

## Biogenicity of the Soil Natural Grasslands in Western and South-Western Serbia

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### SUMMARY

Natural grasslands are an important resource for agriculture and cover almost 29 % of total agriculture land in Serbia, distributed within a wide altitudinal range - from lowland areas to the highest alpine zone. This paper is intended to present biological activity of the soil (biogenicity) of grasslands on the basis of presence the total number of microorganisms. During autumn of 2018 soil samples from 14 localities at altitudes between 572 m and 1328 m were taken for microbiological analyses from a depth of 0-25 cm. These grasslands located in the territory Western and Southwestern Serbia. Physical and chemical characteristics of soil are the most important properties that affect the number of microorganisms, especially pH and organic matter content. In our study the smallest number of microorganisms per one gram of absolutely dry soil (log of number) – 5,778 on location Preseka was

– 5.778  
-  
6.505

Preseka.  
–  
Šume.

found. The largest number of total microflora – 6,505 in rizospheric soil of grassland on location Šume was recorded. According to Fishers test some samples of the tested soil are statistically significantly different, but there are there are several homogeneous groups i.e samples of soil among themselves does not show statistically significant differences the total number of microorganisms. Our study shown that the presence of microorganisms in the tested soil samples is influenced by numerous factors and that each soil provides different conditions for the life of the microbe.

**Key words:** grassland, soil, biogenity

## INTRODUCTION

Grassland vegetation of Serbia occupying about 1.5 million ha or 27% of the total agricultural area of the country as the most represented type of the agroecosystem (Simi et al., 2015).

They are not only important as an economic objective, but also from the ecological and biodiversity point for environment preservation and protection of the soil from erosion (Lugi et al., 2010). Serbian grasslands represents rich fund of autochthonous plant genetic resources of forage grasses (Sokolovi et al., 2009). Distribution of grasslands in the region is unequal but there are certain regularities with increase of altitude, also grassland area increase, especially their share in the structure of agriculture soil (Stoši et al., 2005).

Their production potentials are different and under the influence of numerous factors. Dominant influences are the soil, precipitation level and management (Lazarevi et al., 2003).

In the natural environments, plants live in interactions with different microorganisms and such interactions are

1,5 . ha 27%

(Simi et al., 2015).

al., 2010).

(Sokolovi et al., 2009).

2005).

et al., 2003).

(Stoši et al.,

(Lazarevi

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|---|---|
| <p>(Igiehon and Babalola, 2018).</p>                        | <p>important in ecosystem functioning (Igiehon and Babalola, 2018). Microbial parameters are important for soil quality assessment because of their contribution to energy and nutrients flows in ecosystems and their fast responsiveness to changes in the soil environment (Cardoso et al., 2013; Stenberg, 1999).</p>   |
| <p>(Cardoso et al., 2013; Stenberg, 1999).</p>              | <p>Biological activity of organisms play an important role in soil, mainly for decomposition of organic matter in soil and especially of plant rests (Zubarev et al., 2016)</p>   |
| <p>(Zubarev et al., 2016)</p>                               | <p>The rhizosphere, as the functional unit of grasslands, is the site of organic deposition, also and habitat and resource heterogeneity for soil organisms (Stanton, 1988).</p>  |
| <p>(Stanton, 1988).</p>                                     | <p>The number of microorganisms is determined by the nutrient in the soil and the indigenous microbial communities of each soil are unique for the particular soil, as they have been shaped and evolved over time in accordance with the dynamics of the local habitat (Nazir et al., 2013).</p>   |
| <p>(Nazir et al., 2013).</p>                                | <p>Soil reaction is an important agrochemical indicator because it has a strong influence on the growth medium of plants, on the regime and bio-availability of nutrients and on the way nutritive ions get to plant roots. It is due to this fact that soil reaction is considered to be one of the most important characteristics of the environment (Pislea and Sala, 2012).</p> |
| <p>(Pislea and Sala, 2012).<br/>Higashida Takao (1986),</p> | <p>According to research Higashida and Takao (1986) where they are examined the relations between soil microbial activity and soil properties in grassland the low soil pH condition was assumed to depress bacterial activities, and consequently to delay the decomposition of organic matter.</p>  |
| <p>Lauber et al. (2009)</p>                                 | <p>Also, Lauber et al. (2009) is considered pH of soil to be one of the most important factors which inflate abundance of microorganisms, whereby this parameter may function as an integrating variable</p>  |

that provides an integrated index of soil conditions. Any type of soil has its own characteristic microbiocenosis and the way of soil use may have positive or negative effects on microbiological activities and what is directly reflected on soil fertility (Tintor et al., 2009).

The aim of this investigation was to examine the total number of microbes in the soil of natural grasslands in Western and South-Western Serbia.

**MATERIAL AND METHODS**

During autumn of 2018 soil samples from 14 localities at altitudes between 572 m and 1328 m were taken for microbiological analyses from a depth of 0-25 cm. These grasslands located in the territory Western and Southwestern Serbia. Sampling soil was carried out in three sites from different parts of one location, they are mixed and present as one sample. The chemical properties of the soil were determined by standard methods in the chemical laboratory of the Institute for Forage Crops Kruševac. Biological activity of the soil of grasslands was monitored on the basis of presence the total number of microorganisms. In the lab, each of the samples was analyzed in three repetitions. Total number of microorganisms was established with standard microbiological methods of introducing a certain specific quantity of soil suspension diluted  $10^{-6}$ , method of agar panels (Pochon and Tardieux, 1962). The incubation lasted for five days on  $28^{\circ}$ . The number of grown colonies was calculated per 1g of absolutely dry soil (Jarak and Djuri, 2006).

The results were processed by means of STATISTICS 8.0 computer program, using Fisher's LSD test.

**RESULTS AND DISCUSSION**

The results of chemical analyses of soil show that the pH value ranged from

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## RESULTS AND DISCUSSION

The results of chemical analyses of soil show that the pH value ranged from

3.62 6.17.

strongly acidic 3.62 to 6.17, from very strongly acidic to slightly acidic. According to the content of humus, nitrogen, potassium and phosphorus soil samples from different grasslands were under different among themselves (Table1).

1.

( )

**Table 1. The chemical composition and the total microflora (log of number) of studied soils**

| Location | Altitude of location (m) | pH               |      | N %   | P <sub>2</sub> O <sub>5</sub> mg/100g | K <sub>2</sub> O mg/100g | Humus % | The total number of m.o. |
|----------|--------------------------|------------------|------|-------|---------------------------------------|--------------------------|---------|--------------------------|
|          |                          | H <sub>2</sub> O | KCl  |       |                                       |                          |         |                          |
| Brusnik  | 1239                     | 5.79             | 4.59 | 0,164 | 5.10                                  | 28.70                    | 1.60    | 6.041 <sup>h,i</sup>     |
| Vrmbaje  | 719                      | 6.37             | 4.97 | 0,265 | 4.10                                  | 25.82                    | 3.96    | 6.079 <sup>g,h</sup>     |
| Sveštica | 774                      | 4.38             | 3.62 | 0.193 | 31.20                                 | 20.45                    | 3.29    | 5.903 <sup>j</sup>       |
| Dubrava  | 741                      | 4.85             | 4.13 | 0.324 | 4.60                                  | 4.30                     | 3.16    | 6.000 <sup>i</sup>       |
| Preseka  | 1033                     | 4.51             | 3.79 | 0.205 | 7.70                                  | 16.78                    | 2.70    | 5.778 <sup>k</sup>       |
| Šumer    | 703                      | 6.83             | 6.09 | 0.193 | 72.10                                 | 44.06                    | 3.90    | 6.505 <sup>a</sup>       |
| Rosi i   | 572                      | 5.56             | 4,06 | 0.212 | 4.20                                  | 4.27                     | 3.16    | 6.301 <sup>c</sup>       |
| Mr i i   | 685                      | 6.35             | 5.43 | 0.352 | 4.30                                  | 42.38                    | 3.70    | 6.462 <sup>a</sup>       |
| Drenovci | 812                      | 6.83             | 6.17 | 0.235 | 4.20                                  | 23.78                    | 2.75    | 6.380 <sup>b</sup>       |
| Skakavci | 600                      | 6.51             | 5.54 | 0.460 | 6.80                                  | 36.45                    | 4.15    | 6.471 <sup>a</sup>       |
| Božeti i | 1033                     | 4.95             | 4.08 | 0.316 | 18.40                                 | 22.89                    | 4.11    | 6.176 <sup>e,f</sup>     |
| Debelja  | 1064                     | 6.04             | 4.69 | 0.307 | 14.90                                 | 53.21                    | 313     | 6.113 <sup>f,g</sup>     |
| Suvi Do  | 1328                     | 5.29             | 4.18 | 0.415 | 10.10                                 | 16.30                    | 4.21    | 6.279 <sup>c</sup>       |
| Šip e    | 1240                     | 5.39             | 4.45 | 0.447 | 9.60                                  | 33.72                    | 4.56    | 6.204 <sup>d</sup>       |

LSD (p < 0.05)

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test (p < 0.05)

The largest number of samples soil has medium and high humus, phosphorus and nitrogen content. The content of phosphorus in the studied soil generally is low, the exception is several soil samples that have high and very high content of this nutrient. The high content of organic matter accumulated in the surface of grassland soil is considered to result from the large quantity of plant residues reaching this level, combined with a slower rate of decomposition (Whitehead, 1970).

(5.778)

Preseka.

In our study the smallest number of microorganisms per one gram of absolutely dry soil (log of number) – 5.778 on location Preseka was found.

(6.505) Šume. -

Skakavci (6.471) Mr i i (6.462),

(Wardle and Giller, 1996).

(Moussa et al., 2007).

The largest number of total microflora – 6,505 in rizospheric soil of grassland on location Šume was recorded. A somewhat smaller number of microorganisms compared to this sample have samples from the location Skakavci – 6.471 and location Mr i i – 6.462, but statistically they do not differ among themselves. Further, according to Fishers test there are few homogeneous groups i.e. samples of soil among themselves does not show statistically significant differences the total number of microorganisms as well as some samples of the tested soil are statistically significantly different (Table 1). Our study shown that the presence of microorganisms in the tested soil samples is influenced by numerous factors and that each soil provides different conditions for the life of the microbe. The structure and functioning of microbial communities reflect interactions between a host of biotic and abiotic factors, among the most important of which is the quality of organic substrates available to it (Wardle and Giller, 1996). Microbial metabolism in soil is limited by the availability and types organic substrates, and the quantitative and qualitative differences in substrates supply between grasslands are responsible for the variation in microbial community (Moussa et al., 2007). Soil microorganisms perform a wide range essential services to the sustainable function of all ecosystems, they decompose organic matter, release nutrients into plant-available forms and degrade toxic residues; they also form symbiotic associations with plant roots, modifying soil physical properties and water regimes, enhancing the amount and efficiency of nutrient acquisition by the vegetation and enhancing plant health.

