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# JOURNAL OF AGRICULTURE AND PLANT SCIENCES

**YEAR 2018** 

VOLUME 16, Number 2

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#### Journal of Agriculture and Plant Sciences, JAPS, Vol 16, No. 2 Successor of the Yearbook of Faculty of Agriculture of GDU, Vol 14

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> ISSN 2545-4447 print ISSN 2545-4455 on line Vol. 16, No. 2, Year 2018

# GOCE DELCEV UNIVERSITY - STIP, REPUBLIC OF MACEDONIA FACULTY OF AGRICULTURE

UDC 63(058) ISSN 2545-4447 print ISSN 2545-4455 on line



# Journal of Agriculture and Plant Sciences, JAPS, Vol 16, No. 2

Successor of the Yearbook of Faculty of Agriculture of GDU, Vol 14

**YEAR 2018** 

VOLUME XVI, Number 2

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# 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 1<sup>st</sup> 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 1<sup>st</sup> 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. 2 contains the presented papers from the 1<sup>st</sup> International Meeting Agriscience & Practice (ASP 2018).

Editorial Board,

Editor in chief,

December, 2018

Prof. Liljana Koleva Gudeva, PhD

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 29.05.2018 Accepted: 05.09.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 633.13-114.7(497.7)"2015/2016" Original scientific paper

# EXAMINATION OF SOME QUALITY FEATURES OF OATS GROWN IN CONDITIONS OF ORGANIC PRODUCTION

#### Adrijana Burovska<sup>1</sup>, Dragica Spasova<sup>1</sup>\*, Biljana Atanasova<sup>1</sup>, Dusan Spasov<sup>1</sup>, Mite Ilievski<sup>1</sup>

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#### Abstract

The research was conducted in 2015 and 2016, at 11 oats genotypes in terms of organic production. Three of the populations were domestic: *Krivogastani, Trebenista* and *Kuceviste*. The rest were introduced varieties, including *Rajac, Slavuj* and *Lovcen* from Serbia and varieties: *Kupa, Baranja, Explorer, Sampionka* and *Istra* from Croatia.

In the first year of the study, the energy of germination of oats seed ranged from 70% in variety *Rajac*, up to 96% for the variety *Kupa*. In the second year of the study, the energy of germination was 81% for the variety *Kupa*, to 96% in variety *Istra*. While comparing the varieties it is pointed out that there are statistically significant differences.

The total germination of oat seeds, cultivated under conditions of organic production, is statistically different between different genotypes, in both years of research. In 2015, the variety *Rajac* showed minimum germination (70%), and in 2016, the variety *Lovcen* (82%). Variety *Krivogastani* showed the greatest over all germination in both experimental years (96% in 2015 and 97% in 2016).

Regardless the year and the mode of production, the best genotype of the tested variants for the highest absolute mass of grain, it proved the variety *Istra* with 34,6g in 2015 and 29,6g in 2016. The smallest absolute mass in both experimental years had the *Krivogastani* population, 12.3g in 2015 and 14.9g in 2016. There is statistically significant difference between varieties.

Key words: variety, population, energy of germination, germination, absolute mass

#### INTRODUCTION

Oat (Avena sativa L.) is one of the oldest cereals, known first as weeds in cereal crops, and then grown as an independent culture, because of its high economic qualities - high protein content with a better balanced amino acid composition (Moudry,1992; Yeoh & Watson,1981; Frey,1977), than other cereals, a favourable relationship between nutritional properties and high digestibility, which determine oats as an indispensable concentrated forage in the diet of domestic animals.

The agricultural significance of the oats is due precisely to the quality of the grain and the small requirements about the conditions of cultivation, so it can succeed in the areas where other cereals produce low yields (Spasojević et al., 1984). With the development of the food industry and the growing need for healthy and dietary food, the significance of the oat for balanced nutrition of people has increased. Oat breeding aims at producing as high yielding varieties as possible, but some irreplaceable properties of the grain are also required in connection with the production of valuable food and nutritional products (Савова, 2007; Георгиев et al., 2003).

Oat is a cereal crop that prefers moist and cold climates, but due to the constant increase in air temperature and uneven precipitation in the last ten years, efforts are being made to create new varieties adapted to the new modified conditions (Кузмова К., 2009). Oat is very sensitive to drought and low temperatures (Božić et al., 2013). Insufficient and irregular

precipitation in spring also negatively affects the yield of oats (Barut, 2003).

Genotypes with a high percentage of total germination and development have a high selectivity value and if involved in the selection process can lead to improved crop growth, its competitiveness and productivity (Шевелуха, 1992; Станков et al., 2010; Станков et al., 2008; Вълчев et al., 2010). The energy of germination and germination rate of the seeds describe their physiological characteristics that enable them to quickly grow in the soil and be tolerant towards the various negative environmental factors (Lekić, 2001; Milošević et al., 2010).

Oat is one of the most suitable cereals for organic production (Lockeretz, 1981). This is in

The tests were carried out in 2015 and 2016, in field and laboratory conditions. Field experiments were placed on the experimental field at the Faculty of Agriculture, "Goce Delcev" University - Stip in Strumica, and the laboratory analysis were carried out at the Faculty of Agriculture laboratories.

11 oats genotypes have been analysed, three of which are domestic populations (*Krivogastani, Trebenishta* and *Kuceviste*), three Serbian genotypes (*Rajac, Slavui, Lovken*) and five genotypes are of Croatian origin (*Kupa, Baranja, Explorer, Championka* and *Istra*).

The trials were set in three repetitions, distributed by the random block system method, with a dimension of the basic parcel of 5 m2.

The distance between the variants was 0.50 m, and between the repetitions 1 m. The

In the period of two-year trials, the meteorological indicators for average monthly air temperatures in Celsius were monitored and monthly sums of precipitation in millimetres.

For the period of ten years, 2004/2014, the average annual temperature in Strumica valley (Table 1) was 13.5°C, and an average fall of 663.9 mm precipitation (Table 2).

The schedule of precipitation (Table 2) by months and by seasons is quite unbalanced. The most robust mass of rain, for a period of ten years, is in October with an average of 80.1mm. The driest month, with the lowest average accordance with the studies and other authors (Galie et al., 2004), who examined varieties of oats in organic production conditions, established that oats are a very suitable cereal organoleptic crop, bearing in mind the high yields they received which ranged from 4 to 5 t / ha.

The purpose of the study was to analyse some biological and physical properties such as the energy of germination, total germination and the absolute mass of oat grains in conditions of organic production, which will determine which variety or population is most suitable for growing in the climatic conditions, which are characteristic for Strumica region.

# **MATERIAL AND METHODS**

distance between rows was 20 cm. The seed rate of 550 grains per 1m2 was used. The basic soil treatment was performed at a depth of 35 cm. Prior to sowing, additional processing and fertilization with 30 t/ha biological fertilizers were carried out, according to the regulations for organic production.

Before the harvest, material from plot of 1m2 for laboratory analysis was taken (30 plants of each parcel were used, i.e. 90 plants of each variant)

The biological and physical properties of the grains (energy of germination, total germination and absolute mass) were examined according to international methods of ISTA.

The obtained results were processed statistically according to the method analysis of variance, and the differences were tested according to the LSD test.

# **CLIMATE CONDITIONS**

amount of precipitation is August 39.9mm.

The analysis of the temperatures in the period of research 2015-2016 (Table 1) showed a high similarity with the average annual temperatures in the Strumica valley at the tenyear average. The mean annual temperature in 2015 is 0.3°C higher than the multiyear average, and in 2016 is 0.4°C higher than the average.

It must be noted that the amount of precipitation and temperature smaller or larger than average amount is sufficiently reliable factor for the successful completion of the vegetation, i.e. good yield.

According to the data in Table 1, it can be concluded that the average monthly air temperatures during the spring oats (March - July) vegetation, in the two years of testing, are the lowest in the first month of the oat vegetation, i.e. in March (from 7.2 - 9.5 °C), and the highest in July (25.5 - 26.7 °C). These midmonth temperatures, which prevailed in the two years of testing, are considered good for growing oats.

Spring oat is known as low temperature

sensitive culture and should not be grown in areas where temperatures fall from -10 to -14°C, before forming a snow cover. But the degree of resistance depends on the large extent on the stage development, the stages of organogenesis, the sowing time, the species, the variety, the availability of soil with nutrients, the duration of the low temperatures, the humidity of the soil, etc. The temperature is considered one of the key factors in the germination process (Forcella, 1998).

		Month										Ann.	Average	
Year	I	II	III	IV	v	VI	VII	VIII	IX	x	XI	XII	amount of temp.	ann. temp
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 1. Average monthly temperatures in Celsius

Table 2. Amount of monthly precipitation in mm

	Month										of a- m		
Year	I	II	III	IV	v	VI	VII	VIII	IX	X	XI	XII	Ann. amount o precipita- tion in m
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

For successful vegetation, oats require a lot of water and are therefore considered as cereal that has the greatest need of it.

Critical water periods are the stages of the formation of generative organs (about 15 days before tasselling) and the time of intense growth (until the tasselling) (Vasilevski, 2004).

In Table 2, it can be noted that during the vegetation period of the oats, the precipitation

# **RESULTS AND DISCUSSION**

# **Energy of germination**

Energy of germination defines as the percentage of normally germinated seeds in relation to the number of seeds set for germination, determined after the expiration of the time provided for the first assessment, i.e. for determining the energy of germination.

The energy of germination of the seeds reflects the seed's vitality, so that after the sowing, it yields uniform, healthy and strong holes in a relatively short time and in relatively good scales are relatively good and correspond to the needs of the oats.

Oats have the greatest need for water in relation to other cereals, due to the large leaf mass that it forms during vegetation. The oats transpiration coefficient ranges from 400 to 570, but it largely depends on the variety, agrotechnical measures, the type of soil, relative humidity of the air, etc.

skinning conditions. The energy of germination may vary depending on the action of the seed and the ecological factors (Rajnpreht, 1990).

In the first year of the study, the germination energy of oat grown in organic production (Table 3) ranged from 70% in the *Rajac* variety to 96% in the variety of *Cupa*. In the second year of the study, the germination energy was 81% in the varieties of *Cupa*, up to 96% in the variety of *Istra*. Compared between varieties, there are statistically significant differences.

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# Germination

Determining the seeds germination is one of the most important ways of assessing seed material, and' spinning is the ability of the seed to sprout when it comes in favourable conditions. Germinated seed is the one that in a certain time develops a root and a stem not less than the length of the seed that is considered proper. The term total germination of seeds means the percentage of normally developed germinant in relation to the total number of seeds placed on germination, determined after the expiry of the time provided for the final evaluation. Water, temperature, light, oxygen together with soil characteristics are the factors that have the greatest impact on the germination of seeds (Gorai & Neffati, 2007).

The total germination of the seeds is the most important biological parameter for the quality of the seeds, on which its longevity depends.

This important biological parameter for seed quality (Table 3), in both years of research, is statistically different in different genotypes and ranges from 70% to 96%. In 2015, the variety *Rajac* showed the lowest percentage of germination (70%), and in 2016 it was the *Lovken* variety (82%), while the *Krivogastani* variety showed the highest total germination in both experimental years (96% in 2015 and 97 % in 2016).

The germination of the seeds in the studies of some authors (Спасова, 2008), were slightly higher, ranging from 80% to 99%, and the lowest seeds of the *Rajac* variety (87%) were observed in the first year of the trials.

# Absolute mass of the grain

The absolute mass of the seed is a mass of 1000 air-dry grains and is expressed in grams. It tells us about the nature of the endosperm of the grain or the internal structure of the grain.

Better laxity gives a higher absolute mass, and is important when determining the amount of sowing rate in cereals.

Absolute mass is a feature of the species and variety, but in the same variety, it may be different, depending on the conditions of production (Đekić et al. 2010). In our investigations, the conclusion was confirmed.

Regardless the year and the way of production, the best genotype of the examined variants with the highest absolute mass of grain was the variety *Istra* (34.6 g in 2015 and 29.6 g in 2016). The lowest absolute mass in both years had the *Krivogastani* population, 12.3 g in 2015 and 14.9 g in 2016, as well as the *Kuceviste* variety (14.9 g in 2016). Among the varieties, there is a very significant statistical difference.

Variety / population	Energy of germination (%)	Germination (%)	Absolute mass of grain (g)
2015			
Krivogashtani	94	96	12,3
Trebenishta	86	86	24,7
Kuchevishte	80	88	20,1
Rajac	70	70	17,8
Slavuj	83	84	20,5
Lovken	91	91	25,6
Cupa	96	95	26,4
Baranja	90	90	24,3
Explorer	80	80	27,7
Shampionka	91	91	22,0
Istra	92	92	34,6
LSD 0,05	5.9	4.23	2,77
0,01	8.4	6.0	5,50

Table 3. Quality properties of oats grown in organic production in 2015 and 2016

Variety /	Energy of germination	Germination	Absolute mass of grain		
population	(%)	(%)	(g)		
2016					
Krivogashtani	95	97	14,9		
Trebenishta	89	92	19,9		
Kuchevishte	90	91	14,9		
Rajac	84	85	23,3		
Slavuj	86	88	18,5		
Lovken	90	82	19,3		
Cupa	81	92	22,9		
Baranja	92	93	16,0		
Explorer	82	89	19,3		
Shampionka	91	92	17,6		
Istra	96	96	29,6		
LSD 0,05	2.88	2.62	2,65		
0,01	4.38	3.82	3,78		

# **CONCLUDING REMARKS**

Based on the two-year trials and the obtained results for the quality properties of oats grown in organic production, we can conclude that:

- The lowest energy of germination of the seeds had variety *Rajac* (70%) in 2015 and variety *Kupa* (81%) in 2016, and the highest, variety *Kupa* (2015) and the variety *Istra* (2016) with 96%.
- The *Krivogastani* varieties showed the highest total germination in both experimental years (96% in 2015 and 97% in 2016), and opposite, the lowest total germination had the variety *Rajac* (70%) in
- 2015, and the variety *Lovken* (82%) in 2016.
  The best genotype of the examined variants with the highest absolute mass of grain was the variety *Istra* (34.6 g in 2015 and 29.6 g in 2016), and the most unfavourable for growing in our conditions are *Krivogastani* population, 12.3 g in 2015 and 14.9 g in 2016, as well as the *Kuceviste* with 14.9g in 2016.

Accordingly, the variety *lstra* is the most suitable genotype of the examined 11 varieties for breeding in organic production in the conditions of Strumica region.

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# ИСПИТУВАЊЕ НА НЕКОИ КВАЛИТЕТНИ СВОЈСТВА НА ОВЕС ОДГЛЕДУВАН ВО УСЛОВИ НА ОРГАНСКО ПРОИЗВОДСТВО

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#### Резиме

Испитувањата се вршени во 2015 и во 2016 година, на 11 генотипови овес при услови на органско производство, од кои 3 беа домашни популации: кривогаштани, требеништа и кучевиште, а останатите се интродуирани сорти и тоа: рајац, славуј и ловќен од Србија, и сортите: купа, барања, експлорер, шампионка и истра од Хрватска.

Во првата година од испитувањето, енергијата на 'ртење на семето од овес се движеше од 70% кај сортата рајац, до 96% кај сортата купа. Во втората година од испитувањето, енергијата на 'ртењето изнесуваше од 81% кај сортата купа, до 96% кај сортата истра. Споредено помеѓу сортите, укажува на констатацијата дека постојат статистички многу значајни разлики.

Вкупната 'ртливост на семето од овес, одгледувано при услови на органско производство, и во двете години на испитување е статистички различна кај различни генотипови. Во 2015 година сортата рајац покажа најмала 'ртливост (70%), а во 2016 година сортата ловќен покажа најмала 'ртливост (82%), додека популацијата кривогаштани покажа најголема вкупна 'ртливост и во двете опитни години (96% во 2015 и 97% во 2016 година).

Без оглед на годината и начинот на производство како најдобар генотип од испитуваните варијанти за добивање на висока апсолутна маса на зрно се покажа сортата истра со 34,6 g во 2015 година и 29,6 g во 2016 година. Најмала апсолутна маса и во двете години имаше популацијата кривогаштани и тоа 12,3 g во 2015 г. и 14,9 д во 2016 година заедно со кучевиште. Помеѓу сортите постои многу значајна статистичка разлика.

Клучни зборови: сорта, популација, енергија на 'ртење, 'ртливост, апсолутна маса

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 20.06.2018 Accepted: 27.09.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 633.521-152.61:547.96 (497.742)"2014/2015" Original scientific paper

# PROTEIN PROFILE OF SOME GENOTYPES OF FLAX (Linum usitatissimum L.) IN THE STRUMICA REGION, REPUBLIC OF MACEDONIA

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#### Abstract

Flax (Linum usitatissimum L.) is an industrial crop and has great importance for humans and economy. Analysis of five different flax genotypes, produced in the Strumica region, Republic of Macedonia, in
2014 and 2015 is made, with regard to the content of protein in the flaxseed. The experiment consisted of five variants in three repetitions, divided by the method of randomized block system. During the vegetation, standard farming practices for field flax production were used. Analysis of the protein content in flaxseed was made in the Laboratory for plant and environmental protection, at the Faculty of Agriculture, "Goce Delcev" University – Stip (Standard: ISO 20483:2006). The content of protein in flaxseed was analysed by Kjeldahl method. The results were statistically processed by the method of analysis of variance, and the differences were tested by LSD – test.

The content of protein in flaxseed of the tested genotypes ranged from 26.9% to 29.4%. All flax genotypes had higher percentage of proteins in the seed. But, the genotypes Belinka (29.3%) and Velušina (28.5%) are characterized by the highest average content of protein in the seed, regardless the year of production. Belinka and Velušina are perspective genotypes for the food industry, as they have higher content of protein in the seed than the other analysed genotypes.

Key words: flaxseed, content, variety, percent, year

#### INTRODUCTION

Flax or linseed is among of the oldest crop plant cultivated for the purpose of oil and fiber (Jhala et al., 2010). From the large number of species (200) of flax, the most important for production has one species - Linum usitatissimum L. (Jevtic, 1992). The flax is an industrial crop and it is grown for fibre, seed and combined fiber and seed (Egumenovski P. et al., 2003). Almost every part of the flaxseed plant is utilized commercially, either directly or after processing (Singh et al., 2011). Seeds from flax are crushed to produce linseed oil and linseed meal. Flaxseed is emerging as one of the key sources of phytochemicals in the functional food arena. In addition to being one of the richest sources of  $\alpha$ -linolenic acid oil and lignans, flaxseed is an essential source of high-quality protein and

soluble fibre and has considerable potential as a source of phenolic compounds (Oomah, 2001). In many countries around the world, flax is one of the most important crops in healthy human consumption, due to the high content of dietary fibre, omega - 3 fatty acids and anticancer lignin (Pospišil et al., 2011).

The production of flax in the Republic of Macedonia it is grown on small surfaces for it seed (as bird food) and in 2005-2006 is grown on the Faculty of Agricultural Sciences and Food in Skopje as a research project (Dimov, 2006). Interest for flax in recent years has increased as a result of the increased capacity of oil and protein production. Starting from that point, reintroducing of the flax surfaces in Republic of Macedonia imposed the need for an investigation of the characteristics of certain varieties of flax, their acclimatization, production and quality characteristics.

Main aim of this examination is to determine the content of protein in the seeds of different genotypes of flax, produced in agroecological conditions of the Strumica region of Macedonia and to offer better information to manufacturers and industry who genotype of

# MATERIAL AND METHODS

The research was conducted in the field and laboratory conditions. Field examinations were set up at the experimental field in Strumica at Uniservice - Agro D.O.O.E.L., Faculty of Agriculture, Goce Delchev University - Stip. The research was conducted in the period of two years (2014 and 2015).

As a work material were used five flax genotypes:

- 1. Velušina
- 2. Duferin
- 3. Bellan
- 4. Viking
- 5. Belinka

Four of which are domestic intermediate flax genotypes (Velušina, Duferin, Bellan and Belinka) and one is a French introduced fiber flax variety (Viking).

The experiment consisted five variants in three repetitions, divided by the method of random block system with the basic dimension of the parcel of 10m<sup>2</sup>. The distance between the variants was 50cm and 100cm between repetitions. The distance between rows was 30cm.

The seeding rate was 50kg/ha or 50g per parcel. In two years of testing, a pre-culture of flax was wheat. The soil was prepared in the same way. Primary tillage was ploughing at a depth of 35cm and the surface was fertilized with granulated NPK 15:15:15 fertilizer in an amount of 300kg/ha and also a pre-sowing tillage was performed with a tiller. Sowing was performed manually in rows at a depth of 2-3cm.

After sowing and before germination, the parcels was treating with herbicide DUAL GOLD 960 EC, against certain annual and perennial broadleaf weeds in an amount of 31/ha.

