 1.

Table1. Weight Loss-meat shrinkage in poultry meat during thermal processing

Meat frying	Changes in meat weight	Parameters				
		x	s	Cv	min	max
Lard	Before thermal processing (g)	84.80	8.060	9.504	70.00	94.00
	After thermal processing (g)	46.80	5.741	12.267	42.00	56.00
	Shrinkage (%)	44.00	8.367	19.015	34.00	55.00
Sunflower oil	Before thermal processing (g)	85.60	5.426	6.334	82.00	94.00
	After thermal processing (g)	55.20	5.741	10.400	48.00	64.00
	Shrinkage (%)	35.66	2.494	6.994	32.00	39.00
Palm oil	Before thermal processing (g)	92.40	13.529	14.642	76.00	106.00
	After thermal processing (g)	57.40	10.151	17.684	44.00	66.00
	Shrinkage (%)	38.20	2.315	6.061	35.00	42.00
Lard and sunflower oil	Before thermal processing (g)	83.60	10.072	12.047	70.00	100.00
	After thermal processing (g)	51.60	8.139	15.773	40.00	64.00
	Shrinkage (%)	38.60	3.262	8.450	36.00	43.00
Lard and palm oil	Before thermal processing (g)	104.00	11.662	11.134	92.00	120.00
	After thermal processing (g)	65.60	4.630	7.058	60.00	72.00
	Shrinkage (%)	36.80	3.059	8.314	33.00	42.00
Palm oil and sunflower oil	Before thermal processing (g)	86.80	6.013	6.928	76.00	94.00
	After thermal processing (g)	59.60	9.830	16.494	44.00	68.00
	Shrinkage (%)	31.80	9.064	28.504	23.00	49.00

As seen from data presented in Table 2, the average weight loss during thermal processing of poultry by frying with sunflower oil is 35.66%, by frying with lard is 44.00 % and by frying with palm oil is 38.20%. The frying in a mixture of lard and sunflower oil, lard and palm oil, palm oil and sunflower oil in 3:1 the average weight loss is 35.53%.Shrinkage in poultry meat during thermal processing fried in palm oil and sunflower oil is the lowest and it is 31.80%, and the highest in meat that is fried in combination of lard and sunflower 38.60%. The obtained results in our examinations are in relation to the results stated by other authors. The meat shrinkage during thermal processing depends on the reached temperature in the centre of the product. The weight loss of meat will be as bigger as the temperature of oils in which the meat is thermally processed gets higher (Buchar and Frohlich, 1969; Rohman, A. and Che Man, Y. B. 2009a; Naz Shanina, et al., 2005; Gunston, F.D. 2004). Significant changes of fatty acid composition occur after thermal processing of meat, which is caused by the temperature during thermal processing, as well as the interaction of fats and soluble proteins and the interaction of other meat substances and oils used for thermal processing – frying of meat, (Rashood, et al.,1996; Marina, et al., 2010; Marikkar et al., 2011; Indrasti, et al., 2010).

The changes in the fatty acid content that occur during thermal processing of meat – frying with lard, sunflower oil and palm oil could be seen best from Table 2.

Table 2. Changes in the fatty acid content before and after heat treatment of poultry meat - fried with different types of fats

Fatty acid composition	Different types of fats					
	Lard		Sunflower oil		Palm oil	
	before frying	after frying	before frying	after frying	before frying	after frying
C14:0	1.22	1.43	-	-	0.65	0.75
C16:0	29.55	27.45	10.58	11.57	46.15	44.43
C16:1	2.36	2.79	-	-	-	0.36
C17:0	0.23	0.28	-	-	-	-
C17:1	0.15	0.23	-	-	-	-
C18:0	10.18	7.45	3.49	3.58	3.21	3.25
C18:1n9c	45.84	49.05	45.42	45.51	44.40	44.77
C18:2n6c	9.00	9.31	40.06	38.80	4.06	6.21
C18:3n6	0.39	0.45	-	0.20	-	0.23
C21:0	-	0.45	-	-	-	-
C18:3n3	0.45	0.86	-	0.18	-	0.21
C22:0	-	0.25	-	-	-	-
C20:5n3	-	-	-	0.15	-	-