## CONCLUSIONS

- Results of this study shown that the presence of microorganisms in the tested soil samples is influenced by numerous factors and that each soil provides different conditions for the life of the microbe. This is the beginning of research so it is necessary to continue to obtain study complete information about
- presence of certain physiological and systematic groups of microorganisms in soil of grasslands of this part Republic of Serbia.

## ACKNOWLEDGEMENTS

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(*Vicia faba* L.)

1\* , 2 , 1 , 1 , 1 ,  
 1 , 1 , 1 ,  
 1 , 37251 , 11000 ,  
 2 1

**Variability of Some Agronomic Characteristics of Faba Bean (*Vicia faba* L.) Genotypes in Serbia**

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**SUMMARY**

*faba* L.),

(*Vicia*

In this research we analyzed some agronomic characteristics of the faba bean populations (*Vicia faba* L.) from the collected material of different parts of Serbia. There are a large number of preserved faba bean populations in Serbia that are still only used for farm use. Genotypes with fine-grained seed are selected for this investigation, because they are used more for animal feed. A small-plot trial was carried out in 2016, 2017 and 2018 at the experimental field of the Institute for Forage crops Kruševac. In this trial 10 populations were tested for plant height, number of branches, number of pods per plant, seed number per pod, and 1000 seed weight. Determination of variability among investigated genotypes allows us to make a basis material for future developing cultivars with desired

2016, 2017 2018

10

1000

ANOVA

*Vicia faba*,  
1000

(*Vicia faba* L.)

(Muehlbauer et al., 1997). Prolea (2014)

2012

4.5

75%

34%  
2013 . (FAO, 2014).

(Jensen et al., 2010; McVay et al., 1989).

features.

For most genotypes, statistically significant variability for the investigated properties was determined. The data were statistically analyzed using the ANOVA variance analysis method. Simple correlation coefficients between the investigated properties were calculated.

**Key words:** *Vicia faba*, seed, pod, 1000 seed weight, variability

## INTRODUCTION

Faba bean (*Vicia faba* L.) is an old plant primarily grown for its seeds consumed by humans. Faba bean is now widespread in Europe, North Africa, Central Asia, China, South America, USA, Canada and Australia.

Faba bean is a much appreciated food legume in the Middle-East, the Mediterranean region, China and Ethiopia (Muehlbauer et al., 1997). It is a multipurpose crop used for both food and fodder like hay, silage and straw (Prolea, 2014). The seeds of some varieties are an important livestock feed and some varieties are also grown for fodder. Production for food and feed was 4.5 million t worldwide in 2012.

The 5 top producing countries are China, Ethiopia, Australia, France and United Kingdom (more than 75% of world production). China alone produced 34% of all faba beans in 2013 (FAO, 2014).

However, faba bean utilisation and production has been declining in the last decades due to the increase of industrialized cereal-based systems, and the decrease of traditional cropping systems (Jensen et al., 2010; McVay et al., 1989). Faba bean is grown for green manure production or as a legume ley in cereal/legume rotations (McVicar et al.,

(McVicar et al., 2013; Muehlbauer et al., 1997).

(Eri et al., 2007).

(Trubnikova, 1988).

(McVicar et al., 2013).

(Smith et al., 2013; Jezierny et al., 2010; Blair, 2007).

(McVicar et al., 2013).

(Pettersson et al., 2007)

2013; Muehlbauer et al., 1997).

Faba bean can be a significant source of plant protein for human and animal nutrition. It is less used for human consumption, but in some countries (Egypt) is still the main source of protein for the poor population (Eri et al., 2007).

The importance of cultivation faba beans is multiple due to the composition of organic matter in harvest residues (underground and above ground). This allows nitrogen fixation, improvement of soil biological properties, and possibility of adopting nutrients from deeper layers of soil. Faba bean is not required for the quality of the soil, because it is ensured by the fixation of air the necessary amount of nitrogen for its growth. This culture is unfairly neglected, both in the diet of humans and animals. The grain of the faba bean is of high nutritional value, and the content of lysines is four times higher than the content in the most of bread cereals (Trubnikova, 1988).

Faba bean is used for the production of fodder, seeds and grains. Green fodder is used in mixtures with corn, sorghum or Sudan grass. When faba beans are intended for livestock feeding, small-seed varieties with low-tannin, low vicine-convicine and low-trypsin inhibitor contents are preferred (McVicar et al., 2013). Faba beans have been suggested as an alternative protein source to soybean for livestock in Europe (Smith et al., 2013; Jezierny et al., 2010; Blair, 2007). Faba bean plants can be used to make good quality silage (McVicar et al., 2013). In Sweden, it was used as a lignocellulosic biomass to produce bioethanol and biogas (Pettersson et al., 2007). In Serbia, little has been done to create domestic varieties of faba bean.

Also, it is not widespread in production. Local autochthonous populations of garden faba bean (for human

(  
)  
(Eri et al., 2007).

consumption) are mostly produced in farms. Foreign varieties are produced on small surfaces and have no economic significance (Eri et al., 2007).

Nowadays, when the need for quality foods grows, it is useful to return to some old cultures that can contribute to the production of healthy and quality food for people and animals. At the Institute of Forage Crops there is a collection of heterogeneous faba beans, collected in different parts of Serbia. Part of this collection was included in this research. For the purposes of further selection, genotypes with fine-grained seed and a similar vegetation period were selected.

## MATERIAL AND METHODS

A small-plot trial was carried out in 2016, 2017 and 2018 at the experimental field of the Institute for Forage crops Krusevac. In this trial 10 autochthonous populations were tested for plant height, number of branches, number of pods per plant, seed number per pod, and 1000 seed weight. Indigenous populations originate from different parts of Serbia. The largest number of populations originate from central Serbia (5), three populations are from Eastern Serbia, and two are from Southern Serbia.

Average monthly temperatures ( $^{\circ}\text{C}$ ) and sum of precipitations (mm) data recorded during study period are presented in Table 1.

1. T ( $^{\circ}\text{C}$ )  
P (mm)  
**Table 1. Average monthly temperatures T ( $^{\circ}\text{C}$ ) and monthly sum of precipitations P (mm) during vegetation period**

| /    |   | February | March | April | May   | June | July  | August | September |
|------|---|----------|-------|-------|-------|------|-------|--------|-----------|
| 2016 | T | 8.6      | 8.0   | 14.4  | 15.6  | 21.6 | 22.3  | 20.4   | 17.0      |
|      | P | 48.6     | 86.1  | 63.2  | 144.6 | 77.4 | 102.4 | 70.1   | 49.7      |
| 2017 | T | 4.1      | 10.2  | 11.6  | 16.8  | 22.4 | 23.9  | 23.6   | 18.0      |
|      | P | 24.0     | 44.7  | 61.9  | 77.7  | 51.4 | 15.7  | 41.0   | 16.5      |
| 2018 | T | 5.5      | 4.3   | 13.9  | 18.7  | 23.0 | 22.1  | 23.1   | 18.4      |
|      | P | 13.0     | 32.0  | 33.0  | 12.0  | 0.0  | 145.0 | 69.7   | 9.0       |

30  
20 cm

60 cm

10

1000

(ANOVA)

Fisher 0.01

:  $\geq 0.70$

; 0.30-0.69

;  $< 0.30$

0.0

(

).

STATISTICA.

The field trial was set up by random block system in three replications. For each genotype, 30 plants are planted in two rows with distance of 20cm in the rows and 60cm between rows. For features plant height, number of branches, number of pods per plant, and seed number per plant, all measurements were done on 10 plants. In the maturity phase of the pods, the seeds from all the plants on the parcel were mixed and a unique sample was created from which the weight of the 1000 seeds was determined. Determination of variability among investigated genotypes allows us to make a basis material for future developing cultivars with desired features. For most genotypes, statistically significant variability for the investigated properties was determined. The results were processed by the analysis of variance (ANOVA) by Fisher test at the 0.01 probability level. Simple correlation coefficients between the investigated properties were calculated. The evaluation of the linear correlation coefficients was carried out according to the following scale:  $\geq 0.70$  strong correlation; 0.30-0.69 medium correlation;  $< 0.30$  weak correlation; about 0.0 no linear correlation (does not exclude the existence of a nonlinear form of correlation). The data were statistically analyzed using the STATISTICA software.

## RESULTS AND DISCUSSION

Based on the results of the study (Table 2), there was a significant variation between the tested genotypes for all investigated traits at the level of  $p < 0.01$ . Within the years, there were less significant differences for all investigated features. The smallest height (36.44cm) had the KS44 genotype, and the highest height (85.89cm) achieved genotype SB09; both in the first year of research.

There was very significant variation in height between genotypes, while variance was not significant between the years

( 2)

$< 0.01$ .

(36.44cm)

KS44, (85.89 cm)

SB09.

( <0.01).

KN07 (2.71),  
KS44 (1.60).

NI16 (2.89 cm)  
KS44  
(1.56 cm)

11.22 (AL08  
) 20.78 (KS44  
).

KS44 (3.24),  
KS05 (2.24).

1000  
( 2).  
SB09  
654.33  
655.33

KS44 (447.67  
449.34 1000  
).

(p<0.01). Number of branches per plant varied significantly between investigated genotypes. The largest average number of branches was obtained in the genotype KN07 (2.71), and the lowest in the genotype KS44 (1.60). By year, the largest number of branches had a genotype NI16 (2.89cm) in the third year, and the smallest KS44 (1.56cm) in the second and third year of testing.

The number of pods per plant varied from 11.22 (AL08 in the first year) to 20.78 (KS44 in the first year). These two genotypes had the smallest and highest average value for this feature. For this feature, there was significant variation between the genotypes, while the variation was not significant between the examined years. The highest number of seeds per pod was obtained at KS44 (3.24), and the smallest in KS05 (2.24).

This also applies to the average values for this property. This feature was to a lesser extent varied between genotypes, and there no variation between years. 1000 seed weight varied very significantly between investigated genotypes (Table 2). The largest seed was obtained from genotype SB09 for both: the average number of seeds 654.33, and the highest number of seeds 655.33 in the first year of the investigation. The smallest seed was obtained from KS44 genotype (447.67 in the first year and 449.34 average weight of 1000 seeds for the examined years). For this trait, there was also no variation between the studied years, while the variability between genotypes is very significant.

2.