During the vegetation, standard farming practices for field production of flax were used. Analysis of the protein content in flaxseed flax to be used if the same time protein content in the seed is a priority factor.

On the size of content of protein in the flaxseeds, largely influence had a specificity variety, soil and climate conditions, the applied agro-technical measures, method of storage and more. General average protein content in the seeds of the flax, was 28.3%.

ATERIAL AND METHODS

was made in the Laboratory for plant and environmental protection, at the Faculty of Agriculture, "Goce Delcev" University – Stip (Standard: ISO 20483:2006). The protein content of the flaxseed was analysed by Kjeldahl method. The results were statistically processed by the method of analysis of variance, and the differences were tested by LSD-test.

# **Climatic conditions**

The valley of Strumica is characterized by sub-mediterranean influences from the Aegean Sea to the south, which influence is partially stopped by mountain massif Belasica, Ograzden and Plackovica and northwest of continental climate of Ovche Pole. Compared to other valleys of this area in Strumica the influence of the Mediterranean climate it is enhanced. It is settled on 200-300m above the sea level and it is in the group of Continental-Sub-Mediterranean regions. It is a typically transitive area with combined influences of Mediterranean and East-Continental climate (Filipovski et al., 1996).

During the two-year trials, meteorological indicators for medium temperatures in degrees Celsius and monthly sums of precipitation in mm were monitored. The mean annual temperature in Strumica valley for a period of ten years during the vegetation period of flax, amounted to 18.9 °C (Table 1). For a period 2003/2013 in the valley of Strumica, fall 324.7 mm average rainfall (Table 2).

Schedule of rainfall by months and seasons is quite unbalanced (Table 2). The largest average amounts of rainfall (2003/2013) are registered in May, of 68.1 mm. and the lowest average amount of rainfall in June, with 22.3 mm. The analysis of the temperature in the research period 2014 -2015, showed difference with the average temperatures, in the ten-year average (Table 1).

Year			I	Total (III – IX)	Average (III – IX)				
icai	111	IV	v	VI	VII	VIII	IX	(°C)	(°C)
2014	10.2	12.8	17.4	21.7	24.1	24.0	18.2	3916.2	18.3
2015	7.2	12.4	19.8	21.4	26.7	24.9	20.9	4066	19.0
2003/2013	8.5	13.3	18.4	22.5	25.2	25.0	19.4	4044.6	18.9

Table 1. Average monthly air temperatures during the testing period in degrees Celsius

Table 2. Amount of monthly rainfall during the testing period in mm

Year		Total (III – IX)						
lear	111	IV	v	VI	VII	VIII	IX	in (mm)
2014	71.0	125.5	78.7	56.3	34.4	56.9	100.7	523.5
2015	83.0	16.6	16.1	40.1	6.6	65.6	95.0	323
2003/2013	44.3	35.4	68.1	62.2	22.3	39.3	53.1	324.7

The average temperature in 2014 was about 0.6 °C lower, and in 2015 is 0.1 °C higher than the average. According to the data in Table 1, it can be concluded that the monthly average air temperatures during the vegetation of flax in the period of testing, in both years of testing, are the lowest in the final month and the first month of each year, i.e. March (from 7.2 °C in 2014 to 10.2 °C in 2015) and highest in July (24.1 °C to 26.7 °C). From germination to blooming flax requires temperature in the range of 16 °C to 18 °C. The average monthly temperatures prevailing in the vegetation period are considered as good for growing flax. In Table 2 we can see that the annual amounts of rainfall in the Strumica region, during the test period, is within the optimal needs of flax. In 2015 in the month of July the highest deficit of rainfall is registered (only 6.6 mm), (Table 2). The other months and years, the distribution of rainfall is relatively good to meet the needs of water for growing flax.

# **RESULTS AND DISCUSSION**

In Table 3 and Figure 1 the results of content of flaxseed protein are shown. The ranges of content of proteins was from 26.9 to 29.4%. The general average content of protein in the seeds of the flax, was 28.3%. The size of this parameter largely influence have specificity variety, soil and climate conditions, the applied agro-technical measures, method of storage and more.

In our tests, the genotype for fibre - Viking, had lower content of protein in the seeds of the remaining intermediate genotypes, other than Duferin and Bellan.

In study on Saastamoinen at al. (2013), oil and protein content and their variation on 8 oil and 2 fibre linseed varieties were examined. Fibre varieties 'Belinka' and 'Martta' had higher protein and lower oil contents than oil linseed varieties.

Canadanaa	Ye	<b>A</b>			
Genotype	2014	2015	Average on genotype		
Velušina	28,5+	28,6	28,5		
Duferin	27,8	26,9	27,3		
Bellan	27,8	28,2	28,0		
Viking	27,9	28,4	28,1		
Belinka	29,3	29,4	29,3		
Average on year	28,3	28,3	28,3		
LSD 0,05	n.	S.	General average		
0,01	n.	S.			

In both years of the examination (2014 and 2015), the protein content in the seeds, independent of genotype was 28.3%. The highest protein content in 2014 year of testing had genotype Belinka (29.3%) and the lowest content have Duferin and Bellan (27.8%). Comparing the average protein content in the seeds of flax in 2014 (28.3%) with protein content separately in tested genotypes may be said that Belinka and Velušina have a greater, while Viking, Bellan and Duferin a lower content. In the second year (2015) of the examination, the highest protein content had genotype Belinka (29.4%) and lowest (26.9%), genotype Duferin. The other genotypes (Duferin, Bellan and Viking) had better protein content in the seeds in 2015 (28.6%, 28.2% and 28.4%). All genotypes tested in 2015 had a higher percentage of protein content in the seeds. Comparing the average content of protein in the flaxseeds in 2015 (28.3%) with protein content separately in tested genotypes, can be concluded that Belinka, Velušina and Viking have a greater percentage of protein in the seed in this year, while Bellan and Duferin smaller.

In the general average of genotypes of the two years of testing may be noted that Belinka (29.3%) and Velušina genotype (28.5%) are characterized by the highest average protein content in the seeds.

In the general average of genotypes of the two years of testing may be noted that the lowest protein content in the seeds (27.3%) had genotype Duferin. Regardless of the year of examination, only Belinka (29,3%) had a higher percentage of protein content in the seeds compared to standard Velušina (28.5%). So, Duferin genotype (27.3%) was 1,2% smaller protein content compared to standard Velušina (28.5%), Bellan (28.0%) 0.5% and Viking (28.1%) 0.4%. These obtained differences in percentages of protein in flaxseed in tested genotypes is due to the variety specificity.

In examination on Colovic et al. (2016), the examined linseed cultivars statistically differed ( $p \le 0.05$ ) in the content of protein (from 18.9% to 27.0%) and fat (from 34.1% to 40.7%).

Comparing the average protein content in the flaxseeds from both years (28.3%) with a two-year average protein content separately in tested genotypes, may be said that Belinka and Velušina have a greater percentage of protein in the seeds, while Duferin, Bellan and Viking smaller.

From the received information can be concluded that the greatest percentage of protein in flaxseed had genotype Belinka (29.4%) in 2015 year.

Statistically significant difference on the level of probability of 0.05 and 0.01 does not exist between examined genotypes.

The food industry should be processed and used flaxseed from genotypes Belinka and Velusina, who have more protein content, regardless of the year of manufacture, and which is produced in climate conditions like Strumica region in Republic of Macedonia.

Depending on the years of production, varieties and soil and climatic characteristics of the region, the protein content in the seeds of the flax is 28.3%.



Figure 1. Content of protein in seeds (%) of examination genotypes of flax

#### **CONCLUDING REMARKS**

Based on results of the content of protein in the seeds of the five examined genotypes of flax, produced in 2014 and 2015 in the Strumica region, Republic of Macedonia, may be concluded the following:

The content of protein in the seeds of flax ranges is from 26.9% to 29.4%. On the size of this parameter largely influence had a specificity variety, soil and climate conditions, the applied agro-technical measures, method of storage and more.

The genotypes Belinka (29.3%) and Velušina (28.5%) are characterized by the highest average content of protein in the seeds, regardless of the year of production.

The lowest content of proteins in the seeds had genotype Duferin (27.3%).

Independently of the year of examination, all genotypes have a greater percentage of protein content in the seeds. The differences in the percentages of protein in flaxseed in tested genotypes is due to the variety specificity.

The food industry should be processed and used flaxseed from genotypes Belinka and Velušina, who have more content of proteins, regardless of the year of manufacture, and which is produced in climate conditions like Strumica region in Republic of Macedonia.

The examinations on this field should continue with another genotypes and should give a recommendation for genotypes with better properties for yield and content of proteins for growing in Republic of Macedonia.

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### ПРОТЕИНСКИ ПРОФИЛ НА НЕКОИ ГЕНОТИПОВИ НА ЛЕН (Linum usitatissimum L.) ВО СТРУМИЧКИОТ РЕГИОН, РЕПУБЛИКА МАКЕДОНИЈА

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#### Резиме

Ленот (Linum usitatissimum L.) е индустриска култура и има големо значење за човекот и индустријата. Во периодот 2014 и 2015 година беа извршени испитувања на пет сорти на лен, произведени во струмичкиот регион, Република Македонија во однос на содржината на протеини во семето. Експериментот се состоеше од пет варијанти во три повторувања, поставени по рандомизиран блоксистем. Во текот на вегетацијата беше употребувана стандардна агротехника за производство на лен. Анализата за содржината на протеини во семето од лен е извршена во Лабораторијата за заштита на растенијата и животната средина на Земјоделскиот факултет при Универзитетот "Гоце Делчев" – Штип (Standard: ISO 20483:2006). Содржината на протеини во семето од лен е вршена со Келдал методата. Резултатите се статистички обработени по методот Анализа на варијанса, а разликите тестирани по ЛСД тестот.

Содржината на протеини во семето кај испитуваните сорти се движи од 26.9% до 29.4%. Сите сорти на лен имаа висока содржина на протеини во семето. Но, сортите белинка (29.3%) и велушина (28.5%) се карактеризираат со највисока просечна содржина на протеини во семето, независно од годината на испитување. Во однос на останатите, сортите белинка и велушина се препорачуваат како перспективни сорти за прехранбената индустрија, бидејќи се со највисока содржина на протеини во семето.

Клучни зборови: лен, семе, содржина, сорта, процент, протеини

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 08.06.2018 Accepted: 08.09.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 633.11-293.1(497.11)"2006/2017" Original scientific paper

# EFFECT OF ABIOTIC AND BIOTIC STRESS AND CROP MANAGEMENT ON HEALTH CONDITION AND YIELD OF CEREALS

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#### Abstract

Wheat production has become a global problem due to the climate change impact on wheat farming systems, pest management and control. Yield loss predictions are usually made by using regression models with either biotic or abiotic factors as predictor variables, but only a few of them have considered the combined effects of multiple diseases and climatic conditions. Moreover, efficacy of fungicides in pest control and their effect on yield increase is usually analysed in respect to the level of disease index and yield achieved in untreated plots, without taking into consideration the influence of other environmental elements. This study was conducted in order to determine the combined effects of biotic factors (disease indices) and abiotic factors (climatic elements and efficacy of fungicides) on yield achievements of winter wheat varieties. Field trials were set up under naturally occurring inoculum of the prevalent economic pathogens of wheat in the period 2006-2017. Model varieties Barbee and Durumko, known to have various degrees of susceptibility to wheat pathogens, were used in the study. General linear model function of Minitab 17 (trial version) was used for all the analyses. It was determined that yield loss in untreated plots was significantly influenced by the combined effects of multiple diseases and climatic elements. Moreover, it was determined that the relationship between fungicide efficacy and yield achievements was not straightforward and that it should be analysed in respect to the combined effects of biotic and abiotic factors.

Key words: wheat, fungicide efficacy, yield gain, rusts of wheat, abiotic factors

#### INTRODUCTION

Wheat production has become a global problem due to the climate change impact on wheat farming systems, pest management and control. Analysis of the most influencing factors on yield losses is in the focus of scientific community, knowing that yield is a complex trait. Moreover, agro-ecological conditions, resistant varieties and applied pesticides influence changes in population structure of wheat pathogens making wheat breeding for resistance to economically important diseases very challenging.

The occurrence of obligate parasites (Blumeria graminis f. sp. tritici, Puccinia triticina,

*Puccina striiformis* f. sp. *tritici*) and crop residueborne necrotrophic pathogens (*Zymoseptoria tritici*) in Serbia has been monitored through the decades in experiments related to fungicide efficacy and resistance testing of wheat varieties (Jevtić et al., 2017). Many efforts have been directed to determining the most influencing factors on damage thresholds caused by pathogenic infection and their impact on yield and yield components losses. Although chemical treatments were proven to be a powerful disease-control tool, followed by increases in yield, the question regarding how climate change would influence efficacy of

fungicide applications still remains unanswered (Magan et al., 2011).

Knowing that the relationship between disease rating scale and yield loss is not straightforward (Duveiller et al., 2007; Jevtić et al., 2017), and that fungicide treatment could exhibit negative effect on plant growth when applied under unfavourable environmental

**MATERIAL AND METHODS** 

The fungicide efficacy trials were conducted in the locality Rimski Šančevi (Vojvodina, northern province of Serbia) over the period 2006-2017 using soft wheat variety Barbee (*Triticum aestivum* ssp. *compactum*), and hard wheat variety Durumko (*Triticum turgidum* subsp. *durum*). Variety Barbee is known for its increased susceptibility to wheat rusts and powdery mildew, while Durumko is usually used as a susceptible check for leaf blotch diseases.

#### Field trial and disease assessment

Field trials were set up in a randomized block design in four replicates with plot size of 10 m<sup>2</sup> under naturally occurring inoculum. Fungicides were applied at two growth stages: BBCH 36-37 (flag leaf just visible, rolled) and BBCH 51-59 (inflorescence emergence, heading) (Witzenberger et al., 1989; Lancashire et al., 1991). Usually, ten fungicide-sprayed and non-sprayed check treatments were included in field trial per year. Different types of active ingredients, such as amides, aromatics, azoles, benzimidazoles, morpholines, oxazoles, strobilurins, pyrazoles conditions (Ferree et al., 1999), the main objective of this study was to evaluate the combined effects of biotic factors (disease indices) and abiotic factors (climatic elements and efficacy of fungicides) on yield achievements of winter wheat varieties. The data related to the period 2006 - 2017 were analysed and characterized in terms of agro-ecological conditions of Serbia.

and pyridines, were applied in recommended dosage rates.

Assessments of leaf disease severity were made at the growth stage 71-73 BBCH (kernel watery; early milk) (Witzenberger et al., 1989; Lancashire et al., 1991), known to be highly related to yield (Wegulo et al., 2009). Assessments of powdery mildew, leaf rust, and yellow rust disease severity were made at the growth stage 71-73 BBCH (kernel watery; early milk) (Witzenberger et al., 1989; Lancashire et al., 1991) using modified Cobb's scale (Peterson et al., 1948). Disease severity of Septoria tritici blotch was assessed using the disease rating keys devised by James (1971). The disease indices of obligate and leaf blotch pathogens were calculated by taking into consideration incidence and severity (Cao et al., 2014).

# Yield gain and fungicide efficacy

Yield was measured for each plot at 15 % water content. The yield gain (%) was determined as yield gain in treated plots compared with yield of untreated plots (Eq.1).

 $Y(\%) = ((Y_1 - Y_2)/Y_2) \times 100$ 

Y<sub>1</sub> - grain yield of fungicide treated plot Y<sub>2</sub> - grain yield of the non-sprayed check treatment

Fungicide efficacy was calculated using Abbott's formula (Eq. 2)

Efficacy (%)=((X-Y)/X) x 100

X - disease severity in the non-sprayed check treatment;

Y - disease severity in the treated plot

Yield loss (%) was calculated as yield loss in untreated plots compared with the highest yield response of treated plots.

 $Y(\%) = ((Y_1 - Y_2)/Y_1) \times 100$ 

humidity, and total rainfall (http://www.hidmet.

gov.rs/) together with fungicide efficacy were

considered abiotic predictor variables in building

regression models. General linear model was

performed using Minitab 17 Statistical Software

#### **Statistical methods**

General linear model was used to estimate the relationship between disease indices, abiotic factors and yield gain on fungicide treated plots in the period 2006–2017. Disease indices were considered biotic predictive variables, while monthly averages of temperatures, relative

**RESULTS AND DISCUSSION** 

(2010) (trial version).

Average yield of variety Barbee was 5.4 t/ha on treated and 4.4 t/ha on untreated plots resulting in average yield loss of 18.5 %. Yield of variety Barbee did not change linearly over the period 2006-2017 (Fig.1). The influence of the year (P < 0.000) and treatments (P <0.000) on yield of variety Barbee was determined to be significant. Yield losses caused by obligate parasites varied considerably during 2006-2017 and were in range from 2.7 % in 2012 when average yield on un-treated plots was 7.2 t/ha to 44.2 % in 2009 when average yield on untreated plots was 2.5 t/ha. Jevtić et al. (2017) reported that the most influencing factors on yield loss of Barbee variety in 2006-2013 were disease index of leaf rust and temperature in April. In 2014, yellow rust predominated over leaf rust causing yield loss of 43.4 % on Barbee variety.

Durumko variety is known to have higher yield potential than Barbee variety. Average

yield of Durumko variety on treated plots was 7.1 t/ha in 2006-2017. In untreated plots it was 6.8 t/ha, resulting in average yield loss of 4.2 %. Since difference between yield of treated and untreated plots was not as prominent as it was for variety Barbee, the influence of treatment (treated and untreated) was not recognized as significant, and variations in yield throughout the period were attributed to significant influence of the year (P < 0.000).

# Fungicide efficacy and yield loss of Barbee variety

Chemical treatments were proven to be a powerful tool for disease-control, followed by yield increase, however it was also evident that relationship between yield gain and fungicide efficacy is not always straightforward (Fig.1) and that is related to many factors including difference in disease pressure (Fig.2).



Figure 1. Yield of Barbee and Durumko varieties on treated and untreated plots

In the years when disease pressure of obligate parasites on Barbee variety was below 20%, yield gain in treated plots was not correlated with fungicide efficacy. In 2007 and 2008 when disease index of leaf rust and powdery mildew was below 20 % no evinced difference in yield gain in treated plots (27 % in 2007) and (26 %

in 2008) was observed, although fungicide efficacy in controlling powdery mildew varied from 60 % (2007) to 90 % (2008) (Fig. 2). Average fungicide efficacy in controlling leaf rust in both years was nearly 100 %. Contrary to that, in 2010 disease index of leaf rust in untreated plots was also 20 % and fungicide efficacy was

nearly 100 % as it was is in previous years, but yield gain was more than double higher and reached 63 %. In that year disease pressure of powdery mildew reached 35 % and fungicide efficacy of 70 % brought to yield gain on treated plots of 63 %. These results pointed out that the relation between fungicide efficacy against certain pathogen and yield gain in treated plots is influenced by many abiotic and biotic factors and should be analysed in respect to combined effects of them all. In addition, variability in yield gain in treated plots in the years when disease pressure does not exceed certain limits can be explained by the ability of plants to compensate negative effects of flag leaf infection. El Wazziki et al. (2015) reported that defoliation of flag leaves could improve the photosynthetic activity of the other leaves and that disease severity is not equivalent to the loss of the same percentage of green photosynthetic leaf area.

The fungicidal effect on Barbee yield gain was more prominent in the years when disease pressure took the range between 20-80 % for wheat rusts and 20-44 % for powdery mildew. Leaf and yellow rust with disease indices of 37– 68 % were successfully controlled with fungicide efficacy of 66-79 % resulting in yield gain of 47-54 %. In the years when powdery mildew indices were between 30-35 % (2009-2011), yield gain in treated plots took the range of 47-64 % with evinced correlation with fungicide efficacy. However, it should be pointed out that fungicide efficacy in controlling powdery mildew varied from 7 % in 2011 to 70 % in 2010.

Under high disease pressure, which was on average 80 % for yellow rust in 2015, fungicide efficacy of 47 % resulted in Barbee yield gain of only 7 %. In that year, average powdery mildew disease index also reached the highest value (60 %) comparing with those in twelve-year period and was controlled with fungicide efficacy of 23 %. In 2016, average disease index of powdery mildew was 50 %, which was still higher than twelve-year average, and fungicide efficacy of 29 % resulted in yield gain of 19 %. Those results indicated that under severe disease pressure fungicidal effect could decrease disease index up to 50 %, but it would not be enough to achieve yield gain as in the years when no epidemic invasion occurs.