It is acertained that two fatty acids are formed in poultry fried with lard, such as C21:0 in an amount of 0.45% and C22:0 in an amount of 0.25 %. During the thermal processing of the poultry, the content of C18:1n9c has increased from 45.84% to 49.05%, while C18:0 has decreased from 10.18% to 7.45%, C16:0 has also decreased from 29.55% to 27.45%. These changes are statistically significant at the level of ($p<0.5$).During frying of poultry with palm oil there is minimal decrease of the content of C16:0 from 46.15 % to 44.43 %,and at the expense of it new fatty acids appear, such as C18:3n3 andC18:3n6, which are either result of denaturation changes which occurred in the palm oil during thermal processing of the meat, or extracted from poultry. The changes which occur in fatty acid composition of fats after thermal processing of the meat are minimal and do not show statistical significance.

Most probably, the new fatty acids which appear in fats after thermal processing (most noticeable after frying with sunflower oil) of the meat are either extracted from the meat or newly created fat acids from the existing ones, under influence of high temperature during thermal processing of the meat

The changes in the fatty acid content that occur during thermal processing of meat – frying with combinations of fats could be seen best from Table 3.

Table 3. Changes in the fatty acid content before and after heat treatment of poultry meat - fried with different mixture of fats

Fatty acid composition	Different mixture of fats					
	Lard : sunflower oil (3:1)		Lard : palm oil (3:1)		Palm oil : sunflower oil (3:1)	
	before frying	after frying	before frying	after frying	before frying	after frying
C14:0	0.88	23.81	0.88	23.81	0.59	0.67
C16:0	27.24	1.72	27.24	1.72	42.31	41.99
C16:1	2.23	-	2.23	-	-	0.20
C17:0	0.12	-	0.12	-	-	-
C17:1	-	7.65	0.21	7.65	-	-
C18:0	8.15	51.25	8.15	51.25	3.23	3.48

C18:1n9c	48.05	15.16	48.05	15.16	45.24	41.71
C18:2n6c	12.44	0.20	12.44	0.20	8.42	11.62
C18:3n6	0.36	0.21	0.36	0.21	0.21	0.23
C18:3n3	0.53	-	-	-	-	0.10

Under the influence of high temperature during the frying of poultry in a mixture of lard and sunflower oil, one more significant change is that the content of C14: O increases from 0.88% to 23.81%, and decreases the content of C16:O from 27.24% to 1.72%, C18:O is increased from 8.16 to 51.25%, which is a consequence of the transformation of cis oleic fatty acid. After the heat treatment of poultry meat in the mixture of lard and sunflower oil, a new fatty acid C17:1 occurs in an amount of 7.65%, which we believe has been squeezed out of the meat. During the frying of poultry in a mixture of lard and palm oil,C16: O in the amount of 27.24% after frying decreased to 1.72%, while in oleic fatty acid (C18: O) before frying was represented by 8.15%, and after frying, its presence in fat was 51.25%. A similar situation exists with the fatty acid of C18: 2n6c where its quantity of 12.44% has decreased to 0.20%. After frying of the poultry in this combination of fats there is a new fatty acid C17: 1 which is represented by 7.65%. Other fatty acids have no significant changes, they are almost unchanged. In the third combination of fats pamitic fatty acid (C16: O), which in fats before frying was 42.31% after frying fell to 41.99%, then fatty acid C18: 1n9c which in fat before frying was present in quantity of 45.24% decreased to 41.71%, while fatty acid C18: 2n6c of 8.42% was increased to 11.62%. For other fatty acids in fat before and after frying of poultry meat there are no major changes.

CONCLUSIONS

Based on the investigations carried out on chemical changes that occur in fats after the heat treatment, the following conclusions can be made:

- The average shrinkage during heat treatment of poultry meat in sunflower oil is 35.66%, lard 44.00% and palm oil 38.20%;
- After frying of poultry in lard, there is an occurrence of C18:3n3 with a representation of 0.45% and C22:0 of 0.25% and after frying in palm fat there is a new fatty acid C16: 1 where its presence is 0.36%.

During frying the meat in the mixture of fats, changes that occur in the fatty acid composition of fat in which the meat is processed present the fact that some fatty acids have decreased, others have increased and there have been new fatty acids. The emergence of new fatty acids is considered to be primarily a consequence of the extraction of fatty acids from the meat, and others are a result of the denaturation of fatty acids and the transition into a cis or trans form of the corresponding fatty acid.