(cm),

1000

**Table 2. Plant height (cm), branch number per plant, pod number per plant, seed number per pod and 1000 seed weight for three years**

| Genotype | Year      | Plant height        | Branch number plant <sup>-1</sup> | Pod number plant <sup>-1</sup> | Seed number pod <sup>-1</sup> | 1000 seed weight      |
|----------|-----------|---------------------|-----------------------------------|--------------------------------|-------------------------------|-----------------------|
| SB01     | 1         | 46.11 <sup>k</sup>  | 2.22 <sup>bcd</sup>               | 12.56 <sup>ghij</sup>          | 2.73 <sup>b</sup>             | 528,67 <sup>i</sup>   |
|          | 2         | 46.56 <sup>k</sup>  | 2.67 <sup>ab</sup>                | 12.11 <sup>ghijk</sup>         | 2.63 <sup>bc</sup>            | 536,00 <sup>i</sup>   |
|          | 3         | 48.11 <sup>k</sup>  | 2.22 <sup>bcd</sup>               | 12.11 <sup>ghijk</sup>         | 2.57 <sup>cde</sup>           | 539,60 <sup>hi</sup>  |
|          | / Average | 46,93               | 2.37                              | 12.26                          | 2.64                          | 534.76                |
| JA32     | 1         | 44.67 <sup>k</sup>  | 2.78 <sup>ab</sup>                | 19.78 <sup>ab</sup>            | 2.38 <sup>fghi</sup>          | 454,00 <sup>j</sup>   |
|          | 2         | 45.22 <sup>k</sup>  | 2.67 <sup>ab</sup>                | 19.67 <sup>ab</sup>            | 2.36 <sup>fg hij</sup>        | 454,67 <sup>j</sup>   |
|          | 3         | 45.89 <sup>k</sup>  | 2.56 <sup>abc</sup>               | 19.67 <sup>ab</sup>            | 2.48 <sup>cdef</sup>          | 455,67 <sup>j</sup>   |
|          | / Average | 45,26               | 2.67                              | 19.71                          | 2.41                          | 454.78                |
| AL03     | 1         | 51.44 <sup>ij</sup> | 2.33 <sup>abc</sup>               | 14.00 <sup>ef</sup>            | 2.28 <sup>hij</sup>           | 557,33 <sup>g</sup>   |
|          | 2         | 56.67 <sup>h</sup>  | 2.44 <sup>abc</sup>               | 14.22 <sup>e</sup>             | 2.30 <sup>ghij</sup>          | 559,67 <sup>fg</sup>  |
|          | 3         | 54.00 <sup>hi</sup> | 2.44 <sup>abc</sup>               | 13.34 <sup>efg</sup>           | 2.34 <sup>fg hij</sup>        | 558,00 <sup>g</sup>   |
|          | / Average | 54,04               | 2.40                              | 13.85                          | 2.31                          | 558.33                |
| KS44     | 1         | 36.44 <sup>i</sup>  | 1.67 <sup>de</sup>                | 20.78 <sup>a</sup>             | 2.97 <sup>ab</sup>            | 447,67 <sup>j</sup>   |
|          | 2         | 37.56 <sup>i</sup>  | 1.56 <sup>e</sup>                 | 20.56 <sup>a</sup>             | 3.80 <sup>a</sup>             | 448,67 <sup>j</sup>   |
|          | 3         | 38.00 <sup>i</sup>  | 1.56 <sup>e</sup>                 | 20.33 <sup>a</sup>             | 3.01 <sup>a</sup>             | 451,67 <sup>j</sup>   |
|          | / Average | 37,33               | 1.60                              | 20.56                          | 3.24                          | 449.34                |
| KS05     | 1         | 64.56 <sup>g</sup>  | 2.74 <sup>ab</sup>                | 19.56 <sup>abc</sup>           | 2.28 <sup>hij</sup>           | 584,33 <sup>g</sup>   |
|          | 2         | 66.22 <sup>g</sup>  | 2.67 <sup>ab</sup>                | 19.67 <sup>ab</sup>            | 2.23 <sup>ijk</sup>           | 588,00 <sup>d</sup>   |
|          | 3         | 66.24 <sup>g</sup>  | 2.78 <sup>ab</sup>                | 18.89 <sup>bcd</sup>           | 2.22 <sup>ijk</sup>           | 586,00 <sup>d</sup>   |
|          | / Average | 65,67               | 2.73                              | 19.37                          | 2.24                          | 586.11                |
| NI16     | 1         | 63.78 <sup>g</sup>  | 2.67 <sup>ab</sup>                | 17.67 <sup>d</sup>             | 2.28 <sup>hij</sup>           | 567,33 <sup>ef</sup>  |
|          | 2         | 64.56 <sup>g</sup>  | 2.44 <sup>abc</sup>               | 18.11 <sup>d</sup>             | 2.34 <sup>fg hij</sup>        | 570,00 <sup>e</sup>   |
|          | 3         | 63.44 <sup>g</sup>  | 2.89 <sup>a</sup>                 | 18.33 <sup>cd</sup>            | 2.22 <sup>ijk</sup>           | 563,33 <sup>efg</sup> |
|          | / Average | 63,93               | 2.67                              | 18.04                          | 2.28                          | 566.89                |
| KN07     | 1         | 75.78 <sup>de</sup> | 2.73 <sup>ab</sup>                | 12.44 <sup>ghijk</sup>         | 2.23 <sup>ijk</sup>           | 628,33 <sup>c</sup>   |
|          | 2         | 77.11 <sup>cd</sup> | 2.70 <sup>ab</sup>                | 13.00 <sup>efgh</sup>          | 2.31 <sup>ghij</sup>          | 629,67 <sup>c</sup>   |
|          | 3         | 77.56 <sup>cd</sup> | 2.69 <sup>ab</sup>                | 12.56 <sup>ghij</sup>          | 2.30 <sup>ghij</sup>          | 633,33 <sup>c</sup>   |
|          | / Average | 76,82               | 2.71                              | 12.67                          | 2.28                          | 630.44                |
| AL08     | 1         | 78.67 <sup>cd</sup> | 2.67 <sup>ab</sup>                | 11.22 <sup>k</sup>             | 2.60 <sup>bc</sup>            | 645,00 <sup>b</sup>   |
|          | 2         | 80.33 <sup>c</sup>  | 2.44 <sup>abc</sup>               | 11.44 <sup>jk</sup>            | 2.50 <sup>cdef</sup>          | 650,67 <sup>ab</sup>  |
|          | 3         | 79.78 <sup>c</sup>  | 2.56 <sup>abc</sup>               | 11.78 <sup>hijk</sup>          | 2.40 <sup>fg hi</sup>         | 649,33 <sup>ab</sup>  |
|          | / Average | 79,59               | 2.56                              | 11.48                          | 2.50                          | 648.33                |
| SB09     | 1         | 85.89 <sup>a</sup>  | 2.00 <sup>cde</sup>               | 13.11 <sup>efg</sup>           | 2.42 <sup>efgh</sup>          | 655,33 <sup>a</sup>   |
|          | 2         | 83.44 <sup>ab</sup> | 2.02 <sup>cde</sup>               | 12.89 <sup>fg hi</sup>         | 2.41 <sup>fg hi</sup>         | 653,67 <sup>a</sup>   |
|          | 3         | 85.56 <sup>a</sup>  | 2.10 <sup>cde</sup>               | 11.67 <sup>ijk</sup>           | 2.34 <sup>fg hij</sup>        | 654,00 <sup>a</sup>   |
|          | / Average | 84,96               | 2.04                              | 12.56                          | 2.39                          | 654.33                |
| KN10     | 1         | 72.44 <sup>ef</sup> | 2.78 <sup>ab</sup>                | 13.33 <sup>efg</sup>           | 2.38 <sup>fg hi</sup>         | 591,33 <sup>d</sup>   |
|          | 2         | 70.11 <sup>f</sup>  | 2.67 <sup>ab</sup>                | 13.22 <sup>efg</sup>           | 2.44 <sup>defg</sup>          | 589,67 <sup>d</sup>   |
|          | 3         | 70.56 <sup>f</sup>  | 2.44 <sup>abc</sup>               | 13.22 <sup>efg</sup>           | 2.43 <sup>defg</sup>          | 591,00 <sup>d</sup>   |
|          | / Average | 71,04               | 2.63                              | 13.26                          | 2.42                          | 590.67                |

Note: The same letters in the superscript indicate homology, or the absence of statistically significant differences according to Fischer LSD test ( $p < 0.01$ )

LSD (  $< 0.01$ ).

( 0,27).

- | The correlation (Table 3) between
- | the height of the plant and the number of
- | branches per plant was positive and
- | weak (0.27). Plant height influenced on
- | pod number per plant on negative and

(-0.67),  
(-0.60).  
1000 (0.90).

medium level (-0.67), also on seed number per plant (-0.60).

In this study, plant height influenced very significantly on 1000 seed weight (0.90).

3.

**Table 3. Coefficient of linear correlations between investigated features**

|                                   | Plant height | Branch number plant <sup>-1</sup> | Pod number plant <sup>-1</sup> | Seed number pod <sup>-1</sup> | 1000 seed weight |
|-----------------------------------|--------------|-----------------------------------|--------------------------------|-------------------------------|------------------|
| Plant height                      | *            | 0.27                              | -0.67*                         | -0.60*                        | 0.90**           |
| Branch number plant <sup>-1</sup> |              | *                                 | -0.16                          | -0.82**                       | 0.22             |
| Pod number plant <sup>-1</sup>    |              |                                   | *                              | 0.26                          | -0.82**          |
| Seed number pod <sup>-1</sup>     |              |                                   |                                | *                             | -0.52*           |
| 1000 seed weight                  |              |                                   |                                |                               | *                |

(-0.82).  
(-0.16)  
1000 (0.22).  
(0.26)  
1000 (-0.82).  
(-0.52).  
1000

Number of branches per plant had negative and very significantly correlation with seed number per pod (-0.82). We noticed that in dry years certain number of branches are without pods and, also, if they have pods many of them are without seeds. There was weak negative correlation with pod number per plant (-0.16), and weak positive correlation with 1000 seed weight (0.22).

Pod number per plant had weak positive correlation with seed number per pod (0.26) and strong negative correlation with 1000 seed weight (-0.82). Seed number per pod had negative medium correlation with 1000 seed weight (-0.52).

**CONCLUSIONS**

Investigated features did not varied by year, but significantly varied by genotype. The most variability obtained for the plant height and 1000 seeds



|      |          |          |      |  |
|------|----------|----------|------|--|
| 1000 | .        | .        | -    | weight. A little less variation was obtained for the number of branches per plant, the number of seeds per pod and the pod number per plant.   |
|      |          |          | -    |  |
|      |          |          | -    | The largest positive correlation was between plant height and 1000 seed weight (0.90). The largest negative correlation was obtained between brunch number per plant and seed number per pod (-0.82), and also, between pod number per plant and 1000 seed weight (-0.82). |
|      | (0.90).  | -        | 1000 |  |
|      |          |          | -    |  |
|      | (-0.82), |          | -    |  |
| 1000 |          | (-0.82). | -    | In Serbia there are more populations of large seeds used for human consumption. Therefore genotypes JA32 and KS44 deserve special attention in further selection, because of their small seed.   |
|      |          |          | -    |  |
| KS44 |          |          | JA32 |  |
|      |          |          | -    |  |
|      |          |          | ,    |  |

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(TR-31057, 2011).