**Figure 2.** Relationship between disease index of obligate parasites, fungicide efficacy and yield gain of Barbee variety

In order to determine the most influencing factors on Barbee yield general linear model of Minitab 17 (trial version) was applied on data originating from both treated and untreated plots. Analysis of variance showed significant influence of the year (P<0.001), treatment (P=0.094), and fungicide efficacy against leaf rust (P=0.006) for the period 2006-2017 with the exception of 2014 and 2015 when yellow rust predominated over leaf rust. The regression model accounted for 88.5 % of variation in Barbee yield which is expressed in the term of coefficient of determination (R<sup>2</sup>). In 2014 and 2015, disease index of yellow rust (P=0.016), average temperature in January (P=0.001), fungicide efficacy against yellow rust (P=0.015), and treatment (P=0.001) had significant influence on Barbee yield with R<sup>2</sup> of 67.8 %. Temperature in January was proven to be highly influencing on yellow rust epidemics (Sharma-Poudyal and Chen, 2011). In 2014, winter temperature in Serbia exceeded the tenyear average causing outbreak of Warrior race of

yellow rust (Jevtić et al., 2017), causing Barbee yield loss of up to 43.4 %. The *P*-value (<0.001) for both regression models in the analysis of variance showed that the models are significant at an  $\alpha$ -level of 0.05. An analysis of residuals showed the normal probability plot which evinced an approximately linear pattern that is consistent with a normal distribution.

# Fungicide efficacy and yield loss of Durumko variety

Non-correlated dependence between fungicide efficacy and yield gain of Durumko variety was observed as it was when disease indices of obligate parasites on Barbee variety did not exceed 20 %. In twelve-year period, average disease index of *Septoria tritici* blotch on Durumko did not exceed 24.5 %, fungicide efficacy was higher than 58 %, except in 2016 when it was 2%, however yield gain in treated plots varied from 1 % to 22.5 % and was not correlated with fungicide efficacy (Fig. 3).



**Figure 3.** Relationship between disease index of *Septoria tritici* blotch, fungicide efficacy, and yield gain of Durumko variety

Durumko is known to be less susceptible to obligate parasites than Barbee so the leaf rust and powdery mildew were not considered as additional influencing factors on variation in yield achievements of Durumko. Jevtic et al. (2017) reported that in the period 2006-2013 yield loss of Durumko on untreated plots was 10 % and mostly influenced by disease index of *Septoria tritici* blotch and temperature in June, if agro-ecological conditions of Serbia were taken in consideration. In this study, influencing factors on yield achievements were analysed in respect to both untreated and treated plots, and it was determined that the difference between yield of treated and untreated plots was not as prominent as it was for Barbee. Moreover, in 2012 and 2015 average yield of untreated plots was higher than in treated plots. The possibility of overcoming the yield in treated plots by yield on untreated plots was also reported by Rodrigo et al. (2015). Rodrigo et al. (2015) pointed out that under Mediterranean conditions fungicide application might not be recommended in years drier than average, as severity of *Septoria tritici* blotch would be low and the fungicide application itself could reduce grain yield resulting in lower yield in treated than in untreated plots. In addition, Jevtić et al. (2017) reported that *Septoria tritici* blotch accounted for 37.2 % of variation in Durumko yield loss, which was in accordance with results reported by Berraies et al. (2014). Berraies et al. (2014) noted coefficients of determination of 39% and 44% in two sowing seasons while estimated grain yield losses caused by *Septoria*  *tritici* blotch using 400 lines of durum wheat. Those results indicated that great variation in yield losses can be expected when *Septoria tritici* blotch index does not exceed 25 % and that environmental factors greatly contributed to the final yield achievements. In this study, the factors contributing to yield variation in both treated and untreated plots with R<sup>2</sup> of 71.6 % were: disease index of *Septoria tritici* blotch (*P*=0.005), fungicide efficacy (*P*=0.137), all climatic elements in March (*P*<0.001), and total rainfall in May (*P*<0.001).

# **CONCLUDING REMARKS**

The results of this study indicated that the relationship between fungicide efficacy and yield achievements is not straightforward and is highly influenced by disease pressure.

The combined effects of biotic and abiotic factors influenced yield achievements in the treated plots and should be considered when fungicidal effects are estimated.

Further investigations of potential adverse effects of different types of active ingredients on crop physiology, especially on photosynthesis in changing climate conditions, will provide more information on quality and predictability of fungicidal effects on yield achievements in the future.

# ACKNOWLEDGEMENT

This study was realized as a part of two projects: TR 31066 - Contemporary breeding of small grains for current and future needs and III - 43007- Studying climate change and its influence on environment: impacts, adaptation and mitigation, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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# ВЛИЈАНИЕ НА АБИОТСКИ И БИОТСКИ СТРЕС И МЕНАЏМЕНТ НА КУЛТУРА НА ЗДРАВСТВЕНАТА СОСТОЈБА И ПРИНОСОТ НА ЖИТНИТЕ КУЛТУРИ

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#### Резиме

Производството на пченица стана глобален проблем поради влијанието на климатските промени врз системите за одгледување на пченица, управувањето со штетници и контролата. Предвидувањата за губиток на приносот обично се прават со користење на регресивни модели со биотски или абиотски фактори како променливи варијабли, но само неколку ги земале предвид комбинираните ефекти на повеќе болести и климатски услови. Покрај тоа, ефикасноста на фунгицидите во контрола на штетници и нивниот ефект врз зголемувањето на приносот обично се анализира во однос на нивото на индексот на болеста и приносот постигнат во нетретирани експериметални површини, без да се земе предвид влијанието на другите елементи на животната средина. Оваа студија беше спроведена со цел да се одредат комбинираните ефекти на биотските фактори (индекси на болеста) и абиотските фактори (климатски елементи и ефикасност на фунгициди) врз постиганиот принос кај сорти зимски пченица. Беа поставени ескпериметални површини под инокулум на преовладувачки природно присутни економски патогени на пченицата во периодот 2006-2017 година. Во студијата беа користени модели - сорти Barbee и Durumko за кои се знае дека имаат различни степени на подложност на патогени од пченица. Општа линеарна модел функција на Minitab 17 (пробна верзија) беше користена за сите анализи. Беше утврдено дека загубата на родот во нетретирани експериментални парцели беше значително под влијание на комбинираните ефекти на повеќе болести и климатски елементи. Покрај тоа, беше утврдено дека односот помеѓу ефикасноста на фунгицидот и постиганатиот принос не е јасен и дека треба да се анализира во однос на комбинираните ефекти на биотските и абиотските фактори.

**Клучни зборови:** пченица, ефинаксно на фунгицид, принос на зрно, житни 'рѓи, пепелница, Septoriatriticiblotch, абиотски фактори

Journal of Agriculture and Plant Sciences, JAPS, Vol. 16, No. 2, pp. 25-31

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 22.05.2018 Accepted: 05.06.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 633.71-152.61:575.22(497.775)"2016/2017" Original scientific paper

# STUDY OF QUANTITATIVE CHARACTERISTICS IN ORIENTAL TOBACCO GENOTYPES

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#### Abstract

Ten genotypes of oriental tobacco (three varieties and seven newly created lines) were investigated for some major morphological and agronomic traits: height of the plant without inflorescence, leaf number per plant, length/width of the middle belt leaves and dry mass yield/plant. The trial was set up in the Experimental field of Tobacco Institute–Prilep in 2016 and 2017, in randomized block design with four replications, using traditional agricultural practices. Measurements of the first three traits were made in the stage of butonization and beginning of flowering, while the dry mass yield was recorded during the treatment of cured tobacco. The results were statistically processed.

The aim of the research is to study the quantitative traits of Oriental genotypes, to determine the stability of the population through their variability and to emphasize the best among the selected assortment.

From the set of genotypes we selected three superior lines with a high degree of homogeneity. The coefficient of variation of quantitative traits in these lines was lower than 10%, which is a sign of their stability. After their acceptance by the Commission for recognition and approval of varieties within the Ministry of Agriculture, Forestry and Water Economy of R. Macedonia, these genotypes will be placed on the market.

Key words: tobacco, oriental genotypes, quantitative traits, variability

#### INTRODUCTION

Plant breeding is a creative activity in which the breeder follows his eternal desire to create new varieties more superior than the existing ones. At the Scientific Tobacco Institute - Prilep there is a centuries-old tradition of continuous successive selection work of tobacco varieties of different types, but generally of types Prilep, Yaka, Dzebel and Basmak - more recently. By 2014, the institute owns 10 varieties of Prilep type, 4 varieties of Yaka type, 4 varieties of Dzebel, 3 varieties of the type Otlya, 1 of the type Virginia and 3 of the type Burley (Kotubin - Aleksoska, 2004). Today, the variety fund is drastically increased and exceeds the number of about 60 varieties. The breeders from the Institute have published a number of papers on the topic of tobacco breeding, as for example Aleksoski and Korubin-Aleksoska (2011), studied the mode and level of inheritance of green and dry mass yield per stalk in four parental genotypes (Burley - B 2/93, Suchum - S1, Suchum - S2 and Prilep - P-84) and in their six diallel F1 hybrids, and found positive and negative heterosis. The higher heritability index of both types was recorded for dry mass yield. Mitreski (2012), studied height of the stalk with inflorescence in six oriental tobacco varieties of the type Prilep: P-23, P 12-2/1, NS-72, P 66-9 /7, P-79-94 and Prilep Basma 82. The average values for the

trait ranged from 59,3 cm in Prilep P 12-2/1 to 148,1 cm in Prilep Basma 82. The same author in co-authorship with Korubin-Aleksoska (2014), made tasting of the cigarettes composed from the same varieties and informed that they have good degustation properties that are typical for oriental tobaccos. The authors (2015) of the same varieties examined some morphological traits and announce that Prilep Basma 82 had the highest average leaf number, and the lowest length and width of the middle belt leaves. Korubin-Aleksoska and Aleksoski (2013), presented investigations on the inheritance of length, width and area of middle belt leaves in oriental tobacco varieties Prilep (P0 10-3/2), Djebel (Pobeda P-2) and Yaka (YK 48), and the semi-oriental Forchheimer Ogrodowny - FO, including their six F1 and six F2 hybrids. The regression graphs reveal partially dominant type of inheritance of the characters and absence of interallelic interaction. Dimitrieski and Miceska (2015), offer information about new prospective variety of the oriental Yaka tobacco, and as the most perspective point out Yaka b 65 - 82/1. Korubin – Aleksoska (2016), explores heredity of the more important quantitative traits of four parental genotypes (Prilep P 10-3/2, Djebel A 42/3, Yaka YV 125/3, Floria FL-1) and their dialectic F1, F2 and BC1 generations, through genetic components of variance. The heritability is very high, indicating that the studied traits are highly heritable. Korubin – Aleksoska and Ayaz (2016), investigated height of the stalk with inflorescence, leaf number, length of the middle belt leaves and dry mass yield per stalk in five autochthonous tobacco varieties of types: Prilep (P 10-3/2 and P 12-2/1), Djebel (Dj No 1) and Yaka tobaccos (YK 7-4/2 and KY), and five commercial oriental varieties of Prilep tobacco (P-23, P-84, NS-72, P-66-9/7 and P-79-94). Differences between the genotypes in the investigation period were highly significant, which is a sign

of their mutual genotypic and phenotypic diversity. The same authors (2016), studied ten oriental tobaccos of the types: Basmak (MK-1, MK-2, MB-2, MB-3, MS-8/1, MS-9/3 and YZ-7), Prilep (Prilep Basma-82), Djebel (Xanthi Djebel-1) and Yaka (YV 125/3), for some more important quantitative traits, and gave precise knowledge of the new type Basmak in Macedonia and the Balkans from genetic, morphological and agronomic aspects. All Basmak varieties are characterized by stability and uniformity as a result of their homozygotness. Dimitrieski, Gveroska (2017), studied some morphological traits, length of the growing season and resistance to powdery mildew disease in some oriental tobacco varieties and lines of the type Prilep. Miceska (2017), investigated some morphological, production and guality characteristics in four new lines of the type Prilep obtained by generative hybridization (P .l. 14-65/1,P.I.14-66/7, P.I. 123-82/2,P.I. 14-67/7 ) and the variety P12-2/1 as a standard. Regarding the morphological properties (plant height, leaf number, largest leaf size), all lines showed very low variability, which is an indication of morphological uniformity and stability. Korubin - Aleksoska (2017), studied the oriental varieties in order to obtain data on their tolerance to drought. The highest degree of tolerance to drought was observed in genotypes P - 84 (type Prilep) and P - 2 (type Dzebel). These genotypes can be included in the programmes for improvement of the investigated trait.

The aim of this paper is to show the way of assessment the stability of newly created lines of the Prilep and Yaka types, and then selecting the best for placing them in a comparative trial for varietal confirmation by the Commission for recognition and approval of varieties at the Ministry of Agriculture Forestry and Water Economy of R. Macedonia. The recognized varieties can be put into circulation.

# **MATERIAL AND METHODS**

As a material for work, three oriental varieties were taken from which two of the type Prilep (P-66-9/7 – Fig. 1 and P-84 – Fig. 2) and one of the type Yaka (YV 125/3 – Fig. 3), as well as 7 newly created genotypes, of which four are of the type Yaka (L1 - YK 23-09/07 – Fig. 4, L2 - YK 8-07 / 11 – Fig. 5, L3 - YK 23-12/85 – Fig. 6 and L4 - YK 9-08/80 – Fig. 7) and three of the type

Prilep (L5 – P 6-03/59 – Fig. 8, L6 – P 16-03/63 - Fig. 9 and L7 – P 10-07/57 – Fig. 10). The variety P-66-9/7 due to its mass application in tobacco production in Macedonia is taken to control the comparison with line of the type Prilep. P-84 was taken as a second control because it showed great resistance to drought. YV 125/3 is a control for lines of the type Yaka.

The experiment was set up in 2016 and 2017 on the Experimental Field of the Scientific Tobacco Institute - Prilep after a random block system in four repetitions. From the complete measurements, for this paper are separated: the height of plants without inflorescence, the number of leaves per plant, the length and width of the middle-belt leaves, and dry mass yield per plant. The obtained data are statistically processed through parameters of variability of traits and variance analysis (Najceska, 2002).



Figure 1. Prilep, P-66-9/7



Figure 3. Yaka, YV 125/3



Figure 2. Prilep, P-84



Figure 4. L1 – YK 23-09/07

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Figure 5. L2 – YK 8-07 / 11



Figure 7. L4 – YK 9-08/80



Figure 9. L6 – P 16-03/63



Figure 6. L3 – YK 23-12/85



Figure 8. L5 – P 6-03/59



Figure 10. L7 – P 10-07/57
### **RESULTS AND DISCUSSION**

In order to get acquainted with the genetic stability of the newly created lines, measurements of the quantitative traits were made in 2016 and 2017 and on the basis of the obtained values an analysis and their ranking was performed.

From the results shown in Table 1, the highest height of the plant without inflorescence was observed at the orienteal line of the type Yaka L3 (131 cm - 2016; 128 cm - 2017). This line is higher than the control variety YV 125/3 (118 cm - 2016; 116 cm - 2017), and the difference between them is highly significant. Also, the L4 line is significantly higher than YV 125/3, while L1 and L2 are significantly lower. The smallest height is distinguished in oriental variety of the type Prilep P-84 (62 cm - 2016; 60 cm - 2017), which is significantly lower than the standard variety P-66-9/7 (70 cm - 2016; 62 cm - 2017) taken for control line of the type Prilep. The newly created genotypes L5 and L7 are higher, while L6 is lower than the control variety, and the differences are highly significant. All average values in 2017 are lower compared to those in 2016, due to the fact that 2017 was extremely dry during the vegetation.

The investigations on the variability of the trait in varieties and lines showed low values. The coefficient of variability (CV) ranges from 2.49% (2017) in L3 to 10.82% (2017) in L4. The higher values of lines L4 and L5 point to the fact that they need additional successive selection which will enable their homogenization and stabilization. Lower values in 2017 are a sign of

proper selection, directed to the stabilization of new genotypes. There is an exception in the populations of L4 and L7, but the difference is minimal and due to undefined environmental factors.

The highest number of leaves per plant has P-66-9/7 and L3 (57 - 2016), which can be seen from Table 2. The least leaves have the standard variety YV 125/3 (41 - 2017). The higher number of leaves in the lines from the Yaka type is highly significant (with the exception of L2, where the significance is 5%). From the analysis of the number of leaves per plant in the two years of investigation, small differences can be observed, which points to the fact that it is a high-hereditary trait. The greatest difference occurs in P-66-9/7, from which can be conclude that this variety is sensitive to drought stress, and for its successful cultivation is necessary timely watering.

The values of the variability of the traits in the investigated genotypes are very low. The highest coefficient of variability has line L1 (9.79% - 2016; 7.82% - 2017), and the lowest varieties P-84 (3.02% - 2016) and YV 125/3 (2.18% - 2017). From the newly created Yaka genotypes, the L1 (5.41% - 2016; 3.36% - 2017) has the lowest coefficient of variability, while from Prilep genotypes L6 (4.99% - 2016; 4.59 - 2017). In all newly created variants, the variability in the number of leaves per plant in 2017 is lower than in 2016, which is another confirmation of the proper selection aimed at stabilizing them.

C		201	6		20	2017		
Genotypes	n	$\overline{x} \pm S\overline{x}$ (cm)	σ	CV (%)	$\overline{x} \pm S \overline{x} (cm)$	σ	CV (%)	
1. P-66-9/7 Ø	20	70.49 ± 0.53	2.37	3.41	$62.25 \pm 0.37$	1.66	2.69	
2. P-84	20	61.94 ± 0.63	2.82	4.56	59.65 ± 0.41	1.85	3.11	
3. JV 125/3 Ø	20	117.87 ± 1.82	8.14	6.96	115.8 ± 0.67	3	2.59	
4. L1	20	115.67 ± 1.28*	5.71	4.93	112.39 ± 1.18*	5.28	4.72	
5. L2	20	113.12 ± 1.18**	5.27	4.69	108.77 ± 0.77**	3.44	3.14	
6. L3	20	130.62 ± 0.97**	4.32	3.33	128.12 ± 0.72**	3.20	2.49	
7. L4	20	126.12 ± 2.81**	12.55	10.04	120.75 ± 2.92**	13.06	10.82	
8. L5	20	88.55 ± 2.16**	9.65	10.81	84.84 ± 1.75**	7.83	9.12	
9. L6	20	65.42 ± 0.67**	3	4.59	57.37 ± 0.56**	2.5	4.35	
10. L7	20	92.87±1.29**	5.76	6.18	89.87 ± 1.25**	5.58	6.22	
		LSD <sub>0.05</sub> = 1.73			LSD <sub>0.05</sub> = 2.34			
		$_{0.01}^{0.03} = 3.12$			<sub>0.01</sub> = 4.21			

Table 1. Height of the plant without inflorescence (cm)

Construct		201	6		20	)17	
Genotypes	n	$\overline{x} \pm S\overline{x}$ (cm)	σ	CV (%)	$\overline{x} \pm S\overline{x}$ (cm)	σ	CV (%)
1. P-66-9/7 Ø	20	57.25 ± 0.40	1.78	3.12	$54 \pm 0.56$	2.51	4.65
2. P-84	20	42.45 ± 0.29	1.28	3.02	$42.2 \pm 0.3$	1.33	3.14
3. JV 125/3 Ø	20	41.65 ± 0.28	1.28	3.06	41.3 ± 0.2	0.9	2.18
4. L1	20	45.25 ± 0.55**	2.45	5.41	45.35 ± 0.34**	1.53	3.36
5. L2	20	42.95 ± 0.83*	3.72	8.66	43.6 ± 0.4*	1.8	4.13
6. L3	20	57 ± 1.14**	5.08	8.91	59.2 ± 0.77**	3.43	5.79
7. L4	20	53.5 ± 1.17**	5.24	9.79	54.25 ± 0.95**	4.24	7.82
8. L5	20	49.8 ± 0.65**	2.91	5.84	50.4 ± 0.53**	2.37	4.71
9. L6	20	$48.05 \pm 0.54^{**}$	2.40	4.99	47.35 ± 0.49**	2.17	4.59
10. L7	20	46.3 ±0.73**	3.26	7.04	46.1 ± 0.52**	2.32	5.04
		LSD <sub>0.05</sub> = 1.03			LSD <sub>0.05</sub> = 1.52		
	0.01 = 1.85		$_{0.01}^{0.05} = 2.74$				

 Table 2. Number of leaves per stalk

With the longest length of middle-belt leaves, in both years of investigation, the line L7 is characterized (Table 3). The average value of the trait in 2016 is 31.7 cm, and in 2017 it is 31.65 cm, and the difference in comparison with the control variety P-66-9/7 is highly significant. Also, L5 and L6 lines have significantly longer leaves. The difference between the genotypes from type Yaka is very small, in L1 it is insignificant, and in L2, L3 and L4 the significance is 5%. From the results in the two years of investigations it can be seen that the differences are minimal,

which is a sign of the high inheritance of the trait.