REFERENCES

Gunston, F.D. (2004). Creation and evaluation of a two-dimensional contour plot of fatty acid methyl esters after off-line coupling of reversed-phase HPLC and GC/EI-MS. *Analytical and Bioanalytical Chemistry*, (396), 2695–2707.

Indrasti, D., CheMan, Y.B., Mustafa, S. & Hashim, D.M. (2010). Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry. *Food Chemistry* (122), 1273 - 1277.

Marikkar, J. M. N., Ng, S. L. & Che Man, Y. B. (2011). Composition and thermal analysis of lipids from prefried chicken nuggets. *Journal of the American Oil Chemists' Society*, (88), 749–754.

Marina, A.M., Che Man, Y.B. & Ismail, A. (2010). Use of the SAW sensor electronic nose for detecting the adulteration of virgin coconut oil with RBD palm kernel olein. *Journal of the American Oil Chemists' Society*, (87), 263–270.

Rashood, K. A., Shaaban, R. R. A., Moety, E. M.

A. & Rauf, A. (1996). Triacylglycerols-profiling by high performance liquid chromatography: a tool for detection of pork fat in processed foods. *Journal of Liquid Chromatography*, (18), 2661-2672.

Rohman, A. & Che Man, Y. B. (2009a). Analysis of cod liver oil adulteration using Fourier Transform Infrared (FTIR) spectroscopy. *Journal of the American Oil Chemists' Society*, (86),1149-1153.

Rahelic, S. & Puac, S. (1980–1981). Fibre types in Longissimus dorsi from wild and highly selected pig breeds. *Meat Sci*ence, (5), 439–450.

Ramírez M.R., Estévez M., Morcuende D., Cava R. (2004). Effect of the type of frying culinary fat on volatile compounds isolated in fried pork loin chops by using SPME-GC-MS. Journal of Agricultural Food Chemistry, 52(25),7637-43.

NazShanina, R., Siddiqi Rina Sheikh, S., & AsadSaYeed (2005). Deterioration of olive, corn and soybean oils due to air, light, heat and deepfrying. *Food Research International*, (38), 127-134,

Olsen, E., Vogt, G., Ekeberg, D., Sandbakk, M., Pettersen, J., & Ntlsson, A. (2005). Analysis of the early stages of lipid oxidation in freeze-stored pork back fat and mechanically poultry meat. *Journal Agricultural Food Chemistry*, (53:2), 338-348.

ПРОМЕНИ ВО МАСНОКИСЕЛИНСКИОТ СОСТАВ КАЈ ЖИВИНСКОТО МЕСО ПО ТЕРМИЧКАТА ОБРАБОТКА

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Резиме

Целта на трудот е да се утврдат промените во маснокиселинскиот состав на мастите кај пилешко бело месо по термичката обработка и тоа по пржење во различни видови и комбинации на масти. За испитување се користеше свинска маст, сончогледово масло, палмина маст и комбинација на три дела свинска маст и еден дел на сончогледово масло, три дела на свинска маст и еден дел на палмина маст и три дела на палмина маст и еден дел на сончогледово масло. По пржењето со свинската маст кај месото има поголеми промени во маснокиселинскиот состав каде што масната киселина C18:1n9c од 45.84% која ја имаше пред пржење се зголеми на 49.05%, C18:O се намали од 10.18% на 7.45% и намалување на C16:O од 29.55% на 27.45%. По пржење во сончогледовото масло и палмина маст има формирање на нови масни киселини во незначително количество. Во комбинација од три дела свинска маст и еден дел на сончогледово масло констатирани се минимални промени во маснокиселинскиот состав кој изнесуваат под 1 %. При пржење со три дела свинска маст и еден дел на палмина маст масната киселина C14:O од 0.88% се зголеми на 23.81%, масните киселини C16:O се намалија од 27.24% на 1.72 %, C18:1n9c од 48.05% на 15.16% и C18:2n6c од 12.44% на 0.20%. По термичката обработка на месото во три дела на палмина маст и еден дел на сончогледово масло има зголемување на содржината на масната киселина C18:2n6c од 8.42% на 11.62%.

Клучни зборови: *квалитет, пржење, свинска маст, палмина маст, сончогледово масло*