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**(*Medicago sativa* L.)**

**4**

<sup>1\*</sup>, e <sup>1</sup>, <sup>2</sup>

<sup>1</sup> "7007",  
<sup>2</sup> "1407",

## **Study of the Effect of Biostimulants Application on Green Mass and Dry Matter Yield in Alfalfa (*Medicago sativa* L.) Prista 4 Variety**

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### **SUMMARY**

The study was carried out without irrigation, at the experimental field of the Institute of Agriculture and Seed Science "Obraztsov chiflik" - Rousse, from 2016 to 2018. The aim of the present study was to investigate the effect of L<sub>1</sub> and L<sub>5</sub> biostimulants application, elaborations of the Institute of Cryobiology and Food Technology - Sofia, on the main structural elements of green mass and dry matter yield and forage productivity of Prista 4 alfalfa variety. The field trial included variants with L<sub>1</sub> and L<sub>5</sub> biostimulants application and a control (untreated variant). The grass stands were sprayed twice in each regrowth - at plant height of 10-15 cm and in bud stage, at 300 ml da<sup>-1</sup> application rate per treatment. The foliar application of L<sub>1</sub> and L<sub>5</sub>

|                |                |                |  |
|----------------|----------------|----------------|--|
|                | L <sub>1</sub> | L <sub>5</sub> | biostimulants had a different effect on the morphological traits studied and on the forage productivity of Prista 4 alfalfa variety, in response to the changes in environmental conditions during the regrowths formation.  |
| 4,             |                |                |  |
|                | L <sub>1</sub> | L <sub>5</sub> | The treatment with L <sub>1</sub> and L <sub>5</sub> biostimulants stimulated the development of considerably higher plants. L <sub>5</sub> product contributed to significantly increase the potential for stem formation and the development of grass stands with better density. It had a clearly expressed positive effect on the forage productivity. |
| L <sub>5</sub> |                |                |  |
|                |                | 4,43%          | The green mass and dry matter yields from the treated with L <sub>5</sub> grass stands were higher by 4,43% and 7,37%, respectively than these for the control.  |
|                |                | 7,37%          |  |
|                |                | L <sub>5</sub> | The foliar application of L <sub>5</sub> gives promising results and reason to be used as a corrective component in technology of alfalfa growing for forage.  |
|                |                |                | <b>Key words:</b> alfalfa, biostimulants, yield, dry matter  |

## INTRODUCTION

The growing chemicals application in agricultural sector in recent years considerably increased the chemical burden and has led to a number of negative effects on the natural ecosystems. Taking into consideration the environmental negative effect of chemical products, It is clear that the need for a new concept in agriculture based on reducing the use of chemical products is urgent (Fernández et al., 2013).

One of the possible ways for a sustainable agriculture system is the apply of nutrients as foliar sprays to contribute for increasing plants biological productivity while at the same time reduce environmental pollution (Kannan et al., 2010, Dong et al., 2005).

The foliar fertilization has been the subject of many field researches and has

(Fernández et al., 2013).

(Kannan et al., 2010, Dong et al., 2005).

|   |   |
|---|---|
| <p>(Younus Wani et al., 2017).</p>  | <p>become widely adopted as a standard practice for many crops in a lot of countries. It is theoretically more environmentally friendly, since nutrients can be directly delivered to plant tissues during critical stages of plant growth and development (Younus Wani et al., 2017).</p>  |
| <p>(Georgieva and Nikolova, 2010; Kertikov et al., 2016; Bozhanska et al., 2017 ; El-Refaey et al., 2017; Vasileva et al., 2017; Ivanova-Kovacheva et al., 2018).</p> | <p>In the recent years, the aim of a number of studies has been to investigate the impact of organic products on the crops resilience to environmental stress and increased yield and quality of plant production (Georgieva and Nikolova, 2010; Kertikov et al., 2016; Bozhanska et al., 2017 ; El-Refaey et al., 2017; Vasileva et al., 2017; Ivanova-Kovacheva et al., 2018).</p>  |
| <p>(Mihova et al., 2017; Yakimov and Ivanov, 2017; Enchev et al., 2018).</p>  | <p>The use of foliar fertilizers, growth regulators and biostimulants is one of the perspective guidelines for stimulating the biological potential of plants because they were determined as an supplementary and corrective component of the overall mineral nutrition system and an alternative to chemical fertilizers for more environmentally friendly yields increase in the sustainable agriculture (Mihova et al., 2017; Yakimov and Ivanov, 2017; Enchev et al., 2018).</p> |
| <p>(Du Jardin, 2015; Yakhin, 2017).</p>   | <p>The biostimulants are products derived from organic material containing bioactive substances and/or microorganisms able to stimulate several physiological and biochemical processes that lead to improved uptake and nutrients use efficiency and enhanced tolerance to unfavourable environmental conditions (Du Jardin, 2015; Yakhin, 2017).</p>  |
| <p>(Toscano et al., 2018).</p>  | <p>They ability to increasing plant vigor lead to a reduction in the amount of pesticides and fertilizers used and consequently to an indirect positive impact on the environment (Toscano et al., 2018).</p> <p>The impact of foliar application</p>   |

(Kannan, 2010; Fernández et al., 2013; Bozhanska et al., 2017b).  
(*Medicago sativa* L.)

*Rhizobium meliloti*,  
(Kertikova, 2008).

(Hall et al., 2002; Sevov, 2011; Terzi et al., 2012; Madani et al., 2014).

1,  
(Sevov et al., 2007).  
Total  
Care

fertilizers, growth regulators and biostimulants varies not only between species and varieties but also depends upon phenology and physiological plants status and environment in which they growing (Kannan, 2010; Fernández et al., 2013; Bozhanska et al., 2017b).

Alfalfa (*Medicago sativa* L.) is one of the most important legume forage worldwide as a major source of protein, high productivity, excellent forage quality and adaptability to different climatic conditions. It can be used directly for grazing, hay or conserved as silage. The alfalfa is a reliable forage species with a significant importance to the livestock sector. Environmentally friendly nature of alfalfa cultivation is worth emphasizing for sustainable agricultural production due to the symbiosis with bacteria *Rhizobium meliloti*, converting atmospheric nitrogen to make it available to the plants (Kertikova, 2008).

In order to achieve optimal forage yield the alfalfa requires relatively large amounts of nutrients. In this sense, a number of scientific studies have been carried out, investigating the effect of the application of different agrotechnical practices – optimal density; intensity of utilization; balanced fertilization; application of foliar fertilizer, growth regulators and biostimulants (Hall et al., 2002; Sevov, 2011; Terzi et al., 2012; Madani et al., 2014).

RENI growth regulator applied independently or in various combinations in alfalfa stands of Mnogolistna 1 variety favorably affects the extent of multifoliolate expression, increase forage yield and amino acid content in biomass (Sevov et al., 2007).

The foliar application of organic fertilizers Aminobest and Total Care contribute to development of higher grasses and higher

(Marinova and Ivanova, 2018; Marinova et al., 2019).

L<sub>1</sub> L<sub>5</sub>,

4.

2016-2018 ,

(43°48' N, 26°03' W  
152 m).

4,

2,17%  
5,84-5,94.

L<sub>1</sub> L<sub>5</sub>.

L<sub>1</sub> L<sub>5</sub>

( 2,03%  
0-40 cm)

2

10-15 cm  
300 ml da<sup>-1</sup>

21

2016 .

5 m<sup>2</sup>,  
kg da<sup>-1</sup>  
cm.

2,5  
12,5

10

(2016 .-

yields in alfalfa grown for forage (Marinova and Ivanova, 2018; Marinova et al., 2019).

The aim of the present study was to investigate the effect of L<sub>1</sub> and L<sub>5</sub> biostimulants, an elaboration of the ICFT - Sofia on the main structural elements of green and dry mass yield and the forage productivity of alfalfa Prista 4.

## MATERIAL AND METHODS

The experimental work was carried out from 2016 to 2018, in the Experimental field of the Institute of Agriculture and Seed Science "Obraztsov Chiflik" - Rousse located in the Northern climatic region of the Danube Plain (43°48'N, 26°03'W and altitude 152 m).

The investigation was conducted with Prista 4 variety, without irrigation, on a soil type strongly leached chernozem with low humus content (from 2,03% to 2,17% average for the 0-40 cm layer) and pH 5,84-5,94. The field trial included a control (untreated variant) and two variants with L<sub>1</sub> and L<sub>5</sub> biostimulants application.

L<sub>1</sub> and L<sub>5</sub> products have been elaborated based on solid-liquid extraction of a vermycompost with 2 alkaline extragents at the Laboratory of Biologically Active Substances to the Institute of Cryobiology and Food Technologies - Sofia. The grass stands were sprayed twice in each regrowth - at a plant height of 10-15 cm and in a but stage at 300 ml da<sup>-1</sup> application rate per treatment for the both biostimulants.

The alfalfa was sown on 21 March 2016 in a randomized block design in four replications, at a plot size of 5 m<sup>2</sup>. The sowing rate was 2,5 kg da<sup>-1</sup> and inter-row spacing 12,5 cm. The green mass cutting was carried out at early flowering stage. A total 10 cuttings were made (2016 - two cuts, 2017 and 2018 - four cuts) during

2017 . 2018 . -

:

cm,

3

m<sup>2</sup>,

(50 50 cm)

( kg da<sup>-1</sup>)

( %),

(200 g).

105°

( kg da<sup>-1</sup>).

(ANOVA).

STATGRAPHICS PLUS.

the study period.

An analysis was made of the green mass yield, dry matter yield, dry matter content in green mass and the main yield components plants height and grass stand density.

The grass stand height and grass stand density were determined before each cut. The grass stand height in cm was recorded as the majority of normally developed stems were measured from the surface of the soil to the top. It was done in 3 places in each replication for each variant. The grass stand density trait, expressed by stem number (SN) per m<sup>2</sup> in each harvesting plot for the variants by sampling plot (50 cm x 50 cm) was ascertained.

The yields (in kg da<sup>-1</sup>) were determined at each cut by weighing the green mass of each harvesting plot for the variants. For dry matter content determination (%) 200 g fresh vegetative mass was sampled before each cutting for each variant. The samples were dried to a constant weight in a drying chamber at 105°C and weighed. Green mass yield and dry matter content data were used for dry matter yield (in kg da<sup>-1</sup>) to be counted.

The experimental data were analysed by the One-way analysis of variance (ANOVA) method. The STATGRAPHICS PLUS product was used.