Low variability of the length of middle-belt leaves is an indicator of the stability of the trait, i.e. the low impact of environmental factors on its magnitude. The highest coefficient of variability has the line L3 (9.85% - 2016; 9.47% - 2017), and the lowest L1 (4.99% - 2017). Newly created lines in 2017 have lower variability than those in 2016, which is an indicator of improving their stability.

Constunes		2016			2017		
Genotypes	Genotypes n		σ	CV (%)	$\overline{x} \pm S\overline{x}$ (cm)	σ	CV (%)
1. P-66-9/7 Ø	20	$20.4 \pm 0.44$	1.96	9.61	20 ± 0.32	1.41	7.07
2. P-84	20	20.6 ± 0.35	1.56	7.58	20.5 ± 0.39	1.73	8.45
3. JV 125/3 Ø	20	$21.82 \pm 0.46$	2.06	9.46	$21.55 \pm 0.24$	1.09	5.08
4. L1	20	21.68 ± 0.42	1.87	8.64	21.5 ± 0.24	1.07	4.99
5. L2	20	$20.65 \pm 0.3^{*}$	1.35	6.55	20.3 ± 0.27*	1.21	5.95
6. L3	20	$20.4 \pm 0.45^{*}$	2.01	9.85	20.2 ± 0.43*	1.91	9.47
7. L4	20	$20.68 \pm 0.36^{*}$	1.59	7.7	20.58 ± 0.34*	1.53	7.42
8. L5	20	22.25 ± 0.38**	1.7	7.64	$22 \pm 0.3^{**}$	1.34	6.1
9. L6	20	22.05 ± 0.54**	1.94	8.78	21.9 ± 0.32**	1.45	6.6
10. L7	20	31.7 ±0.5**	2.24	7.06	31.65 ± 0.48**	2.15	6.8
$LSD_{0.05} = 0.85_{0.01} = 1.53$		LSD <sub>0.05</sub> = 0.85			LSD <sub>0.05</sub> = 0.91		
			<sub>0.01</sub> = 1.63				

Table 3. Length of the leaves from the middle belt (cm)

The results in the width of the leaves from the middle-belt, shown in Table 4, inform about the similarity of the variants in relation to this trait. The broadest leaves have YV 125/3 (11.78 cm - 2016; 11.67 cm - 2017) and the newly created L1 line (11.48 cm - 2016; 11.15 cm - 2017). The smallest width has the leaves on the L7 (8.3 cm - 2016; 8.25 cm - 2017). The differences between the years of investigation are minimal, thus confirming the high inheritance of this trait.

The variability of the width of the leaves from the middle belt is very small, and a confirmation of this is the low value of the coefficient of variability. He ranges from 1.93%

(2016) in YV 125/3 to 13.77% (2016) in L6. The low variability values for trait are the indicator for high inheritance and stability. The newly established lines in 2017 have a lower variability than that in 2016, which is still one indicator for balancing the trait.

Constynes		2016			2017		
Genotypes	n	$\overline{x} \pm S \overline{x} (cm)$	σ	CV (%)	$\overline{x} \pm S \overline{x}$ (cm)	σ	CV (%)
1. P-66-9/7 Ø	20	$10.2 \pm 0.09$	0.41	3.98	$9.96 \pm 0.08$	0.34	3.45
2. P-84	20	$10 \pm 0.13$	0.57	5.67	$9.8 \pm 0.09$	0.41	4.14
3. JV 125/3 Ø	20	$11.78 \pm 0.05$	0.23	1.93	11.67 ± 0.06	0.26	2.21
4. L1	20	11.48 ± 0.27	1.23	10.72	11.15 ± 0.19	0.85	7.65
5. L2	20	10.8 ± 0.14	0.64	5.93	10.68 ± 0.12	0.53	4.97
6. L3	20	10.35 ± 0.21*	0.95	9.18	10.2 ± 0.16*	0.73	7.17
7. L4	20	9.95 ± 0.23**	1.01	10.16	9.72 ± 0.19**	0.84	8.68
8. L5	20	9.15 ± 0.15*	0.67	7.35	8.92 ± 0.12*	0.53	5.95
9. L6	20	9.77 ± 0.3	1.34	13.77	9.55 ± 0.25	1.13	11.81
10. L7	20	8.3 ± 0.22**	0.98	11.8	8.25 ± 0.18*	0.8	9.68
LSD <sub>0.05</sub> = 1.01 $_{0.01}$ = 1.82				$LSD_{0.05} = 1.04$ $_{0.01} = 1.87$			

Table 4. Width of the leaves from the middle belt (cm)

The highest yield on the dry mass per plant among the parental genotypes has P-66-9/7 (20.15 g - 2016; 19.07 g - 2017), while between the newly created genotypes L7 (22.53 g - 2016; 21.37 g - 2017). The difference in values between the control of Prilep type and the line L7 is significant in 2016 and high-significant in 2017. Between the lines of Yaka type, significantly higher yield have L1, L3 and L4. The yield of the whole genotype set in 2017 is lower than that in 2016 (with the exception of L3), but the minimum differences do not have any significance. The data of the dry mass yield in the two years of investigations are shown in Table 5.

L5

19.21

19.12

L6

19.05

18.93

L7

22.53\*

21.37\*\*

Dry mass yield per plant (g)

Years P1 Ρ2 Ρ3 20.28\*\* 2016 20.15 17.89\* 17.52 17.24 2017 19.07 19.87\*\* 17.83\* 2016: LSD <sub>0.05</sub> = 1.40 <sub>0.01</sub> = 2.53 2017: LSD <sub>0.05</sub> = 1.19 <sub>0.01</sub> = 2.15

#### **CONCLUDING REMARKS**

L2

17.74

17.56

L1

Genotypes

L3

21.12\*\*

21.15\*\*

L4

19.7\*

18.91\*

The highest average height in the two years of investigation has the newly created line L3 (131 cm - 2016; 128 cm - 2017). In comparison with YV 125/3, the lines L1 and L2 are significantly lower, and L3 and L4 are significantly higher. The minimum average height has P-84 (62 cm -2016; 60 cm - 2017), and from the lines of Prilep type has L6. The variability YV 125/3 and lines from Yaka type have higher height of varieties and lines from Prilep type. Differences in values between the two years in variants are minimal, which is a sign of a high degree of ecological stability.

Highest number on the leaves has P-66-9/7 (57 - 2016; 54 - 2017) and L3 (57 - 2016; 59 -2017). All lines from Yaka type have significantly higher number of leaves than the standard variety YV 125/3. The lines from Prilep type have significantly lower number of leaves than the control P-66-9/7.

The highest length of leaves from the middle belt, in the control variants has YV 125/3 (22.82 cm - 2016; 21.55 cm - 2017), and in the lines has L7 (31.7 cm - 2016; 31.65 cm -2017). Dimensions for the length of the leaves in the control and the lines of Yaka type are very similar, so the difference are minimal or significant for 5%. The newly created variants of the Prilep type have significantly longer leaves than the two control varieties.

The largest width of the leaves from the middle belt has YV 125/3 (11.78 cm - 2016; 11.67 cm - 2017). L1 has the widest leaves between the lines (11.48 cm - 2016; 11.15 cm - 2017). The tightest leaves has L7 (8.3 cm - 2016; 8.25 cm - 2017).

With highest yield on dry mass per plant among the standard varieties is P-66-9/7 (20.15 g - 2016; 19.07 g - 2017), while among the lines L7 (20.53 g - 2016; 21.37 g - 2017). Genotypes L1, L3 and L4 have a significantly higher yield than YV 125/3.

Two-year investigations for variability of the traits in varieties and lines shows low values. The coefficient of variability (SV) for the height of the plant without inflorescence ranges from 2.59% (YV 125/3 - 2017) to 12.55% (L4 - 2016), for the number of leaves per plant from 2.18% (YV 125/3 - 2017) to 9.79% (L4 - 2016), for the length of the leaves from the middle-belt from 4.99% (L1 - 2017) to 9.85% (L3 - 2016), and for the width of the leaves from the middle-belt from 1.93% (YV 125/3 - 2016) to 13.77% (L6 - 2016). The results indicate high genetic homogeneity, i.e. stability and uniformity of the newly created lines.

The highest yielding among the variants from the type Prilep is line L7 (22.53 g / plant - 2016; 21.37 g / plant - 2017), while between variants from the type Yaka is L1 (20.28 g / plant -2016; 19.87 g / plant - 2017) and L3 (21.12 g / plant - 2016; 21.15 g / plant - 2017). These lines can be entered in the comparative labours for varietal recognition by the Ministry of Agriculture, Forestry and Water Economy of R. Macedonia.

# ACKNOWLEDGEMENT

Appreciation to the employees and postgraduates at the Scientific Tobacco Institute – Prilep.

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#### ПРОУЧУВАЊА НА КВАНТИТАТИВНИТЕ СВОЈСТВА КАЈ ОРИЕНТАЛСКИ ГЕНОТИПОВИ ТУТУН

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#### Резиме

Испитувани беа десет генотипови на ориенталски тутуни, од кои три сорти и седум Испитувани беа десет генотипови на ориенталски тутуни, од кои три сорти и седум новосоздадени линии, за поважните морфолошки и агрономски својства: висина на растението без соцветие, број на листови по растение, должина и ширина на листовите од средниот појас и принос на сува маса по растение. Истражувањата беа направени на опит по случаен блок-систем во четири повторувања, поставен на опитното поле при Научниот институт за тутун – Прилеп во текот на 2016 и 2017 година. Опитот беше одгледуван со примена на вообичаени агротехнички мерки. Мерењата на првите четири својства беа направени во периодот на бутонизација и почеток на цветање, а приносот на сува маса беше евидентиран при манипулацијата на сувиот тутун. Резултатите беа статистички обработени.

Целта на овој труд е да се проучат квантитативните својства кај ориенталските генотипови, преку нивната варијабилност да се одреди стабилноста на популацијата и да се истакне најдоброто меѓу избраниот сортимент.

Од поставените генотипови издвоивме три посупериорни линии кои покажаа висок степен на хомогеност. Коефициентот на варијабилност на квантитативните својства им е понизок од 10%, што е знак за нивната стабилност. По нивното признавање од страна на Комисијата за признавање и одобрување на сорти при Министерството за замјоделство, шумарство и водостопанство на Република Македонија, овие генотипови ќе може да се пуштат во промет.

Клучни зборови: тутун, ориенталски генотипови, квантитативни својства, варијабилност

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 14.11.2018 Accepted: 19.11.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 637.523.043:665.327.3 Original scientific paper

# INFLUENCE OF OLIVE OIL ON THE FATTY ACIDS COMPOSITION OF COARSE CHOPPED BOILED SAUSAGES

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### Abstract

The paper examines the influence of ordinary and cold pressed olive oil on the fatty acid composition of coarse chopped boiled sausages. For this purpose, cold pressed olive oil was added in the production of the Folk sausages and in the production of Kranj sausages was added ordinary olive oil. In both production batches, olive oil is added in the amount of 3, 4 and 5 g/kg. In the examined production batches of Folk sausage, the content of palmitic and stearic fatty acids (C16: 0 and C18: 0) is within the limits of other meat products. A smaller percentage representation is observed in the content of C16: 0, and greater in the content of (C18: 0) in the production batches of Kranj sausages. The ratio of PUFA / SFA in bought production batches of sausages is up to 0.4%, which means that the sausages full field the quality requirements of the product according to the lipid content that means that the addition of olive oil in this type of batches is appropriate.

**Key words:** Folk sausage, Kranj sausage, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, quality

### INTRODUCTION

Meat and meat products are considered essential in the diet of the population. The main ingredients of the meat, among the water are: proteins, fats, vitamins and minerals, which give the meat a high degree of biological and nutritional value. In the context of the abovementioned, particularly important sources, types of meat are fatty acids (especially fish meat), B-vitamins and iron content.

Besides the nutritional value, meat and meat products have an important social role in modern society. The increase in population, urbanization, economic growth and the development of markets have led to an increase in demand for meat and products of animal origin (Costales et al., 2006; Steinfeld et al., 2006 a, b).

Food from animal origin, including meat, is required to act favourably for the preservation of human health. To achieve this goal, different additives of plant origin and vegetable oils are added to the meat and meat products. Some of them have an influence on the oxidative processes, some effect on the microbiological quality of products and some of them can be used as a partial replacement of the fatty tissue that is added to the production of meat products.

Lately, more research has been carried out on the use of different types of vegetable oils in the production of meat products. Muguerza et al. (2001) and Jimenez-Colmenero, (2007) investigated the use of olive oil, Carvalho et al. (2006) and Rubio et al. (2007) investigated the used of sunflower oil in the production process of sausages, Muguerza et al., (2003; 2004) and Rubio et al. (2007) investigated the used soybean oil, Valencia et al. (2006; 2007) fish oil and algae oil, Vasilev et al. (2011) investigated the use of palm oil in the production of different types of meat products.

Hyun-Jen Lee et. al., (2015) investigated the effect of lard substitution with vegetable oils on

the quality properties of sausages. The authors concluded that with the replacement of lard with mixed vegetable oils there were no adverse effects on the quality of sausages. The reduced composition of saturated fatty acids, authors said that made the quality product.

Luruena-Martinez et al. (2004) and Muguerza et al., (2002) state that the addition of olive oil does not cause significant differences in the shrinkage of heat treatment but gives them more yellow colour.

Bloukas et al. (1997) found that greater olive oil content causes greater weight loss, probably due to higher amounts of added water.

The aim of our research was to investigate the influence of different quantities of cold pressed and ordinary olive oil on the fatty acid composition of semi-permanent, coarse chopped boiled sausages.

### **MATERIAL AND METHODS**

### **Production of Coarse Chopped Boiled sausages**

The Kranj and Folk sausage served as a material for examination. According to the Rulebook for demands regarding quality of minced meat, meat preparations and meat products (Official Gazette of Republic of Macedonia No. 63 from 29.04.2013), these sausages belongs to the group of semipermanent, coarse chopped boiled sausages. The production of the sausages was in compliance with all sanitary and veterinary regulations applicable in Macedonia.

The cold press olive oil (Santorina extra virgin, Nutria C.A, Greece), was added during preparation of the mix for Folk sausages and ordinary olive oil (Olio Di Sansa Di Oliva) was added during preparation of the mix for Kranj sausages. The mix is stuffed in pork small intestines. Four groups of bacon – Folk and Kranj sausages were prepared for the experiment:

- l group without addition of cold press olive oil (control group)
- II group with addition of 3gr/kg cold press olive oil
- III group with addition of 4gr/kg cold press olive oil
- IV group with addition of 5gr/kg cold press olive oil

### Examination of fatty acid composition

Fatty acid composition of groups of sausages was determined by gas chromatography. Method of work with the sample is AOAC 996.06 GC-FID-7890 A with the device Gas chromatograph with flame ionization detector. The fats from analysed samples of sausages have been extracted by hydrolysis (acid hydrolysis). The Folk sausages were manufactured according to the following technological recipe: chicken MOM 3 kg; chicken mess 12 kg; pork trimming 35 kg; hard fatty tissue 40 kg; leek 3 kg; nitrite salt 1.7 kg; spice "Koleks" 0.400 kg; polyphosphate 0.500 kg; emulsifier 2.0 kg; hard water 20.0 kg; cold pressed olive oil Santorina (extra virgin).

The recipe used for production of the sausage is as follows: 0.7 kg emulsifier, 2.0 kg polyphosphate, 1.8 kg nitrite salt, 0.4 kg spice for Kranj sausage, ordinary olive oil Olio Di Sansa Di Oliva 3g/kg; 4g/kg; 5g/ kg.

Olive oil in both groups of sausages was added during the cure of the mixture. After preparation and mixing, the mix is stuffed in natural pork intestines with diameter 32-34mm. After stuffing and pressing, the sausages were thermally processed, according to the following formula: 35 minutes drying at 62°C, 20 minutes smoking at 62 °C, 35 minutes boiling at 78 °C. After thermal processing, the sausages were vacuumed with vacuum machine Vebomak. After vacuuming, the sausages were stored at refrigerator at temperature of +4 °C.

Pyrogallic acid is added in order to avoid fatty acids release. Methylation of fatty acids was conducted according to AOAC GC - FID - 7890. The obtained fatty acid methyl esters (FAMES) were analysed with a gas chromatograph with flame ionization detector and capillary column (SP 2560 100 mx 0.25 mm to 0, 25  $\mu$ m).

Working conditions: injection temperature

225°C, detector 285°C. The initial temperature at 100°C is maintained 4 minutes, increased by 3°C each minute, up to final temperature of 240°C, leave 15 minutes. The carrier gas had helium flow of 0.75 ml/min. Certain FAMES from the analysed groups of sausages are identified partially, on the basis of comparison between their retention times (characteristic for their molecular mass as identification parameter) and FAMES retention standards (which include cis and trans isomers

of fatty acids). Analysed content of each fatty acid is expressed in percent (%).

### **Statistical processing**

Each parameter was determined after three repetitions, and the results are presented as mean value  $\pm$  Sd. The obtained results are mathematically and statistically processed in Microsoft Excel 2003.

### **RESULTS AND DISCUSSION**

### Fatty acid composition of production batches of Fok sausages

The results of the studies on the fatty acid composition of the investigated samples of Folk and Kranj sausages are given in Tables 1,2,3,4.

Fatty acids (%)	l batch	ll batch	III batch	IV batch
C14:0	1.23	1.25	1.20	1.19
C16:0	23.87	23.64	24.05	23.89
C16:1	2.37	2.41	2.14	2.49
C17:0	0.59	0.54	0.61	0.56
C17:1	0.32	0.28	0.32	0.30
C18:0	13.10	12.88	13.81	13.06
C18:1n9c	44.09	43.62	44.35	43.35
C18:2n6c	12.60	13.48	11.85	13.59
C183n6	0.42	0.44	0.26	0.33
C183n3	1.11	1.15	1.10	0.95
C20:2	0.30	0.31	0.30	0.29

Table 1. Fatty acid composition of Folk sausages (%)

**Table 2.** Saturated, polyunsaturated, monounsaturated and unsaturated fatty acids in the production series of Folk Sausage (%)

Fatty acids (%)	l batch	ll batch	III batch	IV batch
Saturated	38.79	38.31	39.67	38.70
Polyunsaturated	14.13	15.07	13.21	14.87
Monounsaturated	47.09	46.62	47.12	46.43
Unsaturated	61.22	61.69	60.33	61.30

According to Table 1 and 2, it is seen that the content of C16:0 ranges from an average of 23.64% to 24.05% in batches with added olive oil compared to 23.87% in the control. The content of C18: 0 ranges from 12.88% to 13.81% for batches with added olive oil in relation to 13.10% in the control group sausages. The content of these saturated fatty acids is within the limits of other meat products. During production of Folk sausages the content of C16:0 has decreased, while C18:0 is increased compared to the results for Kranj sausages, which is consequence of the different types of meat for sausage production and fat content.

Pork meat normally contains a greater amount of palmitic and oleic acid due to the type of meat and its structure of lipids and poultry meat is significantly comes with lower content of stearic acid. Although in the recipe of the Folk sausage, MOM chicken is involved and digested, yet the percentage of pork trimming content and fatty tissue provide most of the content of steric acid.

The ratio of PUFA /SFA to these series of sausages is 0.4%, which indicates that this group satisfies the quality requirements of the product according to the lipid content. The greater difference in the ratio of PUFA / SFA between

this type of sausages and the previous (Kranj) is due to the higher percentage of fatty tissue (40%: 20%).

From 7-13 g olive oil can be added to 100 g sausages as a substitute for animal fats. However, the purpose of replacing animal fat with olive oil is to produce products with low fat content (Jiménez-Colmenero et al., 2007). One of the basic strategies for developing a healthy lipid formula is to concentrate active ingredients in food products to allow consumption at the recommended levels of intake with normal sizes of portions. Recommendations for the diet by the World Health Organization (2003) were that, MUFA should be the main dietary fatty acids. If MUFAs are the predominant fatty acids in the products, the total fat intake will not be significant (Pérez-Jiménez et al., 2007).

Ansorena and Astiasaran, (2004), in their research studied Dry fermented sausages produced by a partial substitution of pork back fat with pre-emulsified olive oil. The authors established that After 5 months of storage at 4 °C, the combination of the increase in oleic acid and the preservation of PUFA by the antioxidant activity of the olive oil emulsion and antioxidants (when added), lead to better MUFA+PUFA/SFA ratios in olive oil containing sausages (1.90-1.98 g/100 g fatty acids) and particularly in antioxidants containing sausages (2.02-2.16 g/100 g) than in control ones (1.72 g/100 g). Vacuum packaging of the piece was the best method to minimize formation of lipid oxidation volatile compounds.