## RESULTS AND DISCUSSION

The grass stand height is one of the main structural components of forage productivity, which determines to a large extent the green mass and dry matter yields. The natural plants height is a quantitative variety trait and degree of phenotypic expression of the controlling it genes is changes under the influence of different environmental conditions.



L<sub>5</sub>

4,

L<sub>1</sub>

During the study period, there were found differences in the effect of L<sub>1</sub> and L<sub>5</sub> biostimulants application on the grass stand height of Prista 4 alfalfa variety, by regrowths and years.

Data showed the both biostimulants had a positive influence on the plants height in first regrowth in the year of alfalfa stand establishment (Table 1).

1.

4

**Table 1. Grass stands height in Prista 4 alfalfa variety treated by biostimulants**

| Variants       | m / Real plant height, m |        |         |          |          |                                   |
|----------------|--------------------------|--------|---------|----------|----------|-----------------------------------|
|                | 2016                     |        |         |          |          |                                   |
|                | I cut                    | II cut |         |          | Mean     | (+/-), cm<br>Difference (+/-), cm |
| / Control      | 30,00                    | 60,12  |         |          | 45,06 c  |                                   |
| L <sub>1</sub> | 39,75                    | 65,04  |         |          | 52,40 b  | + 7,34                            |
| L <sub>5</sub> | 46,25                    | 65,00  |         |          | 55,63 a  | + 10,52                           |
|                |                          |        |         | LSD 5%   | 2,88     |                                   |
|                |                          |        |         | LSD 1%   | 4,13     |                                   |
|                |                          |        |         | LSD 0.1% | 6,07     |                                   |
| 2017           |                          |        |         |          |          |                                   |
|                | I cut                    | II cut | III cut | IV cut   | Mean     | (+/-), cm<br>Difference (+/-), cm |
| / Control      | 48                       | 60     | 59      | 31       | 49 b     |                                   |
| L <sub>1</sub> | 66                       | 70     | 71      | 30       | 59 a     | + 10                              |
| L <sub>5</sub> | 71                       | 69     | 62      | 25       | 57 a     | + 8                               |
|                |                          |        |         |          | LSD 5%   | 3,11                              |
|                |                          |        |         |          | LSD 1%   | 4,47                              |
|                |                          |        |         |          | LSD 0.1% | 6,57                              |
| 2018           |                          |        |         |          |          |                                   |
|                | I cut                    | II cut | III cut | IV cut   | Mean     | (+/-), cm<br>Difference (+/-), cm |
| / Control      | 92,75                    | 75,75  | 83,75   | 26,50    | 69,69 a  |                                   |
| L <sub>1</sub> | 90,00                    | 72,25  | 80,75   | 23,75    | 66,69 a  | - 3                               |
| L <sub>5</sub> | 88,00                    | 73,75  | 85,50   | 23,50    | 67,69 a  | - 2                               |
|                |                          |        |         |          | LSD 5%   | 4,36                              |
|                |                          |        |         |          | LSD 1%   | 6,70                              |
|                |                          |        |         |          | LSD 0.1% | 9,33                              |

C

P 0.05

Values followed by the same letter are not significantly different at P 0.05

(39,75 m),

L<sub>5</sub> (46,25 m) L<sub>1</sub>

L<sub>5</sub>,

30 m.

L<sub>1</sub> L<sub>5</sub>

The reported values for the sprayed variants with L<sub>5</sub> (46,25 cm) and L<sub>1</sub> (39,75 cm) showed pronounced stronger stimulating effect of L<sub>5</sub>. The plants height for the untreated control was 30 cm. In a second regrowth, the positive impact of the products studied was kept. It were also found the plants treated with L<sub>1</sub> and L<sub>5</sub> were equally high. Mean for the first year the grass stands of

10,52 cm -  
 L<sub>1</sub> (7,34 cm).  
 L<sub>5</sub>  
 L<sub>1</sub>.

L<sub>5</sub> L<sub>1</sub>  
 23 m 17 m.

L<sub>1</sub> L<sub>5</sub>

L<sub>1</sub> L<sub>5</sub>

L<sub>5</sub>

m<sup>2</sup>, 2,

the variant including L<sub>5</sub> application was with 10,52 cm higher than those of the control. A considerable exceeding was reported for L<sub>1</sub> (7,34 cm), too. Data also showed season significantly stronger stimulating effect of L<sub>5</sub> biostimulant on the phenotypic expression of the trait compared to L<sub>1</sub> during first growing.

The tendency outlined for positive impact of the biostimulants on the indicator was kept in the second year. The reported values indicated the studied products contributed to the higher grass stands in all regrowths, except the fourth one.

The strongest positive effect of biostimulants was found in the first cut. The excesses for L<sub>5</sub> and L<sub>1</sub> versus the control were 23 cm and 17 cm, respectively. There was established decrease of the stimulating impact of L<sub>5</sub> in the following regrowths while effect of L<sub>1</sub> was kept. Mean values for trait in the second productive year indicated the grass stands responded positively to the foliar treatment with L<sub>1</sub> and L<sub>5</sub>, developing significantly higher plants than they of the control. The results showed the biostimulants studied not contributed to higher grass stands in all regrowths during the third alfalfa growing season. Slight stimulating effect was observed for L<sub>5</sub> in a third cuts. The reported differences between mean values for the variants were not statistically significant for the year.

Data of grass stand density, expressed by stems number per m<sup>2</sup>, present in Table 2, showed a different by power and character effect of the biostimulants studied in regrowths and years.

**Table 2. Grass stands density in Prista 4 alfalfa variety treated by biostimulants**

| Variants       | m <sup>2</sup> / Stems number per m <sup>2</sup> |   |          |        |                             |        |          |        |                             |
|----------------|--|---|----------|--------|-----------------------------|--------|----------|--------|-----------------------------|
|                | 2016   |   |          |        |                             |        |          |        |                             |
|                | / I cut  | I | / II cut | Mean   | (+/),<br>Diference (+/-), N |        |          |        |                             |
| /Control       | 164  |   | 195,62   | 179,81 | ab                          |        |          |        |                             |
| L <sub>1</sub> | 154  |   | 180,65   | 167,25 | b                           | -25,81 |          |        |                             |
| L <sub>5</sub> | 140  |   | 244,52   | 192,26 | a                           | +12,45 |          |        |                             |
|                |  |   | LSD 5%   | 13,65  |                             |        |          |        |                             |
|                |  |   | LSD 1%   | 19,61  |                             |        |          |        |                             |
|                |  |   | LSD 0.1% | 28,00  |                             |        |          |        |                             |
| 2017           |  |   |          |        |                             |        |          |        |                             |
|                | / I cut  | I | / II cut | II     | /III cut                    | V      | /IV cut  | Mean   | (+/),<br>Diference (+/-), N |
| /Control       | 534  |   | 489      |        | 510                         |        | 419      | 488    | a                           |
| L <sub>1</sub> | 448  |   | 441      |        | 454                         |        | 397      | 435    | b                           |
| L <sub>5</sub> | 506  |   | 491      |        | 476                         |        | 431      | 476    | a                           |
|                |  |   |          |        |                             |        | LSD 5%   | 27,06  |                             |
|                |  |   |          |        |                             |        | LSD 1%   | 38,88  |                             |
|                |  |   |          |        |                             |        | LSD 0.1% | 57,19  |                             |
| 2018           |  |   |          |        |                             |        |          |        |                             |
|                | / I cut  | I | / II cut | II     | /III cut                    | V      | /IV cut  | Mean   | (+/),<br>Diference (+/-), N |
| /Control       | 434  |   | 412      |        | 391                         |        | 290      | 381,75 |                             |
| L <sub>1</sub> | 507  |   | 489      |        | 420                         |        | 397      | 453,25 | +71,5                       |
| L <sub>5</sub> | 492  |   | 441      |        | 400                         |        | 358      | 422,75 | b                           |
|                |  |   |          |        |                             |        | LSD 5%   | 25,12  |                             |
|                |  |   |          |        |                             |        | LSD 1%   | 35,94  |                             |
|                |  |   |          |        |                             |        | LSD 0.1% | 52,88  |                             |

C

P 0.05

Values followed by the same letter are not significantly different at

P 0.05

L<sub>5</sub> (12,45 /m<sup>2</sup>)

L<sub>1</sub>

In the first cut in the first growing season, the alfalfa stands were not reacted to the foliar treatment with biostimulants. It was determined a significant stimulating impact of L<sub>5</sub> on the potential for stems formation in the second cut. The mean trait values indicated that the grass stands of Prista 4 variety sprayed with L<sub>5</sub> exhibited the high st productivity of stems per unit area during the first productive year. The excess of 12,45 SN per m<sup>2</sup> compared to the untreated stand was statistically proven. In the second year, in all regrowths the foliar applicaation of L<sub>1</sub> biostimulant not influenced on the extent of the trait phenotypic exspression. The treatment with L<sub>5</sub> had a slight positive impact on trait in the last cut. During the last year in first regrowth of study the reported values ranged from 507 stems number per unit area (L<sub>1</sub>) to 434 SN

( ) . 507 . (L<sub>1</sub>) 434 .

489 ./m<sup>2</sup> 441 ./m<sup>2</sup>, L<sub>1</sub> L<sub>5</sub> 77 29

- , L<sub>1</sub> L<sub>5</sub>

- .

L<sub>1</sub> L<sub>5</sub> , L<sub>1</sub>

, L<sub>5</sub>.

,

kg da<sup>-1</sup>, 600

575 kg da<sup>-1</sup> 535 kg da<sup>-1</sup> ( L<sub>1</sub>, 3).

(control). In the second one the biostimulants application again contributed to increase the productivity of stems.

The sprayed grass stands with L<sub>1</sub> and L<sub>5</sub> formed 489 SN/m<sup>2</sup> 441 SN/m<sup>2</sup>, respectively and which was 77 and 29 stems above the untreated variant. The tendency for stimulating effect of the products studied was kept in the next regrowths. The highest positive influence of L<sub>1</sub> and L<sub>5</sub> was observed in the fourth regrowth, when the phenotypic expression of the genes controlling stem formation was the lowest. The statistical analysis results for the third vegetation indicated significant differences between the control and the treated variants which were in favour of L<sub>1</sub> and L<sub>5</sub> biostimulants. It was found L<sub>1</sub> had greater positive impact on the indicator than L<sub>5</sub>.

The alfalfa forage productivity is the result of the complex interaction between the variety genetic composition and the influence of all environmental factors.

Data showed the L<sub>5</sub> biostimulant application in the first regrowth during the first year of study resulted in 600 kg da<sup>-1</sup> green mass yield, at reported values for untreated variant and L<sub>1</sub> 575 kg da<sup>-1</sup> and 535 kg da<sup>-1</sup>, respectively (Table 3).

**Table 3. Green mass yield in Prista 4 alfalfa variety at biostimulants treatment**

| Variants       | , kg da <sup>-1</sup> / Green mass yield, kg da <sup>-1</sup> |   |                     |         |           |        |          |         |   |
|----------------|---|---|---------------------|---------|-----------|--------|----------|---------|---|
|                | 2016  |   |                     |         |           |        |          |         |   |
|                | / I cut   | I | / II cut            | / Total | %         |        |          |         |   |
| / Control      | 575,00  |   | 632,50              | 1207,50 | b         |        |          |         |   |
| L <sub>1</sub> | 535,00  |   | 666,40              | 1201,40 | b         | 99,49  |          |         |   |
| L <sub>5</sub> | 600,00  |   | 687,75              | 1287,75 | a         | 106,65 |          |         |   |
|                |   |   | LSD <sub>5%</sub>   | 67,90   |           |        |          |         |   |
|                |   |   | LSD <sub>1%</sub>   | 97,55   |           |        |          |         |   |
|                |   |   | LSD <sub>0.1%</sub> | 143,51  |           |        |          |         |   |
| 2017           |   |   |                     |         |           |        |          |         |   |
|                | / I cut   | I | / II cut            | II      | / III cut | V      | / IV cut | / Total | % |
| / Control      | 2995  |   | 2505                |         | 2940      |        | 810      | 9250    | b |
| L <sub>1</sub> | 3195  |   | 2140                |         | 3330      |        | 890      | 9555    | b |
| L <sub>5</sub> | 3365  |   | 3090                |         | 3400      |        | 845      | 10700   | a |
|                |   |   | LSD <sub>5%</sub>   |         |           |        |          | 384,97  |   |
|                |   |   | LSD <sub>1%</sub>   |         |           |        |          | 553,01  |   |
|                |   |   | LSD <sub>0.1%</sub> |         |           |        |          | 813,62  |   |
| 2018           |   |   |                     |         |           |        |          |         |   |
|                | / I cut   | I | / II cut            | II      | / III cut | V      | / IV cut | / Total | % |
| / Control      | 3580  |   | 3395                |         | 2980      |        | 250      | 10205   | a |
| L <sub>1</sub> | 3475  |   | 2810                |         | 2370      |        | 210      | 8865    | c |
| L <sub>5</sub> | 3350  |   | 3275                |         | 2730      |        | 235      | 9590    | b |
|                |   |   | LSD <sub>5%</sub>   |         |           |        |          | 331,87  |   |
|                |   |   | LSD <sub>1%</sub>   |         |           |        |          | 476,77  |   |
|                |   |   | LSD <sub>0.1%</sub> |         |           |        |          | 701,38  |   |

C P 0.05  
Values followed by the same letter are not significantly different at P 0.05

2017 . L<sub>5</sub> .  
L<sub>5</sub> L<sub>1</sub> .  
a ,  
L<sub>5</sub> - .  
L<sub>1</sub> , L<sub>5</sub>  
L<sub>5</sub> L<sub>1</sub> ,  
3330 kg da<sup>-1</sup> ,  
2940 kg da<sup>-1</sup> .  
L<sub>1</sub>  
- L<sub>5</sub>

In a second cutting the grass stands sprayed with the both products were more productive. The values for the total annual fresh forage yield showed significant stimulating effect for L<sub>5</sub>. There were observed differences in the power and character of impact of L<sub>5</sub> and L<sub>1</sub> biostimulants by regrowths in 2017. In the first cut, both products contributed to increase the productivity of fresh biomass but the positive effect of L<sub>5</sub> was more pronounced. The application of L<sub>1</sub> in the second regrowth not resulted in higher yield while L<sub>5</sub> again exhibited strong stimulating effect. In third cut the grass stands sprayed with the both biostimulants had an equal phenotypic expression of the trait. At L<sub>5</sub> and L<sub>1</sub> application were obtained green mass yield 3400 kg da<sup>-1</sup> and 3330 kg da<sup>-1</sup>, respectively at 2940 kg da<sup>-1</sup> for untreated variant. The reported differences between the variants showed that L<sub>1</sub> contributed to relatively higher dry mass yield than L<sub>5</sub> in

the last regrowth. The statistical analysis results confirmed the tendency for a powerful positive impact of L<sub>5</sub> on the productive potential of Prista 4 alfalfa variety.

4.

Data for the total annual yield showed that, unlike the first and second alfalfa growing season, treatment with the both biostimulants not contributed to higher green mass yield during the last year.

From the results for the dry matter content in the green mass, that determine dry matter yield (hay) it was established the indicator was not influenced of the foliar treatment during the first alfalfa growing season (Table 4). In 2017 more significant variability of the dry matter content between variants was observed.

Recorded values by regrowths and the mean values for the second growing season showed that the biostimulants application not contributed to increase the dry matter content in the fresh mass.

In the last year of the study, the grass stands responded positively to treatment with the products studied in the first cut, with reported values for L<sub>1</sub> and L<sub>5</sub> 28% and 27%, respectively, at 24% for the control. The positive impact of the biostimulants was kept in the next regrowths. A stimulating effect was established for L<sub>5</sub> (29%) in the third cut and for L<sub>1</sub> (34%) in fourth one. The mean values showed that in the third productive year the foliar application of the biostimulants was contributed to increase of the dry matter content in the fresh mass.

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4.  
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**Table 4. Dry matter content and dry matter yield in Prista 4 alfalfa variety at biostimulants treatment**

| Variants       | , % / Dry matter content,%                                    |            |              |            |           |                       |       |  |
|----------------|---|------------|--------------|------------|-----------|-----------------------|-------|--|
|                | 2016  |            |              |            |           |                       |       |  |
|                | / I cut   |            | I / II cut   |            | Mean      | (+/-) Diference (+/-) |       |  |
| /Control       | 30  |            | 28           |            | 29,0      |                       |       |  |
| L <sub>1</sub> | 30  |            | 28           |            | 29,0      | 0                     |       |  |
| L <sub>5</sub> | 30  |            | 29           |            | 29,5      | +0,50                 |       |  |
| Variants       | 2017  |            |              |            |           |                       |       |  |
|                | / I cut   | I / II cut | II / III cut | V / IV cut | Mean      | (+/-) Diference (+/-) |       |  |
|                | /Control  | 25         | 23           | 30         | 27        | 26,25                 |       |  |
| L <sub>1</sub> | 18  | 23         | 29           | 28         | 24,50     | -1,75                 |       |  |
| L <sub>5</sub> | 22  | 24         | 28           | 28         | 25,50     | -0,75                 |       |  |
| Variants       | 2018  |            |              |            |           |                       |       |  |
|                | /Control  | 24         | 26           | 26         | 31        | 26,75                 |       |  |
|                | L <sub>1</sub>  | 28         | 29           | 24         | 34        | 28,75                 | +2,00 |  |
| L <sub>5</sub> | 27  | 29         | 29           | 32         | 29,25     | +2,50                 |       |  |
| Variants       | , kg da <sup>-1</sup> / Dry matter yield, kg da <sup>-1</sup> |            |              |            |           |                       |       |  |
|                | 2016  |            |              |            |           |                       |       |  |
|                | / I cut   |            | I / II cut   |            | /Total    | %                     |       |  |
| /Control       | 172,50  |            | 177,10       |            | 349,60 b  |                       |       |  |
| L <sub>1</sub> | 160,50  |            | 181,20       |            | 341,70 b  | 97,74                 |       |  |
| L <sub>5</sub> | 174,00  |            | 199,45       |            | 373,45 a  | 106,82                |       |  |
|                |   |            |              | LSD 5%     | 19,82     |                       |       |  |
|                |   |            |              | LSD 1%     | 28,46     |                       |       |  |
|                |   |            |              | LSD 0.1%   | 41,86     |                       |       |  |
| Variants       | 2017  |            |              |            |           |                       |       |  |
|                | I cut   | I II cut   | II III cut   | V IV cut   | /Total    | %                     |       |  |
|                | /Control  | 748,75     | 576,15       | 882,00     | 218,70    | 2425,60 b             |       |  |
| L <sub>1</sub> | 575,10  | 492,20     | 965,70       | 249,20     | 2282,20 b | 94,08                 |       |  |
| L <sub>5</sub> | 740,30  | 741,60     | 952,00       | 236,50     | 2670,40 a | 110,09                |       |  |
|                |   |            |              | LSD 5%     | 445,24    |                       |       |  |
|                |   |            |              | LSD 1%     | 639,64    |                       |       |  |
|                |   |            |              | LSD 0.1%   | 940,97    |                       |       |  |
| Variants       | 2018  |            |              |            |           |                       |       |  |
|                | I cut   | I II cut   | II III cut   | V IV cut   | I cut     | I II cut              |       |  |
|                | /Control  | 859,20     | 882,70       | 774,80     | 77,50     | 2594,20 b             |       |  |
| L <sub>1</sub> | 973,00  | 814,90     | 568,80       | 71,40      | 2428,10 c | 93,60                 |       |  |
| L <sub>5</sub> | 904,50  | 949,75     | 791,70       | 75,20      | 2721,15 a | 104,89                |       |  |
|                |   |            |              | LSD 5%     | 85,29     |                       |       |  |
|                |   |            |              | LSD 1%     | 122,53    |                       |       |  |
|                |   |            |              | LSD 0.1%   | 180,25    |                       |       |  |

C P 0.05  
Values followed by the same letter are not significantly different at P 0.05

It can be noted that the difference in effect power and character of the biostimulants on the dry matter content indicator, by regrowths and years, determined the amount of dry matter yield produced. Data for the variants including L<sub>1</sub> and L<sub>5</sub> biostimulants treatment in the

year of alfalfa establishment were one-way with those for green mass yield and showed a strong positive impact of L<sub>5</sub> on annual dry matter yield of Prista 4 grass stands (Table 4).

The tendency of stimulating effect of L<sub>5</sub> was kept during the second growing season of alfalfa. It was found that during the third year of study despite the relatively lower green mass yields at biostimulants foliar application, the yield of dry matter of the variant which including treatment with L<sub>5</sub> was higher than the control.

The effect of L<sub>5</sub> on dry mass yield was expressed in a yield of 791,70 kg da<sup>-1</sup> at 774,90 kg da<sup>-1</sup> for the untreated variant. The reported values for the indicator indicated that the treatment of grass stands with L<sub>1</sub> product not had a positive effect on the trait phenotypic expression in all regrowths, respectively total for the growing season.

The comprehensive evaluation of the results obtained showed that biostimulant L<sub>5</sub> had a stronger positive effect on the studied quantitative indicators compared to L<sub>1</sub> under the specific soil and climatic conditions of IASS "Obraztsov chiflik" (Table 5).