Six treatments of Chorizo de Pamplona, traditional Spanish fermented sausage, were manufactured under usual commercial conditions by replacing 0, 10, 15, 20, 25 and 30% of pork back fat by pre-emulsified olive oil with soy protein isolate. Sausages with 20-30% replacing level had higher (p<0.05) protein content than control as a result of the addition of soy protein isolate. The oleic acid increased (p<0.05) in sausages with 15-30% replacing level, and linoleic acid increased in sausages with 10-25%. Sausages with 10-25% of substitution had lower total SFA-stearic and higher (p<0.05) total MUFA, total PUFA, (MUFA+PUFA)/ (SFA-stearic), and PUFA/(SFA-stearic), reported (Muguerza et al., 2001).

Backes et al., (2017) in Italian type of sausages with addition of vegetable oils, found lower levels of saturated fatty acids (SAFAs), higher levels of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The results showed that the replacement of pork with emulsified vegetable oil improved the nutritional values of salami of the Italian type.

Kaayardi et al. (2003) examined the effect of olive oil on the chemical and sensory characteristics of the Cabanosi sausage made from soy sauce, cottonseed oil, in which three samples were made 0.1%, 1.0% and 2 %. They found that as the percentage of olive oil increased, the total saturated fatty acids increased as the total polyunsaturated fatty acids acids decreased.

The content of fatty acids, the structural and percentage distribution of sausages in both types (Kranj and Folk), using olive oil, showed that olive oil has an influence on the PUFA / SFA ratio, and so on the quality of the produced sausages in the ratio of the lipid content. The produced sausages have a quality fatty acid profile, primarily because of the lower percentage of saturated fatty acids compared to non-saturated fatty acids.

Comparatively, our results with the literary results showed similarity and appropriate correlation with regard to the structure of the fatty acid composition and the manner of storage.

Table 5. Fatty acto composition of Manj sausages (%)							
l batch ll batch		III batch	IV batch				
1.43	1.41	1.40	1.39				
25.72	25.26	25.97	25.19				
3.03	3.10	2.91	3.00				
0.49	0.52	0.47	0.51				
0.52	0.55	0.47	0.53				
12.57	12.47	13.30	13.17				
44.57	44.27	43.02	43.70				
10.54	10.87	11.02	11.17				
0.40	0.68	0.69	0.56				
0.73	0.85	0.75	0.78				
	l batch 1.43 25.72 3.03 0.49 0.52 12.57 44.57 10.54 0.40	I batch         II batch           1.43         1.41           25.72         25.26           3.03         3.10           0.49         0.52           0.52         0.55           12.57         12.47           44.57         44.27           10.54         10.87           0.40         0.68	I batch         II batch         III batch           1.43         1.41         1.40           25.72         25.26         25.97           3.03         3.10         2.91           0.49         0.52         0.47           0.52         0.55         0.47           12.57         12.47         13.30           44.57         44.27         43.02           10.54         10.87         11.02           0.40         0.68         0.69				

Table 3. Fatty acid composition of Kranj sausages (%)

Fatty acids (%)	l batch	ll batch	III batch	IV batch
Saturated	40.21	36.66	41.14	40.26
Polyunsaturated	11.67	12.40	12.46	12.51
Monounsaturated	48.12	46.40	47.92	47.27
Unsaturated	59.79	60.32	58.86	59.74

**Table 4.** Saturated, polyunsaturated, monounsaturated and unsaturated fatty acids in the production series of Kranj Sausage (%)

From the table of contents for the fatty acid composition of the Kranj sausage, it can be determined that the content of palmitic acid (C16: 0) ranges from 25.19% to 25.26% in the batches with added olive oil in relation to 25.72% in the control, respectively 12.47% to 13.30% stearic (C18: 0) for the batches with added olive oil in relation to 12.57% in the control. The content of these saturated fatty acids is within the limits of other meat products. Oleic acid accounts for 92% of MUFA in food, and 60-80% of oleic acid comes from olive oil (Pérez-Jiménez et al., 2007), as confirmed by our research.

Olive oil is a vegetable oil with the highest level of monounsaturated fatty acids (MUFA) and has attracted attention as a replacer for animal fat in processed meat products. Olive oil has a high biological value due to a favourable mix of predominantly MUFA and naturally occurring antioxidants including vitamin E, vitamin K, carotenoids and polyphenols such as hydroxytyrosol, tyrosol and oleuropein, (Moon et al., 2009). Since 3, 4 and 5 gr / kg of olive oil were added in the produced sausages, the content of monounsaturated fatty acids increased adequately with the addition: 46.40% for added 3 gr/kg olive oil, 47.92% for added 4 gr / kg olive oil and 47.27% for added 5 gr / kg olive oil.

Therefore, the replacement of animal fat with olive oil or its addition to a greater quantity of 4 or 5 gr / kg (as in our research) can produce the products with a healthier composition of lipids (higher MUFAs, predominantly oleic acid) without greater deterioration of the diet quality.

According to Ansorena and Astiasaran (2004), the PUFA / SFA ratio is one of the parameters currently used to assess the quality of foods in terms of lipid content. According to the same authors, this ratio should not exceed 0.4%, in order to reduce the negative effects of saturated fatty acids. In our research in all three groups, the ratio of polysaturated to saturated fatty acids is 0.3%, and in the control is 0.2%, which means that the addition of olive oil in this type of batches is appropriate.

## CONCLUCIONS

Adding of olive oil more than 4 or 5g/ kg (according to our research), allows greater production of products containing healthy amount of lipids (higher MUFA, mainly oleic acid ) without any significant deterioration of food guality.

The content of fatty acids, the structural and percentage distribution of sausages in both types (Kranj and Folk), using olive oil, showed that olive oil has an influence on the PUFA / SFA ratio, and so on the quality of the produced sausages in the ratio of the lipid content. The produced sausages have a quality fatty acid profile, primarily because of the lower percentage of saturated fatty acids compared to non-saturated fatty acids. Comparatively, our results with the literary results showed similarity and appropriate correlation with regard to the structure of the fatty acid composition and the manner of storage.

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### ВЛИЈАНИЕ НА МАСЛИНОВОТО МАСЛО ВРЗ МАСНО КИСЕЛИНСКИОТ СОСТАВ НА ГРУБО ИСИТНЕТИ БАРЕНИ КОЛБАСИ

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#### Резиме

Во трудот е испитувано влијанието на обично и ладно цедено маслиново масло врз маснокиселинскиот состав на грубо иситнети барени колбаси. За таа цел во производството на Народен колбас е додадено ладно цедено маслиново маслово, а во производството на Крањски колбас е додадено обично маслиново масло. И во двете производни серии додадено е маслиново масло во количина од 3,4 и 5 g/кg. Кај испитуваните серии на Народен колбас содржината на палмитинската и стеаринската масна киселина (С16:0 и С18:0) е во границите како и кај останатите месни преработки. Помала процентуална застапеност е забележан во содржината на С16:0, а поголема во содржината на (С18:0), кај крањските серии колбаси. Односот на PUFA / SFA, кај двете производни серии колбаси изнесува до 0.4%, што значи дека колбасите ги задоволуваат условите за квалитет на производот според содржината на липиди, односно дека потполно е оправдано додавањето на маслиновото масло кај овој тип на барени колбаси.

Клучни зборови: народен колбас, крањски колбас, мононезаситени масни киселини, полинезаситени масни киселини, заситени масни киселини, квалитет

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 10.12.2018 Accepted: 18.12.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 633.11-153.1.011.3(497.7)"2018" Original scientific paper

# INFLUENCE OF THE SEED SIZE AND VARIETY ON THE SEEDLING VIGOUR AND GERMINABILITY IN THREE VARIETIES OF SOFT WHEAT SEED

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#### Abstract

The objective of this research is to determine the effect of the seed size and variety on the germinability of the wheat (*Triticum aestivum* L.). Three fractions of seed size (>3 mm, 2-3 mm and <2 mm) and three varieties of soft wheat (*radika, amazon 150* and *pobeda*) were included as experimental factors. The seed was produced for commercial purposes from the category first generation certified seed (C1) in 2018. The laboratory examinations are conducted in the "Unilab" laboratory at the Faculty of Agriculture in Shtip. The laboratory is accredited pursuant to the MKC EN ISO/IEC 17025:2006 standard for several methods among which the method for examining the quality of the seed of agricultural crops. The amazon 150 variety had the lowest seedling vigour (87,75%), but the highest total germinability (94,33%). Contrary to amazon 150, the *pobeda* variety had the loggest seedling vigour (92,08%), but the lowest level of total germinability (93,25%). The differences of the seed ling vigour and the germinability between the varieties did not show any statistical justifiability. The middle fraction (2-3 mm) had significantly higher seedling vigour than the other two fractions. The first and the middle fraction (>3 mm and 2-3 mm) had a significantly higher total germinability compared to the third fraction (<2 mm). The seed sized proved to have the biggest and most important effect on the total germinability (49,32%). The variety did not prove to be a significant factor that influences the total germinability.

Key words: total germination, seedling vigour, variety, fraction, seed

### INTRODUCTION

The success of the production is under the influence of the genotypes, localities and cultivating technology, as well as of the environmental conditions (Đekić et al., 2014). Today's varieties of wheat are genetically highly selected and advanced biotechnical materials present in the mercantile production in terms of all the bases and all the requests (Sabovljević et al., 2010). It is generally known that the seed is the main factor for the transfer of the genetic characteristics of the variety and the improvement of the quality and the yield. A high quality and measured seed is a basic prerequisite for a successful production. The seed quality depends on the variety, i.e. it is genetically determined and, to a great extent, it is also defined by the cultivation conditions

and the physiological processes. The research on different types of plants show that even though the seed quality is mainly hereditary, the agricultural and environmental conditions, the agriculture technology that has been applied, the quality of the seed material approbation, the further processing of the seed, storage, keeping, etc. may have great influence on the seed quality.

The genetic variability is the cause for the variability of the seed size among the varieties. However, the differences of the seed between one variety and another regarding the size and the weight are a result of the conditions of the external environment and the agriculture technology that has been applied. The variability of the seed size is under a huge influence of the conditions of the external environment because in unfavourable cultivation conditions the participation of the lower fractions of the seed is significantly increased (Jevtić et al., 1985).

Seed size is an important physical indicator of seed quality that affects vegetative growth and is frequently related to yield, market grade factors and harvest efficiency (Rukavina et al., 2002). Most investigators have reported a positive relationship between seedling vigour, improved stand establishment and higher productivity of cereal crops with plants originating from large seed compared to those grown from smaller seed (Mohsen et al., 2011). Smaller seeds generally germinate faster providing greater competitive advantage especially in early successional stages (Baskin & Baskin, 1998). Nevertheless, larger seeds, although germinating slowly, often have higher percentage of germination than small seeds (Harper, 1977).

The seed with high seedling vigour and high germinability determines the potential for fast and equal germination and growth. These are the basic prerequisites that provide crops with good structure and strong growth and enable having a high yield.

The germinability of the seed is one of the most significant indicators for the seed quality, i.e. the living ability on which its used value depends on (Poštić et al., 2010). The germinability is ability of the seed, in favourable conditions, to provide a normal growth and development of sprouts from which the future plants will be developed.

However, apart from the high overall germinability, it is very important for the seed to have a high seedling vigour that, also, is a significant indicator for the living ability of the seed. In the production of every crop the fast and equal germination has a huge importance that depends directly on the seedling vigour of the seed (Poštić et al., 2010). The faster germination of the seed in minor conditions will provide better and more equal germination of the seeds, stronger development, better resistance to the conditions of the external environment, illnesses and pests since more developed plants have better resistance.

The seedling vigour represents the number of normal sprouts compared to the number of the seeds planted to germinate determined by the time foreseen for the first assessment, i.e. for the determination of the seedling vigour. The germinability of the seed represents the number of normal sprouts compared to the total number of seeds planted to germinate determined by the time foreseen for the final assessment has passed (Official Gazette of the Republic of Macedonia, No.61.2007).

Numerous researches show that the size or the weight of the seed and the contents of the nutritious elements in it have a great influence on the seedling vigour, the germinability and the growth potential in the germination stage. The results of the researches vary to a great extent from one type to another, even among varieties from the same type. According to this, the knowledge about which fraction of seed within a party of the seed has a better and which one has worse seedling energy and total germinability is of important practical value. By further procession of the seed the seedling energy and the total germinability of the seed can be improved.

The basic objective of this research is to determine the influence of the seed size on the seedling vigour and on the total germinability in laboratory conditions in three varieties of soft wheat. The varieties subject to research play an important role in the wheat production in our country. Hence, the knowledge about the influence of the variety on the seedling vigour and on the total germinability would have practical bearing.

# MATERIAL AND METHODS

The research encompasses three varieties of wheat: radika, amazon 150 and pobeda. All three varieties are widely present in the wheat production in the Republic of Macedonia. The examinations have been conducted on average samples of parties of further processed seed from the category of first regeneration certified seed (C1) for each of the above stated varieties. The laboratory analyses have been conducted in the "Unilab" laboratory within the Plant and Environment Protection Department of the Faculty of Agriculture in Shtip. The laboratory is accredited in accordance with the MKC EN ISO/ IEC 17025:2006 standards for several methods among which the methods for examining the seed quality of agricultural crops.

The division of the seed into different fractions according to the size is performed with laboratory sieves with rectangular openings the size of which is 3mm and 2 mm. Three fractions have been set apart from the seed of each variety, seed larger than mm (>3 mm), seed with a size between 2 and 3 mm (2-3 mm) and a seed smaller than 2 mm (<2 mm).

A hundred seeds have been set apart four times from each variety and fraction to determine the seedling energy and the total germinability of the seed, i.e. a total number of nine variations with four repetitions have been created. In order to eliminate the influence of any potential seed infections it has been treated with 1% solution of Na hypochlorite. Standard methods prescribed by the ISTA Rulebook (2010) and by the Rulebook on the mode of work, the equipment of the room and the technology of the authorized laboratories and methods for examining the seed material quality of agricultural crops (Official Gazette of the Republic of Macedonia No.61.2007) have been applied in order for the seedling vigour and the total germinability to be determined.

On the basis of the examined samples, the average values for each variety and fraction have been calculated. The mean values are statistically processed by using the basic parameters of the descriptive statistics. 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**

### **Seedling vigour**

The seedling vigour, after the sowing determines the living ability of the seed, i.e. the uniform growth of healthy and strong sprouts. The seed size of the wheat is positively related to the living ability of the seed, which means **Table 1.** Seedling vigour (%)

that a larger seed has a tendency to produce more vital sprouts (Ries & Everson, 1973).

In Table 1 the average values of the seedling vigour of the examined varieties and fractions of wheat seed are presented.

Devenenter	Seed fraction			Average veriety		
Parameter	>3	2-3	<2	Average variety		
х	88,75	91	89	89.58		
min	85	88	87			
max	92	93	91			
I.V.	7	5	4			
CV (%)	0,04	0,03	0,02			
х	83	94,75	85,5	87.75		
min	75	93	83			
max	94	97	89			
I.V.	19	4	6			
CV (%)	0,12	0,02	0,04			
х	92,25	92,75	91,25	92.08		
min	87	89	89			
max	95	95	95			
I.V.	8	6	6			
CV (%)	0,04	0,03	0,03			
Average fraction		92.83	88.58			
LSD <sub>0.05</sub>		Variety=4.03				
	min max I.V. CV (%) x min max I.V. CV (%) x min max I.V. CV (%) x cV (%) raction	Parameter         >3           x         88,75           min         85           max         92           I.V.         7           CV (%)         0,04           x         83           min         75           max         94           I.V.         19           CV (%)         0,12           x         92,25           min         87           max         95           I.V.         8           CV (%)         0,04           raction         88	Parameter         >3         2-3           x         88,75         91           min         85         88           max         92         93           I.V.         7         5           CV (%)         0,04         0,03           x         83         94,75           min         75         93           max         94         97           I.V.         19         4           CV (%)         0,12         0,02           x         92,25         92,75           min         87         89           max         95         95           I.V.         8         6           CV (%)         0,04         0,03           raction         88         92.83	Parameter         >3         2-3         <2           x         88,75         91         89           min         85         88         87           max         92         93         91           I.V.         7         5         4           CV (%)         0,04         0,03         0,02           x         83         94,75         85,5           min         75         93         83           max         94         97         89           I.V.         19         4         6           CV (%)         0,12         0,02         0,04           x         92,25         92,75         91,25           min         87         89         89           max         95         95         95           I.V.         8         6         6           CV (%)         0,04         0,03         0,03           raction         88         92.83         88.58		

x - arithmetic mean; min - minimum; max-maximum; I.V. - interval of variation; CV - coefficient of variation.

The lowest seedling vigour (83%) is shown to have the variety amazon 150 for the fraction >3 mm, while the highest seedling vigour (94,75%) of the same variety is found in the fraction 2-3 mm. Regardless of the fraction, the variety pobeda has shown to have the highest seedling vigour (92,08%), while the variety amazon 150 has the lowest seedling vigour (87,75%). The difference of the seedling vigour between these two varieties is statistically important.

Regardless of the variety, the highest seedling vigour (92,83%) is found in the middle fraction (2-3 mm) the value of which is significantly different compared to the one of the two other fractions. The other two fractions have almost the same seedling energy (88% of the fraction >3 and 88,85% of the fraction 2-3 mm).

The variation coefficient is more or less equal in all the varieties and fractions and varies from 0,02 to 0,04%, with the exception of the variety amazon 150 for the fraction >3 mm, where it is 0,12%. The same variety has the highest variation interval (19). All the other varieties have relatively equal seedling vigour.

The results that have been obtained show that the smaller seed has a greater seedling vigour, i.e. it germinates faster than the larger seed.

### **Total germination**

In order to provide larger wheat production and to achieve stable yield, first and foremost it is necessary for high quality seed to be used in the sowing process, which is the primary task of the distribution and sale of seeds. The declared seed has high variety purity and a high percentage of germination (Savić et al., 2000).

The average values of the total germination of the examined varieties and fractions of wheat seed are given in Table 2.

Maniatur	Deverseter		1. Seed frac	tion	Average	
Variety	Parameter	>3	2-3	<2	variety	
	х	96,5	92,5	92,5	93.83	
	min	94	90	91		
Radika	max	98	95	95		
	I.V.	4	5	4		
	CV (%)	2	2,25	2,07		
	х	95,5	97,75	89,75	94.33	
A 100 0 0 0 0	min	94	96	87		
Amazon 150	max	98	100	92		
150	I.V.	4	4	5		
	CV (%)	2,01	1,75	2,3		
	х	94,5	93	92,5	93.25	
	min	91	90	90		
Pobeda	max	97	95	97		
	I.V.	6	5	7		
	CV (%)	2,65	2,32	3,36		
Average	e fraction	95.42	94.42	91.58		
LSD <sub>0.05</sub>		Variety=5.55 Fraction=2.16				

**Table 2.** Total germination (%)

x – arithmetic mean; min - minimum; max - maximum; I.V. - interval of variation; CV - coefficient of variation.

The lowest total germination (89,75%) is determined with the variety amazon 150 for the fraction <2 mm, while the highest (97,75%) of the same variety is found to be with the fraction 2-3 mm. Regardless of the fraction, the highest total germination (94,33%) is obtained with the variety amazon 150, while the lowest (93,25%) has been obtained with the pobeda variety. The differences in the total germination between all three varieties that have been examined do not have the statistical justifiability.

Regardless of the variety, the fraction >3 mm (2-3 mm) has the highest total germination (95,42), while the fraction <2 mm has the lowest one (91,58%). The values that have been obtained are statistically different. The middle fraction (2-3 mm) has a total germination of 94,42% and it is not significantly different from the fraction >3 mm, while compared to the fraction <2 mm it is significantly higher.

In this case as well, the coefficients and the

variation intervals of the varieties and fractions of wheat seed subject to examination were minor and relatively equal (Tab. 2).

The results show that the larger seed has a higher total germination. These results are in accordance with the research conducted by Stevanović et al., 2018, Farahani et al. 2011, and many more authors.

With the objective to see the influence of the variety and of the fraction on the seed and their interaction on the germinability of the seed of the varieties of wheat subject to examination, a two-way analysis of the variance has been conducted (Tab. 3). From the Table 3 it can be seen that the seed size has the biggest influence, i.e. the fraction of the seed (49,92%), while the share of the interaction between the variety and fraction of the seed is 47,01%. In this research it was shown that the variety, as a factor, is the least influential when the germinability of the seed is concerned (3.67%).

Factor	SS	df	MS	F	η			
Total	192.389	8	24.049	5.103				
A	7.056	2	3.528	0.749	3.67			
В	94.889	2	47.444	10.067	49.32*			
AxB	90.444	4	22.611	4.798	47.01			
Error	12.250	27	4.713					

Table 3. Effect of variety and seed size on total germination

A - factor variety; B - factor seed fraction; A X B - interaction between variety and seed fraction; SS - sum of square; df - degrees of freedom; MS - mean square; F - F test;  $\eta$  - effect of factor; \* - level of significant p< 0.05.

Farahani et al. (2011), found that the effect of seed size significantly affected the germination of wheat. In the researches of Zareian et al.

CONCLUDING REMARKS

On the basis of the results that have been obtained from the examinations, it can be concluded that the seed size has a direct influence on the seedling vigour and on the total germination of the seed. The variety, as a factor, has not shown to have a significant influence on the seedling vigour and on the total germinability. The smaller seed had higher values of seedling vigour, while the larger seed had larger values of total germination. According to this, the smaller seed germinates faster compared to the larger seed, while the larger seed produces higher number of sprouts. All the varieties and fractions of the seed that were subject to examination showed high values of seedling vigour and total germination, which means that the seed with a size within the examined fractions can give good results in the production. With the aim for the seed quality to be further improved, the equipment for further processing of the seed should be adjusted so that the smaller seeds can be divided.

(2013), the seed size did not have a significant

influence on the germinability percentage.

55 .....

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## ВЛИЈАНИЕТО НА ГОЛЕМИНАТА НА СЕМЕТО И СОРТАТА ВРЗ ЕНЕРГИЈАТА НА 'РТЕЊЕ И **РТНОСТА КАЈ ТРИ СОРТИ МЕКА ПЧЕНИЦА**

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### Резиме

Целта на ова истражување беше да се утврди ефектот на големината на семето и сортата врз ртноста кај пченицата (Triticum aestivum L.). Како експериментални фактори беа вклучени три фракции според големината на семето (>3 mm, 2-3 mm и <2 mm) и три сорти мека пченица (радика, амазон 150 и победа). Семето беше произведено за комерцијални цели, од категоријата сертифицирано семе од прва генерација (С1), во 2018 година. Лабораториските испитувања се спроведени во лабораторијата "Унилаб", на Земјоделскиот факултет во Штип. Лабораторијата е акредитирана согласно стандардот МКС EN ISO/ IEC 17025:2006 за повеќе методи, меѓу кои и методите за испитување на квалитет на семе од земјоделски растенија. Сортата амазон 150 имаше најмала енергија на 'ртење (87,75%), но најголема вкупна 'ртност (94,33%). Спротивно од амазон 150, сортата победа имаше најголема енергија на 'ртење (92,08%), но најмала вкупна 'ртност (93,25%). Разликите за енергијата на 'ртење и 'ртноста помеѓу сортите не покажаа статистичка оправданост. Средната фракција (2-3 mm) имаше значајно поголема енергија на 'ртење од останатите две фракции. Првата и средната фракција (>3 mm и 2-3 mm) имаа значајно поголема вкупна 'ртност во однос на третата (<2 mm). Најголем значаен ефект врз вкупната 'ртност имаше големината на семето (49,32%). Сортата како фактор не покажа значајно влијание врз вкупната 'ртност.

Клучни зборови: вкупна 'ртност, енергија на 'ртење, сорта, фракција, семе

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 29.08.2018 Accepted: 20.09.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 636.2.09:[618.19-002:618.19-083 Original scientific paper

# MONITORING UDDER HEALTH AND MILK HYGIENE ON-FARM USING QUICK SCREENING METHODS

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### Abstract

In this paper the use of on-farm screening methods for monitoring udder health and milk quality are discussed. Special attention was given to the evaluation of the usefulness of California mastitis test (CMT) as quick field screening test for detection of udder quarters with an intra-mammary infections caused by major mastitis pathogens. Application of CMT in dairy herd health management in period of early lactation is illustrated through the two years cross sectional study that was carried out to screening the quarter milk samples with abnormal milk secretion (AMS) and using of microbiological culture for detection of inframammary infections (IMI). The quarter milk samples were obtained in two periods of early lactation: the period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. The quarter level prevalence of AMS and IMI in the first 21 days in lactation was 5.33% and 4.03%, and up to the 42 days in lactation the prevalence of AMS and IMI was 5.45% and 4.38%, respectively. The prevalence of AMS and IMI from udder quarters that show a positive reaction on CMT in the first 21 days in lactation was 56.96 and 55.42; and 55.42 and 44.58 in the period from 22nd to 42nd day in lactation was 56.96 and 55.42; and 55.42 and 44.58 in the period from 22nd to 42nd day in lactation was 56.96 and 55.42; and 55.42 and 44.58 in the period from 22nd to 42nd day in lactation was 56.96 and 55.42; and 55.42 and 44.58 in the period from 22nd to 42nd day in lactation, respectively. The results indicated that positive CMT reaction in early lactation may be a good indicator for IMI; there was a significant association between the frequency of isolation of major pathogens and the CMT score in milk samples obtained in the period of early lactation (Pearson's  $\chi$ 2=240.031, df=9, P<0.001).

Key words: California mastitis test, dairy cows, intra-mammary infection

### INTRODUCTION

Milk composition and microbiological characteristics are important factors for the dairy farmer (raw milk quality), dairy industry (technological process and quality of dairy products), and consumer (nutritional quality and safety). In most dairy systems, it is assumed that farmers, informed by the official organization in their country or region, have the responsibility to deliver milk that is of sufficient quality (Hogeveen et al., 2010). Although regulations dealing with milk quality standards differ between dairyproducing countries, the general agreement is that abnormal milk, as the presence of flakes, clots, or other gross alterations in milk appearance, or highly elevated Somatic Cell Count (SCC) or injured udders should be excluded from milk supplied for human consumption.

Control of inflammation of the mammary gland and the reduction in their appearance in the herd is based on the number of somatic cells in milk and the incidence of clinical mastitis (IDF, 1997). The measurement used most commonly to detect subclinical mastitis is the SCC of milk. Thus, in order to minimize the appearance of mammary inflammation, the main goal should be the number of somatic cells below 200.000 / ml of milk, and the incidence of clinical mastitis below 20% (Pyorala, 2003; Schukken et al., 2003). According to the National legislation in the Republic of Macedonia (FVA, 26/2012) milk from uninfected mammary glands contains ≤100.000 somatic cells per millilitre. A milk SCC from 200.000 - 400.000/ml is a clear indication that milk has reduced manufacturing properties, which means is not for consummation. This, an increase in the SCC of milk is a reasonably good indicator of inflammation in the udder.

Identifying cows with clinical mastitis (CM) involves visual inspection of the udder and manual check of the foremilk from each quarter at each milking, and regular implementation of screening methods for early detection of cows with elevated SCC, like California Mastitis Test (CMT), measurements of milk conductivity, and the presence of chlorides and sodium in milk as results of udder inflammation (Sharma et al., 2011). However, increasing herd sizes, reliance on less-skilled labour, and an increasing emphasis on lowering bulk milk SCC levels (Lacy-Hulbert et al., 2010) are all factors contributing to an increased demand for more consistent and less labourintensive methods to help farmers manage mastitis and bulk milk SCC levels. Comparing to culturing methods and determination of SCC, the field screening methods for diagnosis of mastitis are easy and routine methods that give prior information for antibiotic treatment of infected udder quarters and early drying off. Among the others, CMT is widely used for on-farm detection of mastitis in dairy herds (Sharma et al., 2011). Calderon and Rodrigues (2008) reported some insufficiency of the CMT regarding their sensitivity and specificity in the determination of IMI. The main weakness of the CMT is its low specificity for determination of udder quarters infected with major or minor mastitis pathogens. The CMT is a rapid and inexpensive test to determine indirectly the somatic cell concentration in milk and is a practical, easy method for demonstrating IMI by testing milk samples on-farm. In general, there is no ideal screening test for prompt and quick diagnosis of IMI. Culturing examination is the "gold standard" for detection of infected udder quarters, but very often these methods are very expensive, time consuming for routine screening followed by lack for on-farm assessment

(Sargeant et al., 2001). An ideal screening method would have maximum sensitivity to minimize the proportion of false-negative results, and a reasonable degree of specificity to reduce the number of false positive results (Dingwell et al., 2003; Midleton et al., 2004). Accurate mastitis detection and effective mastitis control strategies have an influent economic impact on dairy farms followed by sustainable milk production (Wallace et al., 2002).

The intensive systems for breeding of dairy cows in the Republic of Macedonia practice a free posture system, in accordance with the National legislation for Animal Welfare (FVA, 149/2014). Such breeding technology requires the existence of central milking parlour on farms. This segment of complex objects of farms for milking cows that is the one of the key control points for reduction of incidence of mammary gland inflammation. Trajchev and Nakov (2009) suggested mastitis programme that outlines control these procedures: systematic examination of herd for detection clinical and subclinical forms of mastitis; laboratory tests for identification of mastitis causative microorganisms and selection of most appropriate antimicrobial substance for cow therapy; application of suitable therapy and procedures for mastitis cow treatment; and preventive measures on farms, which is consist of standard methods and procedures, with main goals to prevent or reduce the influence of new intramammary infections in the herds.

The purpose of the study was to determine whether or not individual cow quarter milk CMT score, collected in the period of early lactation, from calving to 42nd day in, could indicate whether or not these quarters were infected by pathogens associated with subclinical mastitis. Testing the cows in one dairy herd, quarter milk samples were subjected to CMT under field (cowside) conditions and the same samples were examined microbiologically.

## MATERIALS AND METHODS

A two year cross sectional survey was carried out in a commercial dairy farm localized in the southeaster part of Macedonia. Cows were kept in a loose housing system and were feeding with an ordinary diet which differed according to the stage of pregnancy and lactation period. The feeds included corn and grass silage, hay, commercial concentrate (maize, wheat, barley) as well as vitamin and mineral premixes. Data were collected from 211 cows that were all starting their 1<sup>st</sup> or a subsequent lactation, and that had at least 3 functional quarters and a total of 844 quarter CMT and culture results were available for analysis. Milking of cows was performed twice daily in milking parlour with exception of cows in the first 45 days in lactation which were

milked three times daily. Pre-milking and postmilking hygiene measurements were practiced permanently. The udder health status was followed by calving to 42<sup>nd</sup> day in lactation. This period of early lactation was subdivided into 2 periods: the period from calving until 21st day in lactation and period from 22<sup>nd</sup> to 42<sup>nd</sup> day in lactation. The screening of udder health status was done on a quarter level using California Mastitis Test (CMT) as predicted tool for detection of quarters with abnormal milk secretion (Schalm и Noorlander, 1957). The test was performed at cow-side by mixing an equal volume of milk with a 1:1000 dilution of 3% sodium lauryl sulfate and bromocresol purple. Each quarter milk sample from the cow was placed in one clean well of a white plastic test paddle divided into four separate wells, one for each guarter sample. As the plate was rotated gently, any colour changes or formation of a viscous gel were interpreted by the authors above: scores were given within the range 0-4, with 0 for no reaction, 1 for a trace, 2 a weak positive, 3 a distinctly positive and 4 a strong positive.

Samples for bacteriological culture from each quarter positive to CMT were collected aseptically

in sterile 10 mL tubes, without additives according to the National Mastitis Council (NMC, 2001) and kept at 4°C during transportation. Samples were analysed within 12 hours of collection. Bacterial species were identified according to the standard microbiological procedure using a certificated methodology based on the National Mastitis Council standards (NMC, 2001). A minimum of five colonies of the same type of bacterium was recorded as bacteriological positive, and growth of more than two types of bacterial colonies was categorized as mixed growth. No bacterial growth was recorded when fewer than five colony-forming units were detected during 48 h of incubation.

Based on results from screening using CMT and bacteriological culturing, all cows in the observed population were allocated into three groups: healthy cows without udder health problems, cows with persistent abnormal milk secretion (AMS) and cows with persistent intramammary infection (IMI). The difference in prevalence of udder quarters with AMS and IMI and their significance was estimated by the Chisquare test.

## **RESULTS AND DISCUSSION**

The results from a quick screening test and the prevalence of udder quarter health disorders

in an observed population of dairy cows during the early lactation are shown in Table 1.

Table 1. The quarter leve	l prevalence of abnc ا	ormal milk secretion	n (AMS) and intra-mamı	mary infections (IMI) in
dairy herd during the pe	riod of early lactatior	n		

Time of sampling	Total quarters tested	*CMT(-)	quarters		MT(+) arters	A	MS		MI
	n	n	%	n	%	n	%	n	%
***Day_21	844	765	90.64	79	9.36	45	5.33	34	4.03
****Day_42	844	761	90.17	83	9.83	46	5.45	37	4.38

\*negative reaction on California mastitis test (CMT)

\*\* positive reaction on California mastitis test (CMT)

\*\*\* period in lactation from calving until 21<sup>st</sup> day in lactation

\*\*\*\* period in lactation from 22<sup>nd</sup> to 42<sup>nd</sup> day in lactation

Based on the results from the field screening test (CMT), the total prevalence of udder health disorders on the quarter level was 9.36% and 9.83%, respectively for the period from calving until 21<sup>st</sup> day in lactation and period from 22<sup>nd</sup> to 42<sup>nd</sup> day in lactation. Overall, the prevalence of

udder quarters with AMS in these data was 5.33% and 5.45%, while the prevalence of quarters with IMI was 4.03% and 4.38%, respectively for the period from calving until 21<sup>st</sup> day in lactation and period from 22<sup>nd</sup> to 42<sup>nd</sup> day in lactation.

In Table 2 are shown the ratios between udder quarters with AMS and IMI versus udder

quarters that have positive CMT reaction.

<b>Table 2.</b> The ratio of udder quarter with AMS and IMI relating to the udder quarters with positive reaction on
California mastitis test (CMT+)

Time of compling	CMT(+) quarters	AMS	AMS	IMI	IMI
Time of sampling	n	n	%	n	%
Day_21	79	45	56.96	34	43.04
Day_42	83	46	55.42	37	44.58

The prevalence of quarters with IMI from the screened udder quarters that were showed a positive reaction on CMT was high, 43.04% in the period from calving until the 21<sup>st</sup> day in lactation and 44.58% in the period from 22<sup>nd</sup> to 42<sup>nd</sup> day in lactation. There was a significant association between the frequency of isolation of major pathogens and the CMT score in milk samples obtained in the period of early lactation ( $\chi$ 2=240.031, df=9, P<0.001).

A major health problem in the dairy industry in the Republic of Macedonia is mastitis, either clinical or subclinical. Therefore, one of the most important objectives of the mastitis control programme in dairy herds is the control of raw milk quality and safety. Milk with clinical mastitis is easy to recognize, but when the milk of cows with subclinical mastitis without visible changes, is accidentally mixed into bulk milk, it enters the food chain and can be hazardous to human's health.

In the recent years, there is an increasing focus on milk quality and hygiene in the dairy industry. The main goal of legislation for milk quality is to stimulate farmers to produce hygienically proper milk. The basic criteria for assessing the safety of raw cow's milk are lying down in the "legislation on the special requirements for safety and hygiene and the manner and procedure for performing official controls of milk and dairy products" (FVA, 2012). According to national legislation in the Republic of Macedonia for the quality of raw milk (FVA, 2012), the milk is classified in terms of the total number of bacteria and the total number of somatic cells. According to the total number of microorganisms, raw milk is classified into three classes: extra class (≤100.000/ml), first class (100.001/ml - 700.000/ml) and second class (700.001/ml - 1.500.000/ml). According to the total number of somatic cells, raw milk also is classified into three classes: extra class

 $(\leq 400.000/ml)$ , first class (400.001/ml - 500.000/ml) and second class (500.001/ml - 600.000/ml). Increased SCC is associated with reductions in casein, milk fat, and lactose; increased enzymatic activity; and reduced quality and yield of dairy products (Schukken et al, 2003).

Mammary gland infection is the most important factor affecting SCC in milk in the subclinical mastitis by increasing the number of somatic cells in milk (Bachaya et al., 2011). As IMI is usually followed by an influx of leucocytes and other macrophages into the milk, an increase in its SCC has been used widely as indicating mastitis. Implementing good management practices at the begging of lactation is essential for good milk quality in the subsequent period of lactation and also, for evaluating udder health before the administration of antibiotic therapies. Preventing clinical mastitis in early lactation, decreasing the amount of discarded milk, and reducing the bulk milk SCC are some of the potential benefits. However, as there is growing consumer resistance to using antibiotics in all food animal production systems, it may be desirable to reduce the number of cows that need treatment by identifying and treating only those with IMI.

An ideal diagnostic tool to select quarters or heifers for pre-calving treatment should be quick, easy to perform on-farm, reliable and inexpensive. Milk bacteriology does not correspond to those criteria. The CMT is a rapid and inexpensive test to indirectly determine the somatic cell concentration in milk (Midleton et al., 2004) and is a practical, easy method for demonstrating IMI by testing milk samples onfarm (Dingwell et al., 2003). However, differences in CMT or SCC scores could also be associated with other factors such as the age of cows and environmental factors. Previous research had suggested that SCC is related with parity, a period of lactation, milking frequency and milk yield (Hand et al., 2012). Some other researchers did not find any relationship between the physiological status of cow and SCC in milk from healthy cows (Charffeddine et al., 1997).

Whilst an increase in CMT score corresponds with an increase in SCC, it is uncertain whether or not CMT or SCC scores can reflect accurately IMI due to specific pathogens. If either SCC or CMT could be used reliably to identify subclinical mastitis in lactating cows, they might be useful in identifying such affected quarters that require antibiotic treatment and early drying off (Barkema et al., 1998).

The sensitivity of CMT on the guarter level in the period of early lactation according to the results obtained in our research was 43.04% and 44.58%, respectively for the period from calving until the 21<sup>st</sup> day in lactation and the period from 22<sup>nd</sup> to 42<sup>nd</sup> day in lactation. The sensitivity and specificity of CMT reported in the literature is variable (Pyorala, 2003). For example, at 3 days in milk (DIM), Sargeant et al. (2001) found that sensitivity and specificity to identify any IMI at a quarter level were 57% and 56%, respectively. On the other hand, Vijaya Reddy et al. (1998) reported a sensitivity of 71% and a specificity of 75%. Sensitivity and specificity are improved when only major pathogens are considered (Sargeant et al., 2001; Dingwell et al., 2003).

In the present study, we hoped to determine whether the CMT scores for individual quarter milk samples could be used as a screening method to identify mammary glands with subclinical infections. Our results suggest that overall there was a significant association between the frequency of isolation of major and minor pathogens and the CMT score in milk samples obtained in the period of early lactation. The present results also indicated that quarters infected with a major pathogen were more likely to have higher CMT scores than those infected with either minor pathogens or uninfected (previously published results Trajchev et al., 2017). These results agree with those reported by Kivaria et al. (2004), which suggested that CMT scores of 2+ or more were associated with an increased risk of infection with S. aureus. Sargeant et al. (2001) reported that the CMT had a useful surveillance role in dairy herd monitoring programs to detect cows with IMI caused by major pathogens. The results obtained by Saidi (2013) showed a good correlation between the results of CMT and isolation for the identification of intra-mammary infections in cows.

Ruegg and Reiman (2002), Kivaria et al. (2004) and Rasmussen et al. (2005) summarized that the CMT is still the superior screening diagnostic aid for subclinical mastitis, while bacteriological examination is still the most suitable for identifying mastitis but not feasible as a routine test to identify subclinical mastitis because some logistical and financial constraints limit its use especially in developing countries.

#### **CONCLUDING REMARKS**

All farmers in the Republic of Macedonia should aspire to the legislation for milk quality control to produce more quality milk. The milk producer or, where appropriate, the operator who collects the milk should ensure compliance with the specific safety requirements for the production of raw milk: animal health requirements for the production of raw milk for human consumption, requirements for premises and equipment of hygiene of the holdings for

during milking, collection and transport and requirements for raw milk. Producing high quality milk requires effective udder health programmes at a herd level.

production of raw milk, hygiene requirements

The early mastitis detection in the dairy herd has many potential benefits, out of which it will allow implementation of proactive management strategies that will avoid negative effects of disease and will lead to better milk quality.