4,

**2016-2018 .**  
**Table 5. Effect of biostimulants treatment on main quantitative indicators in Prista 4 alfalfa variety, average for 2016-2018**

| Variants       | Grass stand height, m | Stems number per m <sup>2</sup> | Green matter yield  |        | Dry matter content, % | Dry matter yield    |        |
|----------------|-----------------------|---------------------------------|---------------------|--------|-----------------------|---------------------|--------|
|                |                       |                                 | kg da <sup>-1</sup> | %      |                       | kg da <sup>-1</sup> | %      |
| Control        | 54,60 b               | 336,52 b                        | 6887,5 b            | 100,00 | 27,33                 | 1789,80 b           | 100,00 |
| L <sub>1</sub> | 59,40 a               | 351,83 ab                       | 6540,5 c            | 94,96  | 27,42                 | 1684,00 c           | 94,09  |
| L <sub>5</sub> | 60,10 a               | 363,67 a                        | 7192,6 a            | 104,43 | 28,08                 | 1921,67 a           | 107,37 |
| LSD 5%         | 2,29                  | 15,08                           | 282,67              |        |                       | 93,21               |        |
| LSD 1%         | 3,30                  | 21,67                           | 406,36              |        |                       | 145,18              |        |
| LSD 0.1%       | 4,85                  | 31,88                           | 597,71              |        |                       | 184,73              |        |

C P 0.05  
 Values followed by the same letter are not significantly different at P 0.05



|                |                |                |   |                |
|----------------|----------------|----------------|---|----------------|
|                |                | L <sub>5</sub> | - |                |
|                | L <sub>5</sub> |                |   |                |
|                |                | 4,             | - |                |
|                | L <sub>5</sub> | 7,37%          | - |                |
|                |                |                |   |                |
|                | L <sub>1</sub> | L <sub>5</sub> |   |                |
|                |                |                |   |                |
|                |                | 4,             |   |                |
|                |                |                |   |                |
| L <sub>1</sub> | L <sub>5</sub> |                |   | L <sub>5</sub> |
|                |                |                |   |                |
|                |                |                |   |                |
|                |                |                |   |                |
|                |                | 4.             |   |                |
|                | L <sub>5</sub> |                |   |                |
| 4,43%          | -              |                |   |                |
| 7,37%          | -              |                |   |                |
|                |                |                |   |                |
|                |                |                |   | L <sub>5</sub> |

The treatment with L<sub>5</sub> contributed to development of significantly higher and with better density grass stands than the control. The L<sub>5</sub> product had a clear expressed stimulating impact on the forage productivity. The mean annual dry matter yield at the stand of Prista 4 variety treated with L<sub>5</sub> biostimulant was 7,37% higher compared to the control and the difference was statistically significant.

## CONCLUSIONS

The foliar application of L<sub>1</sub> and L<sub>5</sub> biostimulants had a different effect on the morphological traits studied and on the forage productivity of Prista 4 alfalfa variety, in response to the changes in environmental conditions during the regrowth formation.

The treatment with L<sub>1</sub> and L<sub>5</sub> biostimulants stimulated the development of considerably higher plants. L<sub>5</sub> product contributed to significantly increase the potential for stem formation and the development of grass stands with better density. It had a clearly expressed positive effect on the forage productivity in Prista 4 alfalfa variety. The green mass and dry matter yields from the treated grass stands with L<sub>5</sub> were higher by 4,43% and 7,37%, respectively than these for the control.

The foliar application of L<sub>5</sub> gives promising results and reason to be used as a corrective component in technology of alfalfa growing for forage.

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**(*Vicia sativa* L.)**

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**Production of Forage from Spring Vetch (*Vicia sativa* L.)  
cv. "Tempo" Depending on the Technology of Cultivation**

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**SUMMARY**

The aim of the experiment is to establish the phenology, the changes in some quantitative indicators and the yield of spring vetch (*Vicia sativa* L.) cv. "Tempo" depending on the technology of cultivation. The experiment was conducted during the period 2011-2013 on soil subtype slightly-leached chernozem. Variants of cultivation: 1. At conventional technology – control; 2. Without use of preparations of inorganic origin; 3. Treatment only with bio insecticide "Ecofil P". It was found that, depending on the methods of cultivation and the influence of agro-meteorological conditions, the vegetation period for forage production ranges from 93 to 105 days. The crop cultivated in the conventional technology has a shorter phenological development period. With the highest number of plants, root weight and number of nodules per m<sup>2</sup> is the crop harvested by standard technology. When growing a spring vetch cv. "Tempo" for forage production by the biological method and by treatment with the bio insecticide "Ekofil P", the obtained forage

The aim of the experiment is to establish the phenology, the changes in some quantitative indicators and the yield of spring vetch (*Vicia sativa* L.) cv. "Tempo" depending on the technology of cultivation. The experiment was conducted during the period 2011-2013 on soil subtype slightly-leached chernozem. Variants of cultivation: 1. At conventional technology – control; 2. Without use of preparations of inorganic origin; 3. Treatment only with bio insecticide "Ecofil P". It was found that, depending on the methods of cultivation and the influence of agro-meteorological conditions, the vegetation period for forage production ranges from 93 to 105 days. The crop cultivated in the conventional technology has a shorter phenological development period. With the highest number of plants, root weight and number of nodules per m<sup>2</sup> is the crop harvested by standard technology. When growing a spring vetch cv. "Tempo" for forage production by the biological method and by treatment with the bio insecticide "Ekofil P", the obtained forage

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2017; 2018).

yields are significant lower than that obtained with the standard technology of cultivation.

**Key words:** spring vetch, technology, bio insecticide, phenology, forage, yields

## INTRODUCTION

Common vetch (*Vicia sativa* L.) is well known in our country forage legumes. It is important for solving the protein problem in the country, although less disseminated than other legumes (Kertikov, 2010). Scientific studies with this culture are both – breeding and agro-technical. The influence of some biotic and abiotic factors on seed productivity, yields of grain and forage in individual and mixed crops has been proven (Jimenez, 1999; Mousa et al., 1997).

An important part is the study of the quantitative and qualitative parameters of the yield (Orak, 2000; Kertikov, 2003), seed and fertilizer rates (Kertikov, 2000), the nitrogen-fixing ability of liquid chromatography have its effect on the soil nutritive regime (Yankov et al., 1995). Were conducted a thorough evaluation of the use of biologically active substances to control growth, development and increased yield of spring vetch (Katayama, 1991; Kertikov and Vasileva, 2000; Vasileva and Kertikov, 2007).

As a result of comprehensive studies on the cultivation and use of new varieties and intensive breeding-improvement work created a new variety of spring vetch - Tempo (Kertikov, 2005; Kertikova et al., 2012).

Some qualitative and quantitative changes in grain (cv. Tempo) have been identified, depending on applied cultivation technology (Kertikov and Kertikova, 2017; 2018).

*sativa* L.)

(*Vicia*

The aim of the study is to establish phenology, changes in some quantitative indicators and the forage yield of spring vetch (*Vicia sativa* L.) cv. Tempo depending on the technology of cultivation.

## MATERIAL AND METHODS

The experiment was conducted during the period 2011-2013 in the second experimental field in Institute of Forage Crops with spring vetch cv. Tempo. The variety is a good productivity, earliness, resistant to lodging and good adaptability. It is suitable in the direction of grain and green mass (Kertikova et al., 2012). The study was conducted on the soil subtype slightly leached chernozem, without irrigation. It used the split plot method with four repetitions of the variants and a size of 10 m<sup>2</sup> of harvest plot. Variants of the field experiment: Variant 1 control – a conventional technology (Kostov and Pavlov, 1999), including fertilization and treatment with herbicides and insecticides; Variant 2 – without the use of preparations of inorganic origin (biological); Variant 3 – treatment only with bio insecticide ("Ecofil P") of organic origin. Treatment with bio preparation "Ecofil P" is performed in phenophase full flowering at a dose of 3,5 l/da. Agro-meteorological and phenological observations and readings were carried. The following indicators were recorded: yield of fresh and dry mass (kg.da<sup>-1</sup>); number of stems (m<sup>2</sup>); weight roots (g/m<sup>2</sup>); number of nodules (m<sup>2</sup>). The harvesting of the forage was carried out in phenophase full bottom pods with a small-scale mower. The data were processed with the software STATGRAPHYCS plus for Windows Version 2.1.

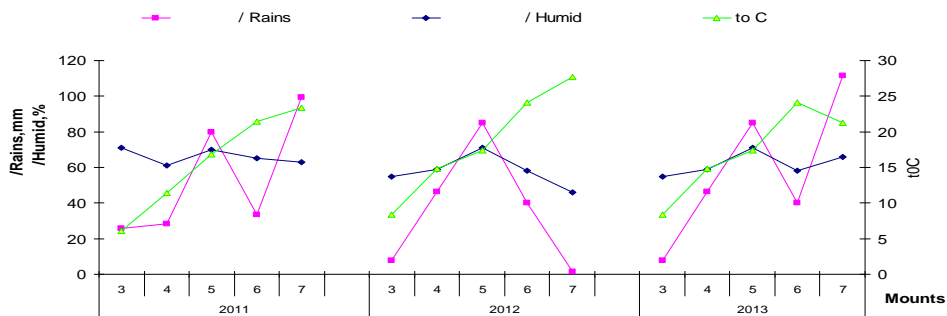
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## RESULTS AND DISCUSSION

From the meteorological data (Figure 1) covering rainfall, atmospheric humidity and average spring daily air temperatures during spring growing

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(March to July), it appears that appropriate conditions for sowing the crop over the three experimental years are present during the third ten-day period of March (Table 1); at the earliest opportunity for work under field conditions.



**Fig. 1. Klimatogram for the vegetation period**

In general, weather conditions are characterized as favourable, with normal temperatures and rainfall during the cultivation of spring vetch. In March the amount of precipitation is sufficient, but not evenly distributed.