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# СЛЕДЕЊЕ НА ЗДРАВСТВЕНИОТ СТАТУС И ХИГИЕНАТА НА МЛЕЧНАТА ЖЛЕЗДА СО КОРИСТЕЊЕ НА БРЗИ СКРИНИНГ ТЕСТОВИ

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#### Резиме

Во овој труд беше дискутирана употребата на брзите скрининг тестови на фарма за млечни крави како алатка за следење на здравствениот статус и квалитетот на добиеното сурово млеко. Направени се двегодишни проспективни истражувања за оценување на карактеристиките на Калифорнија маститис тестот (КМТ) во периодот на рана лактација како брз теренски скриниг тест за откривање на инфицираните четвртинки од млечната жлезда со патогени микроорганизми кои предизвикуваат мастит. Истражувањето опфаќаше скрининг на пробите млеко од четвртинките на млечната жлезда на кравите со цел откривање на четвртинките со нарушена секреција на млеко (КМТ+) и употребата на микробиолошки методи за откривање на инфицираните четвртинки од млечната жлезда (ИМИ). Пробите млеко беа собирани во два периода од лактацијата на кравите: периодот од почетокот на лактацијата до 21. ден во лактацијата и периодот од 22. до 42. ден во лактацијата. Преваленцијата на КМТ+ и ИМИ во периодот од почетокот на лактацијата до 21. ден во лактацијата изнесуваше 5,33% и 4,03%, соодветно, а во периодот од 22. до 42. ден во лактацијата преваленцијата на КМТ+ и ИМИ изнесуваше 5,45% и 4,38%, соодветно. Преваленцијата на КМТ+ и ИМИ од четвртинките на млечната жлезда кои покажаа позитивна реакција на КМТ во периодот од почетокот на лактацијата до 21. ден во лактацијата изнесуваше 56,96 и 55,42; додека преваленцијата на КМТ+ и ИМИ во периодот од 22. до 42. ден во лактацијата изнесуваше 55,42 и 44,58; соодветно. Добиените резултати укажуваат дека позитивната реакција на КМТ во периодот на раната лактација кај млечните крави претставува добар индикатор за откривање на инфицираните четвртинки на млечната жлезда. Постоеше статистички значајна поврзаност помеѓу присуството на патогени микроорганизми во пробите млеко од четвртинките на млечната жлезда земани во периодот на рана лактација и позитивната реакција на Калифорнија маститис тестот (x<sup>2</sup>=240,031; df=9, P<0,001).

Клучни зборови: Калифорнија маститис тест, млечни крави, инфекција на млечната жлезда

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received: 14.06.2018 Accepted: 10.09.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 635.91-134.45(497.742) Original scientific paper

# CULTIVATION AND PROTECTION OF SEASONAL FLOWERS IN GREENHOUSES IN STRUMICA

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#### Abstract

For the period 2014 – 2015, different varieties and hybrids of seasonal flowers were examined in greenhouses in Strumica. Nine flowering varieties were studied in the 2 year period: Verbena (*Verbena officinalis* L.); Salvia (*Salvia officinalis* L.); Aliciaum (*Alyssum martimum* Lam. Synonym *Lobularia maritima*); Petunia (*Petunia* sp.); Begonia (*Begonia* sp.); Tagetes (*Tagetes* sp.); Portulaca (*Portulaca grandiflora*); Impatiens (*Impatiens* sp.) and *Lobelia* sp. L. The technology and conditions of production were examined, including the conditions of seed production, seedling production, and planting and care during the whole vegetative stage, such as spraying, nutrient supplementation and preventive measures against diseases and pests. All examined variants were compared with untreated controls to assess for higher yield, quality and an earlier arrival time at market.

Five fungicides were used for preventative care: Ridomil Gold MZ 68 WG, Score 250 EC, Switch 62.5 WG, Bravo 500 SC and Topas 100 EC. In addition, 5 different insecticides were assessed: Actara 25 WG, Confidor SL 200, Karate Zeon 5 CS, Chess 50 WG and Pirimor 50 WG. Five variant solutions were studied, each consisting of 2 fungicides and 1 insecticide. All examined variants were highly effective. However, the best performance in 2014 was achieved with a variant containing Bravo 500 SC, Topas 100 EC and Chess 50 WG, and 1 containing Topas 100 EC, Switch 62.5 WG and Pirimor 50 WG. Both of which had a coefficient of efficiency of 99.6%. The index of disease in 2014 in the control variant was 48.2%. In 2015, the control index variation was 54.1%, and the highest biological efficiency was achieved with the variant containing Bravo 500 SC, Topas 100 EC and Chess 50 WG, with a coefficient of efficiency of 98.4%.

In addition to these experiments, our examination was supplemented by surveys. From the polls it was found that flower growing in greenhouses was at an early stage of development, requiring appropriate resources, commitment and time, and that it was still a family business. It also required the state to provide a strategy for the development of floriculture and to invest in it, such that it could contribute to the growth of the national economy.

Key words: fungicides, insecticides, surveys

### INTRODUCTION

Throughout the world, floral species are studied in order to create new genotypes with higher values. Floriculture in the Republic of Macedonia also re presents a large part of the agricultural production, having a significant role in teconomic development. Лозановски и Јанкуловски (1994) estimated that it оссирies 10% of the total cultivation area, about 65,000 ha. A 2-year trial (2014 - 2015) was conducted in order to assess relevant indicators in hybrid flower production. In particular, characteristics during cultivation in greenhouses, including appropriate fungal and insect protection, were determined.

On the basis of these results recommended could be made for wider production. Hybrids that are selected for cultivation should be

characterized by quality, yield and tolerance of certain diseases (Evens & Hensley, 2004; Carvalho et al., 2005).

In addition to analysing the cultivation and disease and insect protection of flowers in

Seed materials were hybrids of foreign origin. The 2 years of research took place in greenhouses of an individual producer in Vladevci, Strumica.

For both years of the study, 9 flowering species were examined:

- 1. Verbena (Verbena officinalis L.)
- 2. Salvia (Salvia officinalis L.)
- 3. Alyssum (*Alyssum martimum* Lam. Synonym *Lobularia maritima*)
- 4. Petunia (Petunia sp.)
- 5. Begonia (Begonia sp.)
- 6. Tagetes sp.
- 7. Portulaca grandiflora
- 8. Impatiens (Impatiens sp.)
- 9. Lobelia sp. L.

The harvested hybrids were placed in 4 repetitions of 10 plants each.

In order to ensure normal growth and development of the test hybrids, adequate production methods were implemented in the greenhouses. For plant protection, fungicides and insecticides were used as preventative care. During 2014 and 2015, 5 fungicides were examined, Ridomil Gold MZ 68 WG, Score 250 EC, Switch 62.5 WG, Bravo 500 SC and Topas 100 EC. In addition, 5 different insecticides were also assessed, Actara 25 WG, Confidor SL 200, Karate Zeon 5 CS, Chess 50 WG and Pirimor 50 WG.

In this study, the phenophases of growth and plant development were followed and the nutrient status and flowering of plants were assessed.

By phenological observation, the occurrence and duration of individual phenophases were determined. The recording of the appearance and duration of each phenophase from sowing seeds to flowering was done in order to show the seasonal greenhouses, we also assessed the cultivation of flowers as a family business, and its contribution to the development of the regionand Macedonia (Caves, 1982).

# **MATERIAL AND METHODS**

The examined variant mixtures, a combination of 2 fungicides and 1 insecticide, were used to treat plants; non-treated plants were used as controls.

The experiment included a total of 5 variant treatments and an untreated control. Each variant treatment was performed on 10 plants in 4 repetitions, for a total of 40 plants. The first treatment was carried out on seedlings 4 days prior to planting. Another 3 treatments were performed at intervals of 10 days. The efficacy of the preparations was evaluated 10 days after the last treatment and was calculated according to the formula of Abbott (1925). The level of infection was scored on a scale of 0 - 5, and the disease index was calculated according to the Townsen-Heuberger formula (1943).

The research was also supplemented by a survey, which used basic research methods of comparative analysis. Methods and techniques for data collection were also used, including a method for analyzing document content and a test method for applying a survey to respondents. The survey was completed by 65 respondents, 15 flower producers, 25 flower shop workers and 25 flower consumers. As an instrument for conducting the research, a questionnaire was used. The respondents answered the questions with an appropriate form in which there was a brief explanation on how the questionnaire should be filled out, as well as general questions for the respondent.

## **RESULTS AND DISCUSSION**

cultivation of the studied flowering species in a protected area, greenhouses in the Strumica region. In addition, comparisons were made between treated plants and their controls for each species. This analysis determined how and to what extent the application of appropriate production techniques, including the protection of plants from fungi and insects, was a crucial factor in the cultivation of flower crops in obtaining higher and better quality yields. Chronologically ordered phenological and quantified results are given in Tables 1 - 4. There were visible differences in the number of days from planting to 20% flowering, and also to 70% flowering. This was significant because when a product arrives at market earlier it can command a higher price. Control plants that were not fed and treated with plant protection products showed much fewer flowers of poorer quality. In addition, many of them lagged behind in their growth and development, with some dying due to diseases and pests.

Flower culture	Variants	Sowing date	Date of sprouting	Date of planting	Date of flowering 20%
Verbena	control, Ø	20.11.2014	30.12.2014	15.01.2015	20.02.2015
officinalis	treated	20.11.2014	30.12.2014	15.01.2015	10.02.2015
Salvia	control, Ø	20.11.2014	25.12.2014	25.01.2015	10.03.2015
splendens	treated	20.11.2014	25.12.2014	25.01.2015	28.02.2015
Alyssum	control, Ø	20.11.2014	25.12.2014	30.01.2015	15.02.2015
martimum	treated	20.11.2014	25.12.2014	30.01.2015	05.02.2015
Petunia sp.	control, Ø	25.11.2014	30.12.2014	25.01.2015	10.03.2015
	treated	25.11.2014	30.12.2014	25.01.2015	25.02.2015
Description	control,Ø	25.11.2014	10.01.2015	25.02.2015	10.04.2015
Begonia sp.	treated	25.11.2014	10.01.2015	25.02.2015	25.03.2015
T	control,	25.11.2014	20.12.2014	15.01.2015	02.03.2015
Tagetes sp. L.	treated	25.11.2014	20.12.2014	15.01.2015	20.02.2015
Portulaca	control, Ø	25.11.2014	30.12.2014	25.01.2015	25.03.2015
grandiflora	treated	25.11.2014	30.12.2014	25.01.2015	10.03.2015
luon ations an	control, Ø	25.11.2014	30.12.2014	25.01.2015	15.03.2015
Impatiens sp.	treated	25.11.2014	30.12.2014	25.01.2015	01.03.2015
Labelia en l	control, Ø	25.11.2014	30.12.2014	15.02.2015	20.03.2015
Lobelia sp. L.	treated	25.11.2014	30.12.2014	15.02.2015	10.03.2015

Table 1.	Phenological	observations	durina 2014
	riteriological	0000110110110	

Flower culture	Variants	Days from sowing to sprouting	Days from sprouting to planting	Days from planting to flowering
Verbena officinalis	control, Ø	40	16	36
verbena omemans	treated	40	16	26
Calvia colondone	control, Ø	35	31	44
Salvia splendens	treated	35	31	34
Abussium in sutingung	control, Ø	35	36	16
Alyssum martimum	treated	35	36	6
Detunian	control, Ø	35	31	44
Petunia sp.	treated	35	31	31
Decemieum	control, Ø	46	46	44
Begonia sp.	treated	46	46	28
Tractor on I	control, Ø	25	26	36
Tagetes sp. L.	treated	25	26	26
Dentul and energy differen	control, Ø	35	26	31
Portulaca grandiflora -	treated	35	26	16
loop ations on	control, Ø	35	26	49
Impatiens sp.	treated	35	26	35
Labalia en l	control, Ø	35	47	33
Lobelia sp. L.	treated	35	47	23

Flower culture	Variants	Sowing date	Date of sprouting	Date of planting	Date of flowering 20%
Verbena	control, Ø	20.11.2014	30.12.2014	15.01.2015	20.02.2016
officinalis	treated	20.11.2014	30.12.2014	15.01.2015	10.02. 2016
Salvia splendens	control, Ø	20.11.2014	25.12.2014	25.01.2015	10.03. 2016
	treated	20.11.2014	25.12.2014	25.01.2015	28.02. 2016
Alyssum	control, Ø	20.11.2014	25.12.2014	30.01.2015	15.02. 2016
martimum	treated	20.11.2014	25.12.2014	30.01.2015	05.02. 2016
Petunia sp.	control, Ø	25.11.2014	30.12.2014	25.01.2015	10.03. 2016
	treated	25.11.2014	30.12.2014	25.01.2015	25.02. 2016
Begonia sp.	control, Ø	25.11.2014	10.01.2015	25.02.2015	10.04. 2016
	treated	25.11.2014	10.01.2015	25.02.2015	25.03. 2016
Tagetes sp. L.	control, Ø	25.11.2014	20.12.2014	15.01.2015	02.03. 2016
	treated	25.11.2014	20.12.2014	15.01.2015	20.02. 2015
Portulaca	control, Ø	25.11.2014	30.12.2014	25.01.2015	25.03. 2016
grandiflora	treated	25.11.2014	30.12.2014	25.01.2015	10.03. 2016
Impatiens sp.	control, Ø	25.11.2014	30.12.2014	25.01.2015	15.03. 2016
	treated	25.11.2014	30.12.2014	25.01.2015	01.03. 2015
Lobelia sp. L.	control, Ø	25.11.2014	30.12.2014	15.02.2015	20.03. 2016
	treated	25.11.2014	30.12.2014	15.02.2015	10.03. 2016

Table 3.	Phenological	observations	durina 2015
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**Table 4.** Phenological observations during 2015

Flower culture	Variants	Days from sowing to sprouting	Days from sprouting to planting	Days from planting to flowering
Verbena officinalis	control, Ø	40	16	36
	treated	40	16	26
Salvia splendens	control, Ø	35	31	44
	treated	35	31	34
Alyssum martimum	control, Ø	35	36	16
	treated	35	36	6
Petunia sp.	control, Ø	35	31	44
	treated	35	31	31
Begonia sp.	control, Ø	46	46	44
	treated	46	46	28
Tagetes sp. L.	control, Ø	25	26	36
	treated	25	26	26
Portulaca grandiflora	control, Ø	35	26	31
	treated	35	26	16
Impatiens sp.	control, Ø	35	26	49
	treated	35	26	35
Lobelia sp. L.	control, Ø	35	47	33
	treated	35	47	23

The results of the biological investigations of fungicides and insecticides for greenhouses production of flowers in Vladevci, Strumica are shown in Tables 5 and 6. All examined combinations of fungicides and insecticides showed high efficacy in protecting the flowering plants grown in greenhouses in 2014 and 2015. In 2014, the highest efficacy was achieved for the combination of Bravo 500 SC, Topas 100 EC and the Chess 50 WG, and for Topas 100 EC, Switch 62,5 WG and Pirimor 50 WG, with scores of 99.6%.

It is important to note that other combinations of fungicides and insecticides also

showed high efficacy in preventing damage by fungi and insects. The disease control index in the control plants reached an extremely high level of 48.2% of disease and leaf mass loss in 2014.

The results from 2015 indicated a high percentage of protection against diseases and pests for the species grown in the greenhouses. The efficacies of the examined combinations were 1 - 2% lower compared to 2014. However,

it is important to note that the control plants had a higher level of infection at 54.1% in 2015 compared with those in 2014 (48.2% index of disease).

The 2 best treatments with efficacies of > 98% were the second variant of Score 250 EC, Switch 62.5 WG and Confidor SL 200 and the fourth variant of Bravo 500 SC, Topas 100 EC and Chess 50 WG (Table 6).

Serial Number	Variants	Index of disease %	Efficacy %
1.	Ridomil Gold MZ 68 WG Topas 100 EC Actara 25 WG	2.2	98.4
2.	Score 250 EC Switch 62,5 WG Confidor SL 200	0.8	99.2
3.	Switch 62,5 WG Bravo 500 SC Karate Zeon 5 CS	0.6	99.4
4.	Bravo 500 SC Topas 100 EC Chess 50 WG	0.2	99.6
5.	Topas 100 EC Switch 62,5 WG Pirimor 50 WG	0.2	99.6
6.	Control (untreated)	48.2	/

Table 5. Efficacy of fungicides and insecticides in flower crop species in 2014

Table 6. Efficacy of fungicides and insecticides in flower crop species in 2015

Serial Number	Variants	Index of disease %	Efficacy %
1.	Ridomil Gold MZ 68 WG Topas 100 EC Actara 25 WG	3.4	96.4
2.	Score 250 EC Switch 62,5 WG Confidor SL 200	2.2	98.1
3.	Switch 62,5 WG Bravo 500 SC Karate Zeon 5 CS	2.6	97.3
4.	Bravo 500 SC Topas 100 EC Chess 50 WG	2.2	98.4
5.	Topas 100 EC Switch 62,5 WG Pirimor 50 WG	2.8	97.8
6.	Control (untreated)	54.1	/

On the basis of the surveys and statistically analysed data contained in the questionnaire, it was found that in the Republic of Macedonia it is relatively difficult to create a greenhouse environment for the commercial production of flowers. The results obtained can be generalized with 99% reliability. Of consumers, 60% believed that conditions have not been developed for flower crop production, while 30% considered that a large part of the development of a flower trade had been achieved. Of these, 10% of consumers considered that conditions for the development of flower trade were difficult. The research found that the development of flower growing in greenhouses in our area is

in the initial phase, consisting mostly of family businesses and most of these were self-financed.

### **CONCLUDING REMARKS**

Based on the results obtained from the 2 years of research on the cultivation and protection of seasonal flowers in greenhouses in Strumica, the following significant conclusions could be drawn.

In the species where appropriate production technniques was applied, with regular feeding of the plants and appropriate and timely application of plant protection products, the occurrence of flowering was earlier. In some floral species such management practices resulted in flowers > 10 days before control plants. Earlier placing of flowers at the market brings a higher selling price, which means greater profit for the producer.

The control variants examined were characterized by significantly higher yield and quality when grown under greenhouse conditions.

All examined variants of fungicides and insecticides in 2014 and 2015 yielded a high percentage of efficacy for plants compared to no treatment. It was best to apply preventive protection of at least 3 - 4 treatments of the fungicide and insecticide mixes. The first must be mandatory in the seedling stage, at least 3 - 4 days before planting, with 3 treatments at intervals of 10 days during the vegetative stage.

The high efficacy of the tested preparations was confirmed when compared with the control variant, where no chemical protection was applied. Control plants showed indexes of disease of 48.2% in 2014 and 54.1% in 2015.

The results of the study indicate that flower plants can not be successfully grown in greenhouses without adequate preventive chemical protection.

From the survey it was established that the development of flower growing in greenhouses in our area is in the initial phase, with self-financed family businesses.

The state needs to develop a strategy for the development of floriculture and provide necessary investment. Such a strategy will contribute to the development of the national economy.

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### ОДГЛЕДУВАЊЕ И ЗАШТИТА НА СЕЗОНСКО ЦВЕЌЕ ВО ОРАНЖЕРИИ ВО СТРУМИЦА

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#### Резиме

Во периодот 2014 и 2015 година се испитуваат различни сорти и хибриди сезонско цвеќе во оранжерии во Струмица.

Во двегодишниот период на испитување имаше 9 цветни сорти: вербена (Verbena officinalis L.); салвиа (Salvia officinalis L.); алиција (Alyssum martimum Lam. синоним Lobularia maritima); петунија (Petunia sp.); бегонија (Begonia sp.); Tagetes (Tagetes sp.); портулака (Portulaca grandiflora); импатиенс (Impatien ssp.) и Lobelias sp. L. Техниката и технологијата на производството биле испитувани во услови на производство, потоа биле испитувани условите за производство на семе, производство на садници, садење и нега во текот на целата вегетација (прскање, негување и превентивна заштита од болести и штетници.

Имено, сите испитани варијанти во споредба со контролната варијанта (нетретирани) се карактеризираат со повисок принос, квалитет и порано пристигнување за пласирање на пазарот.

За превенција се користени пет фунгициди: Ridomil Gold MZ 68 WG, Score 250 EC, Switch 62.5 WG, Bravo 500 SC и Topas 100 EC и пет различни инсектициди: Actara 25 WG, Confidor SL 200, Karate Zeon 5 CS, Chess 50 WG и Pirimor 50 WG. Од сите тестирани фунгициди и инсектициди за испитување, подготвени (се користат) се пет варијанти кои се состојат од два фунгициди и еден инсектицид. Сите испитувани варијанти даваат висок процент на ефикасност. Најефикасна, во 2014 г. се покажала варијантата Bravo 500 SC, Topas 100 EC и Chess 50 WG и Topas 100 EC, Switch 62.5 WG и Pirimor 50 WG, со коефициент на ефикасност од 99.6%.

Индексот на болеста во 2014 во контролната варијанта е 48.2%.

Во 2015 г. индексот на болеста во контролната варијанта беше 54.1%, а најголема биолошка ефикасност е постигната со варијантата Bravo 500 SC, Topas 100 EC и Chess 50 WG, со коефициент на ефикасност од 98.4%. Во прилог на нашите експерименти, нашето испитување беше дополнето со анкети.

Од анкетите беше откриено дека развојот на цвеќареството во оранжерии во нашите простори е во почетна фаза и бара соодветни ресурси, посветеност и време, и дека с уште е во семеен бизнис. Исто така, тоа бара од државата да развие стратегија за развој на цвеќарството и да инвестира финансиски средства, што исто така ќе придонесе за развојот на националната економија.

Клучни зборови: цвеќиња, фингициди, инсектициди, анкети, заштита

#### JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received: 28.11.2018 Accepted: 07.12.2018



In print: ISSN 2545-4447 On line: ISSN 2545-4455 UDC: 634.852/.853-152.61:631.95(497.2) Original scientific paper

#### AGROBIOLOGICAL EVALUATION OF GRAPEVINE VARIETIES FOR WHITE AND RED WINES

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### Abstract

A comparative agrobiological evaluation of grapevine varieties for white and red wines from different ecological-geographic groups have been done through application of mathematical methods - analysis of variance, cluster and factor analysis using R language for statistical processing. It has been established that the varieties Fetească albă, Fetească regală, Pamid, Dimiat and Mavrud differ materially according to almost all examined characteristics. The analysed indicators in the structure of the yield in the case of the oriental varieties are summarized in five factors, in the case of those from the Black Sea – in four, which are enough to explain more than half of their total variability. The indicators from factor F3are with the biggest essential direct impact on the yield in the case of all varieties from the first group, and in the case of those from the second – F1. Other factors, which are not analysed, have also an impact on the formation of their yield.

Key words: analysis of variance, cluster analysis, agrobiological indicators, wine grape varieties

### INTRODUCTION

The agrobiological characteristics of each vine variety contain extremely important indicators revealing its economic value. The most popular and most spread local varieties for white and red wines in Bulgaria are mainly from the Black Sea ecological-geographic group (convarietas pontica Negr.), Balkan subgroup (subconvarietas balcanica Negr.). There are rarely varieties from the Eastern ecologicalgeographic group (convarietas orientalis Negr.) (Bulgarian Ampelography, 1990; Roychev 2012). When grown on appropriate terrains and according to the corresponding technology, they can produce good and quality produce (Donchev 1990). The application of factor analysis in agrobiological studies of wine grape varieties allows economically most important indicators for the formation of their yield to be determined (Simeonov et al., 2015, Simeonov et al., 2016, Roychev 2017). Comparison of different agrobiological factors impact on the yield of grapes in the case of grapevine varieties from different ecological-geographic groups is of interest for ampelography and applied viticulture. The purpose of this study is to establish the possibilities for application of factor analysis in comparative agrobiological studies of wine grape varieties.

### **MATERIAL AND METHODS**

Two varieties for white wines from the Eastern ecological-geographic group - Fetească albă and Fetească regală, two varieties for red wines-Mavrud and Pamid and one for white wines - Dimiat, which are grown in the ampelographic assortment of the Department of Viticulture -Agricultural University-Plovdiv are included in the study. The ampelographic indicators - shoot fertility coefficient (C), millerandage berries (%), average cluster weight (g), cluster length (cm), cluster width (cm), seeds in 100 berries (number), weight of seeds in 100 berries (g), grapevine yield (kg), average weight of 100 berries (g), berry length (mm), berry width (mm), sugars (%), acids (g/dm3) of 25 typical grapevines of each variety in the respective phenophases have been determined in the course of five consecutive years. Experimental data processing has been performed with the functional programming language and environment for statistical data analysis R. The R open source principles allow access to software applications for solving specific statistical tasks. The presence of reliable variance between the varieties in the analysed indicators has been determined by single factor analysis of variance and evaluation of averages according to the Duncan's method. In the case of the Black Sea varieties the cluster analysis has been also applied. The inter-group connection method has been chosen as an agglomeration method for clustering, and a similarity measure is the quadratic Euclidean distance. The distance between two clusters A and B is defined as the average value of nA.nB of number of distances between nA points from A and nB points from B by the formula:

$$D(A,B) = \frac{1}{n_A n_B} \sum_{i=1}^{n_A} \sum_{j=1}^{n_B} d(x_i, x_j) \quad (1)$$

where the sum changes in all xi and xj from A and B. With

$$d(x_{i}, x_{j}) = \sum_{m=1}^{p} (x_{im} - x_{jm})^{2} \quad i, j = \overline{1, n} \quad (2)$$

we denote the quadratic Euclidean distance between two vectors  $x_i(x_{i1}, x_{i2}, ..., x_p)$  and

$$x_{j}(x_{j1}, x_{j2}, ..., x_{jp})$$

With the use of the R language statistical techniques a check for presence of the necessary conditions for application of a factor analysis has been done. A complex study of the factors (indicators) impact on the yield

of grapes per grapevine has been carried out using R. The coefficients of the regression model are statistically significant at rates of statistical significance (Sig.) less than 0,05.

#### **RESULTS AND DISCUSSION**

Data from the multidimensional comparative analysis shows that the studied varieties from the Oriental ecological-geographic group differ materially in almost all biometric indicators, except for the percentage of millerandage berries, yield, berry size and acids (Tab. 1). Fetească albă differs with greater cluster length and seeds in 100 berries, and Fetească regală - with higher values of the indicators fertility coefficient, average cluster weight, cluster width, weight of seeds in 100 berries, sugars content. In the case of the varieties from the Black Sea group, the variances between the studied agrobiological indicators have not been proven only for weight of seeds in 100 berries. Statistical groups two and three are formed in the case of both varieties.

According to the dendrogram-clustering, the Pamid and Dimiat varieties form a cluster of the first level of similarity (Fig. 1). The relative distance between them is from 0,0 to 0,5. They are characterized by a high degree of closeness in the values of the studied indicators. Mavrud differs with a high degree of remoteness from the other two varieties in terms of the values of the studied indicators and is considered different from them. The relative distance between Mavrud and the rest of the varieties is in the range from 0,5 to 25,0.

The analysis of the factor distribution of the obtained agrobiological data shows that on the formation of the yield per grapevine in the case of the varieties Fetească albă and Fetească regală impact five summarizing factors (60,7% and 60,7%), as each of them covers more often two and rarely one indicator (Tab. 2). In F1 in the case of both varieties are included berry length and berry width with factor impact of 15,8% -15,8% and positive impact on the amount of grape yield. In F2 - in the case of the first variety (12,4%) are the average cluster weight and acids, and in the case of the second (15,2%) - cluster length and cluster width. The third factor - F3 covers the following indicators: fertility coefficient (with a negative value), millerandage berries (12,0%) and seeds in 100 berries, weight of seed in 100 berries (15,0%). In F4 the diversity of the studied indicators increases but their factor impact on the formation of the yield decreases - cluster

length, weight of seeds in 100 berries (with a negative value) (10,5%) and millerandage berries, sugars (10,1%). In the case of the first variety in F5 fall the indicators seeds in 100 berries (with a negative value) and sugars (10,0%), and in the case of the second - only average weight of 100 berries (9,9%). The formation of the yield in the case of the studied varieties from the Black Sea group is determined by four factors - Pamid (62,6%), five - Dimiat (61,8%) and seven - Mavrud (83,4%). The correlation coefficients for all the factors of Pamid have positive values, as for Dimiat there is one indicator with a negative value, and in the case of Mavrud - they are three. The agrobiological specificity between these varieties is demonstrated by the fact that the individual indicators fall into different factors. The fertility coefficient in the case of the first and the second varieties is in F4, and in the case of the third one in F6; millerandage berries - only in F6 of Mavrud; average cluster weight - F3 of Dimiat and F7 of Mavrud; cluster length and cluster width - F3 of Pamid and F2 - Dimiat and Mavrud: number and weight of seeds in 100 berries - F1 of Pamid, F5 - Dimiat and F3 - Mavrud; average weight of 100 berries - only in F5 of Mavrud; berry length and berry width - in the case of the three varieties sequentially in F2, F1 и F1; sugars - only in F4 of Mavrud and acids - F4 of Pamid, F3 - Dimiat and F4 - Mavrud.

The direct impact of the factors expressed by the standardized regression coefficient (Beta) shows that with the highest proven positive significance for the yield per grapevine in the case of Fetească albă is F5 (0,013), Fetească regală – F4 (0,173), Pamid - F4 (0,213), Dimiat – F2 (0,020), Mavrud –F7 (0,143) (Tab. 3). The coefficients of the regression model are statistically significant. The low coefficients of determination in the case of all varieties mean that the grape yield is not determined solely by the studied indicators in the summarizing factors.

The analysis of the main components in the varieties from the Oriental group shows that five of them are sufficient to explain 59,8% of the total variation of the studied indicators (Tab. 4). Their relative degree of variation correlates most strongly with the first major component. With berry length and berry width is explained 15,4% of the total variation. The second component explains 13,0% of the total variation, as with the highest correlation coefficients are the fertility coefficient and seeds in 100 berries, which is with a negative sign. The third major component explains 11,3% of the total variation, mostly with cluster length and weight of seed in 100 berries, as the second indicator has a negative impact on the total variation. The fourth component explains 10,1% of the total variation, as with the highest correlation coefficient are millerandage berries and sugars. With the highest correlation coefficients for the fifth major component is the cluster width, which explains 10,0% of the total variation. In the case of the varieties from the Black Sea group, four of the major components are sufficient to explain 63,8% of the total variation of the studied indicators. With average cluster weight, cluster length and cluster width is explained 21,0% of the total variation of the characteristics in the first basic component, in the second - with berry length and berry width (19,6%), in the third - number and weight of seeds in 100 berries (12,2%) and in the fourth - shoot fertility coefficient and millerandage grains (11,0%).

The coefficients of the regression model of variation of the studied indicators Beta show that a negative value and impact in the case of the oriental varieties is found only in F4 (-0,046) и F5 (-0,094) (Tab. 5). With the biggest direct impact on yield are the indicators of the summarized factor F3 (0,179). In the case of the varieties from the Black Sea group, there are no negative values for the summarized factors. Biggest direct impact has F1 – 0,323. The coefficients of the regression model in the case of both groups of varieties are statistically significant. The low values of the coefficient of determination - 4,9% and 15,9% show that on the formation of the grape yield in the case of the varieties from both of the groups impact have indicators other than the studied ones.

## **CONCLUDING REMARKS**

 The wine grape varieties Fetească albă, Fetească regală - Oriental ecologicalgeographic group and Pamid, Dimiat and Mavrud - Black Sea ecological-geographic group differ statistically proven according to almost all studied agrobiological indicators. The varieties Pamid and Dimiat form a cluster of first level of similarity, and Mavrud is farthest from them.

- 2. Economically most important indicators having positive impact on the yield of grape in the case of the studied varieties are grouped in four to seven summarizing factors, as with the greatest proven positive significance are F2, F4, F5, and F7. The yield in the case of most of the varieties is mainly determined by the indicators cluster and berry length and cluster and berry width, followed by average cluster weight.
- 3. The importance of the studied indicators

in the structure of the yield in the case of the oriental varieties is summarized in five factors, in the case of those from the Black Sea – in four, which are enough to explain more than half of their total variability. With the biggest proved direct impact on the yield in the case of all varieties from the first group are the indicators from the summarized factor F3, and from the second group – F1. Other factors which are not analysed have also an impact on the formation of their yield.

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<b>e 1.</b> Multidirectional comparative analysis of fertility and yield indicators of the grapevine varieties studied	
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Table 1. Multidirectional comparative analysis of fertility and yield indicators of the grapevine varieties studied	ctional co	mparative analy	/sis of fertilit)	/ and yield i	indicators c	of the grapev	ine varietie.	s studied					
Group	U	Millerandage berries (%)	Average cluster weight	Cluster length (cm)	Cluster width (cm)	Seeds in 100 berries	Weight of seeds in 100 berries	Yield (kg)	Average weight of 100 berries	Berry length (mm)	Berry width (mm)	Sugars (%)	Acids (g / dm³)
vallety			(6)			(number)	(g)		(g)	``````````````````````````````````````			
				Ō	riental ecolo	Oriental ecologo-geographical group	hical group						
Fetească albă	1,28 <sup>b</sup>	1,41ª	124 <sup>b</sup>	23,18 ª	8,58 <sup>b</sup>	178 <sup>a</sup>	6,33 <sup>b</sup>	6,28 ª	157 b	13,80 ª	14.39 ª	18,75 <sup>b</sup>	6,69 <sup>a</sup>
Fetească regală	1,90 ª	1,46ª	154 <sup>a</sup>	14,42 <sup>b</sup>	9,17 ª	161 <sup>b</sup>	6,81 <sup>a</sup>	6,53 ª	168 <sup>a</sup>	13,98 ª	14,29 ª	19,40ª	6,79 ª
				Bla	ick Sea eco	Black Sea ecologo-geographical group	chical group						
Pamid	1,36 ª	1,76 b	179 ∘	12,80 °	8,37 °	181 <sup>b</sup>	6,81 <sup>a</sup>	5,36 b	214 <sup>b</sup>	13,83 <sup>b</sup>	12,81 °	20,76 ª	6,06 <sup>b</sup>
Dimiat	1,47 <sup>a</sup>	4,10 ª	200 <sup>b</sup>	14,11 <sup>b</sup>	10,14 <sup>b</sup>	211 <sup>a</sup>	6,70 <sup>a</sup>	7,76 ª	297 <sup>a</sup>	16.56 <sup>a</sup>	15,49 ª	18,63 <sup>b</sup>	6,04 <sup>b</sup>
Mavrud	1,08 <sup>b</sup>	2,24 b	357 <sup>a</sup>	17,86 ª	12.83 ª	216 <sup>a</sup>	6,69 <sup>a</sup>	7,78 <sup>a</sup>	181 °	14,35 <sup>b</sup>	14,33 <sup>b</sup>	19,05 b	6,78 <sup>a</sup>
[a, b, c a degree of proof according to the Duncan's method with error $\alpha$ = 0.05	ee of proof	according to the	e Duncan's m	lethod with €	<u>error α = 0.(</u>	05							

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lable 2. Factor distribution of indicators having impact on the yield per grapevine in the case of the studied varieties	distrik	JOITUC		dicato	rs nav	ing in	npact	on une	s yreid	per g	rapev	lue in	the ca.	se of ti	ne stu	died v	/arietiv	SS							
Variety		Fe	tească ș	۱bă			Fete	ască reç	jală			Pam	id				Dimiat		┝			Ma	Mavrud		
Indicators			(60,7%)	(			)	( 60,7%)				(62,6)	2)				(61,8)					(8)	(83,4)		
	щ	F_	Ъ.	F_4	F S	ц,	F_2	Ē	Е4	۳ <sub>2</sub>	ц.	F_2	Ē	<b>Т</b> 4	Ľ.	F_2	Ľ	F 4	Ъ,	ц.	F_F	F <sub>3</sub>	4 F5	ц Ч	F <sub>7</sub>
Shoot fertility coefficient			-0,734										<u> </u>	0,712		<u> </u>	<u> </u>	0,743						-0,813	ε
Millerandage berries (%)			0,765						0,710															0,675	Ŀ0
Average cluster weight (g)		0,793															0,649								0,948
Cluster length (cm)				0,774			0,849						0,831		-	0,668				0,	0,885				
Cluster width (cm)							0,866						0,824		_	0,830				0	0,934				
Seeds in 100 berries (number)					-0,731			0,899			0,919							0	0,708		0,871	371			
Weight of seeds in 100 berries (g)				-0,734				0,896			606'0							0	0,648		0,771	71			
Average weight of 100 berries (g)									-	0,818													-0,895	95	
Berry length (mm)	0,871					0,934						0,875		-	0,964				0,	0,949					
Berry width (mm)	0,902					0,908						0,860		-	0,968				0,	0,930					
Sugars (%)					0,748				0,770													-0,880	80		
Acids (g/dm <sup>3</sup> )		0,639										_		0,619		-	-0,678	_				0,6	0,616		
Factor impact (%)		12,4	15,8 12,4 12,0	10,5	10,0	10,0 15,8 15,2		15,0	10,1	9,9	17,6	16,7	15,2	13,1	17,8	11,3	11,2	11,1 1	10,4 1	16,8 1	14,0 13	13,1 10,5	,5 9,8	9,7	9,5

		Regression	Standardized regression
Variety	Factors	coefficient	coefficient
		(B)	(Beta)
	(Constant)	6,532	
	F <sub>1</sub>	-0,072	-0,113
Fetească albă	F <sub>2</sub>	-0,041	-0,064
l'eteasca alba	F <sub>3</sub>	-0,011	-0,017
	F <sub>4</sub>	-0,083	-0,130
	F <sub>5</sub>	0,008	0,013
Coefficient of determination	R <sup>2</sup> =3,4%		
	(Constant)	6,285	
	F <sub>1</sub>	0,199	0,133
	F,	-0,239	-0,160
Fetească regală	$F_3$	-0,018	-0,012
	F <sub>4</sub>	0,258	0,173
	F <sub>5</sub>	-0,066	-0,044
Coefficient of determination	R <sup>2</sup> =7,5%		
	(Constant)	5,363	
	F,	0,185	0,107
Pamid	F <sub>2</sub>	-0,160	-0,092
	F <sub>3</sub>	0,106	0,062
	F,	0,375	0,213
Coefficient of determination	R <sup>2</sup> =6,7%		
	(Constant)	7,756	
	F,	-0,046	-0,021
	F <sub>2</sub>	0,044	0,020
Dimiat	F <sub>3</sub>	-0,136	-0,062
	F <sub>4</sub>	-0,213	-0,096
	F	0,023	0,010
Coefficient of determination	R <sup>2</sup> =1,4%		
	(Constant)	7,776	
	F,	0,172	0,086
	F,	0,207	0,103
	F <sub>3</sub>	0,246	0,122
Mavrud	F <sub>4</sub>	-0,398	-0,195
	F <sub>5</sub>	-0,089	-0,045
	F	0,030	0,015
	F <sub>7</sub>	0,290	0,143
Coefficient of determination	R <sup>2</sup> =8,8%		

**Table 3.** Coefficients of the regression model of the variation of indicators having impact on the yield per grapevine in the case of the studied varieties

Varieties groups		Orier	ntal varie	eties			Black Sea	a varieties	
Indicators	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>	F <sub>5</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
C Shoot fertility coefficient		0,703							0,690
Millerandage berries (%)				0,812					0,625
Average cluster weight (g)						0,713			
Cluster length (cm)			-0,643			0,838			
Cluster width (cm)					0,611	0,866			
Seeds in 100 berries (number)		-0,750						0,784	
Weight of seeds in 100 berries (g)			0,752					0,891	
Average weight of 100 berries (g)									
Berry length (mm)	0,899						0,902		
Berry width (mm)	0,907						0,916		
Sugars (%)				0,677					
Acids (g / dm³)									
Factor impact (59,8%); (63,8%)	15,4	13,0	11,3	10,1	10,0	21,0	19,6	12,2	11,0

<b>Table 4.</b> General factor distribution of the indicators impact on the yield per grapevine in the case of all
studied grapevine varieties

**Table 5.** Coefficients of the regression model of the variation of indicators having impact on the yield per grapevine in the case of all studied grapevine varieties

	Factors	Regression coefficient (B)	Direct impact of the factors (Beta)	Regression coefficient (B)	Direct impact of the factors (Beta)
		Orienta	al varieties	Black Sea v	arieties
Varieties	(Constant)	6,411		6,965	
Varieties	F <sub>1</sub>	0,086	0,075	0,729	0,323
	F <sub>2</sub>	0,006	0,005	0,468	0,214
	F <sub>3</sub>	0,207	0,179	0,245	0,114
	F <sub>4</sub>	-0,053	-0,046	0,229	0,107
	F <sub>5</sub>	-0,108	-0,094	6,965	
Coefficient of determination	R <sup>2</sup> = 4,9%			R <sup>2</sup> = 15,9%	

**Figure 1.** Dendrogram-clustering of the studied grapevine varieties from the Black Sea ecologo-geographical group

Dendrogram using Average Linkage (Between Groups)

			Rescaled	Distance	Cluster	Combine	
N	um	0	5	10	15	20	25
Pamid Dimiat Mavrud		]					

# АГРОБИОЛОШКА ЕВАЛУАЦИЈА НА СОРТИ ГРОЗЈЕ ЗА ПРОИЗВОДСТВО НА БЕЛИ И ЦРВЕНИ ВИНА

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#### Резиме

Компаративна агробиолошка евалуација на сорти за производство на бели и црвени вина од различни еколошко-географски групи беше изработена со примена на математички методи и тоа: анализа на варијанса, кластер анализа и фактор анализа со користење на Р јазик за статистичка обработка. Утврдено е дека сортите Fetească albă, Fetească regală, Pamid, Dimiat и Mavrud се разликуваат според речиси сите испитувани карактеристики. Анализираните индикатори во структурата на приносот во случај на ориенталните сорти се сумирани во пет фактори, оние од Црното Море - во четири, кои се доволни да објаснат повеќе од половина од нивната вкупна варијабилност. Со најголемото суштествено директно влијание врз приносот во случај на сите сорти од првата група се индикаторите од факторот F3, а во случај на оние од вториот - F1. Други фактори кои не се анализираат, исто така, имаат влијание врз формирањето на нивниот принос.

Клучни зборови: анализа на варијанса, кластер анализа, агробиолошки показатели, вински сорти грозје