**Table 1. Phenological development of spring vetch cv. „Tempo” depending on the applied agro technical factors, 2011-2013**

| Variants                                    | / Phenological development |                  |                          |               |
|---|----------------------------|------------------|--------------------------|---------------|
|   | /sowing                    | /emergency       | /growth                  | /buttoning    |
| 1. ( )                                      | 21.03 - 28.03              | 02.04 - 12.04    | 16.04 - 25.04            | 12.05 - 23.05 |
| 1. In technology (control)                  | 21.03 - 28.03              | 02.04 - 12.04    | 16.04 - 25.04            | 12.05 - 23.05 |
| 2. Without preparations of inorganic origin | 21.03 - 28.03              | 02.04 - 12.04    | 16.04 - 25.04            | 12.05 - 23.05 |
| 3. " " " Treatment with "Ecofil P"          | 21.03 - 28.03              | 02.04 - 12.04    | 16.04 - 25.04            | 12.05 - 23.05 |
| Variants                                    | full flowering             | full bottom pods | vegetation period (days) |               |
| 1. ( )                                      | 30.05 - 28.06              | 04.06 - 12.06    | 93 - 101                 |               |
| 1. In technology (control)                  | 05.06 - 05.07              | 10.06 - 14.06    | 100 - 104                |               |
| 2. Without preparations of inorganic origin | 03.06 - 03.07              | 08.06 - 15.06    | 98 - 105                 |               |
| 3. " " " Treatment with "Ecofil P"          | 03.06 - 03.07              | 08.06 - 15.06    | 98 - 105                 |               |

3-4  
50%  
93 105

- In view of the increasing average  
 - daily soil and air temperature in April, full  
 germination in plants of all three variants  
 was registered at the beginning of the  
 second ten days of the month. The period  
 from phenophase germination to 3-4 leaf  
 and the beginning of buttoning is  
 characterized by frequent rainfall, good  
 humid and optimal temperatures for  
 development of the crop. This period is  
 the most intense in the development of  
 spring vetch. In the case of phenophase,  
 - 50% of the flowering of vetch in the three  
 studied variants, there are no reported  
 differences in their development.  
 Differences in phenology depending on  
 applied cultivation methods are reported  
 in full flowering phases - beginning of pod  
 formation - the end of May and beginning  
 of June. The crop from the first variant  
 (conventional technology) passes said  
 two phases in a shorter period of time.  
 - Compared to the second variant  
 (biological), it is from 3 to 7 days and  
 from 4 to 5 days compared to the third  
 variant. This tendency persists until the  
 harvesting of the spring vetch for the  
 forage production. The reported  
 vegetation period ranges from 93 to 105  
 days.

2.

, 2011-2013

**Table 2. Changing some quantitative indicators under influence on the applied agro technical factors, 2011-2013**

| Variants                                    | Number of plants m <sup>2</sup> | Deviation (%) +/- | Weigh of roots g/m <sup>2</sup> | Deviation (%) +/- | Number of nodules m <sup>2</sup> | Deviation (%) +/- |
|---|---------------------------------|-------------------|---------------------------------|-------------------|----------------------------------|-------------------|
| 1. In technology (control)                  | 396,1 <sup>a</sup>              | -                 | 55,25 <sup>a</sup>              | -                 | 117,28 <sup>a</sup>              | -                 |
| 2. Without preparations of inorganic origin | 354,7 <sup>b</sup>              | - 13,09           | 36,18 <sup>b</sup>              | - 34,52           | 94,34 <sup>b</sup>               | - 19,53           |
| 3. Treatment with "Ecofil P"                | 356,3 <sup>b</sup>              | - 13,44           | 40,28 <sup>b</sup>              | - 27,10           | 114,74 <sup>a</sup>              | - 2,16            |

a, b, c, - P<sub>0,05</sub>  
 a, b, c, - statistically proven differences in P<sub>0,05</sub>



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 ) (m<sup>2</sup>) 13% -  
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 m<sup>2</sup>, -  
 - , " " -  
 27,10%, -  
 36,18%. -  
 (m<sup>2</sup>) 117,28. -  
 " " -  
 2,16%. , -  
 (19,53%) -  
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In tracking the change in some quantitative indices under the influence of the applied methods of cultivation (Table 2) revealed significant deviations from those in the control (standard technology) crop.

The number of plants reported per unit area (m<sup>2</sup>) is more than 13% higher in harvesting of the control variant compared to the crops treated with bio insecticide "Ecofil P" and growing a biological method. The difference is very good mathematical significant. Crops placed under organic cultivation and treatment with bio insecticide "Ecofil P" account the higher level of weed infestation are a reduced number of plants per m<sup>2</sup>. This fact has a significant negative impact on the amount of forage yield. In the results for the weight of the roots of m<sup>2</sup>, the tendency mentioned above is again observed, here it is even more pronounced.

In the crop treated with bio insecticide "Ecofil P", the weight loss of the roots compared to the control crop is 27,10%, while in the case of the biological crop, the decrease in the weight of the roots reaches 36,18%. When growing spring vetch by standard technology, the number of nodules reported per unit area (m<sup>2</sup>) reached 117,28. In crops treated with bio insecticide "Ekofil P", the number of nodules reported was almost the same as those reported in crops grown under standard technology, with a reduction of only 2,16%.

The decline in nodules (19,53%) recorded in the harvesting of crops grown by the biological method is significant. The analysis of the results shows that the highest number of plants, weight of the roots and number of nodules per m<sup>2</sup> are found in the crops grown under standard technology, followed by treatment with bio insecticide "Ecofil P".

The applied different technologies for growing spring vetch for forage production have a significant impact on the quantity of green mass produced as well as on the dry mass yield (Table 3). From the data, both in years and average for the period of the study show that the results for green and dry matter are highest in crops grown on standard technology.

3.

, kg.da<sup>-1</sup>

**Table 3. Fresh and dry mass yields under the influence on the applied agro technical factors, kg.da<sup>-1</sup>**

| Variants  | 2011                 | 2012                  | 2013                 | mean                 | Deviation (%) +/- |
|---|----------------------|-----------------------|----------------------|----------------------|-------------------|
|   | /Fresh mass          |                       |                      |                      |                   |
| 1. ( )<br>1. In technology (control)              | 1936,22 <sup>a</sup> | 1872,04 <sup>a</sup>  | 2044,93 <sup>a</sup> | 1951,06 <sup>a</sup> | -                 |
| 2.<br>2. Without preparations of inorganic origin | 1357,18 <sup>b</sup> | 1752,53 <sup>a</sup>  | 887,08 <sup>c</sup>  | 1332,26 <sup>b</sup> | - 31,72           |
| 3. " "<br>3. Treatment with "Ecofil P"            | 1428,32 <sup>b</sup> | 1564,26 <sup>b</sup>  | 1156,22 <sup>b</sup> | 1382,93 <sup>b</sup> | - 29,12           |
| LSD <sub>99,5%</sub> (kg.da <sup>-1</sup> )       | 89,782               | 134,638               | 91,777               | 97,102               |                   |
| / Dry mass  |                      |                       |                      |                      |                   |
| 1. ( )<br>1. In technology (control)              | 452,92 <sup>a</sup>  | 424,92 <sup>a</sup>   | 458,88 <sup>a</sup>  | 445,57 <sup>a</sup>  | -                 |
| 2.<br>2. Without preparations of inorganic origin | 298,57 <sup>b</sup>  | 394,90 <sup>a,b</sup> | 198,88 <sup>b</sup>  | 297,45 <sup>b</sup>  | - 33,24           |
| 3. " "<br>3. Treatment with "Ecofil P"            | 328,51 <sup>b</sup>  | 352,15 <sup>b</sup>   | 225,10 <sup>b</sup>  | 301,92 <sup>b</sup>  | - 32,23           |
| LSD <sub>99,5%</sub> (kg.da <sup>-1</sup> )       | 89,782               | 51,214                | 42,817               | 38,580               |                   |

In the other two variants, in the first and second years, the yields of forage (green and dry mass) in crop grown by treatment with bio insecticide "Ecofil P" is higher compared to those obtained from organic fertilizer cultivation.

31,72%  
33,24%

29,12%  
32,23%

(Kertikov and Kertkova, 2017).

In the second experimental year, the organic cultivation method of the crop has given better results compared to the treatment of the crop with bio insecticide "Ecofil P".

Average over the period of this study, from the crop grown by standard technology to give a higher yield from 29,12% to 31,72% green mass and from 32,23% to 33,24% dry matter as compared to the forage obtained from cultivation of the crop by biological method and treatment with bio insecticide "Ecofil P". It appears that standard spring vetch cultivation technology is more effective in producing forage compared to biological grown or treatment with bio-insecticide "Ecofil P". Similar results are also reported in the grain yield (Kertikov and Kertkova, 2017).

## CONCLUSIONS

Depending on the cultivation technology and under the influence of the various agro-meteorological conditions during the study period, the vegetation period of the spring vetch for the production of forage ranges from 93 to 105 days. The crop grown on conventional technology has the shortest period of phenological development.

With the highest number of plants, weight of the roots and number of nodules per  $m^2$  is the crop harvested by standard technology, followed by treatment with bio insecticide "Ecofil P".

When growing a spring vetch cv. "Tempo" for forage production by the biological method and by treatment with the bio insecticide "Ecofil P", the obtained forage yields are significant lower than that obtained with the standard technology of cultivation.

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## (*Vicia sativa* L.)

# Breeding Assessment of Vetch Cultivars (*Vicia sativa* L.) in Organic Farming Conditions

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### SUMMARY

- Modern selection programs are directed at developing adaptive cultivars characterized by both high productivity and stability with respect to the main yield components. For the purpose of a breeding assessment of common vetch cultivars in organic production conditions, a field trial was conducted during the period 2012-2014 with one Bulgarian (Obrazets 666) and four introduced cultivars (Moldavskaya, Liya, Lorina, Vilena).
- The methods of dispersion and regression analysis were used to determine the effect of main quantitative traits on the seed yield. The results showed that in the yield formation, determining traits were seed weight per plant ( $R=27.97$ ), number of seeds per pod ( $R=7.69$ ) and pods per plant ( $R=5.29$ ).
- The effect of pod length ( $R=1.85$ ), 1000 seeds weight ( $R=1.08$ ) and plant height ( $R=0.20$ ) was weaker pronounced. The

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 (Nikolova, 2015)  
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 (Abbasi et al., 2014).

- vetch cultivars exhibited higher adaptability on the traits of plant height, number of seeds per plant and 1000 seeds weight, and low adaptability – regarding seed weight per plant, pod length and seed yield.  
 -  
 - In terms of individual productivity, cultivar Lorina was the desired breeding compromise: with seed weight per plant above the average value for the group and an average level of adaptability.  
 - Cultivars Liya and Lorina represented a selective value due to the good combination of high seed yield with low variability and good adaptability.  
**Key words:** vetch, regression, productivity, adaptability

## INTRODUCTION

The *Vicia* genus has been widely used in agroecosystems as a cover crop, for green manure and increasing soil fertility (Cheminingwa and Vessey, 2006; Campiglia et al., 2010; Mothapo et al., 2013).

*Vicia sativa* L., known as common vetch, is one of the most cultivated vetch species because of its high nutritional value, as well as its ability to grow in a wide range of climatic and soil conditions (Hueze et al., 2011; Abbasi et al., 2014).

The common vetch is used mainly for animal nutrition (Bet et al., 2016). The seeds of this leguminous plant contain a relatively high quantity of minerals, protein (Georgieva and Nikolova, 2011), starch (Bet et al., 2016). Vetch biomass is also high-quality (Nikolova, 2015) and used for pasture, silage, and hay (Hueze et al. 2011; Sullivan 2003). Common vetch is a valuable plant in crop rotation due to its limited vegetation period, and the possibility of being sown at different planting times (Abbasi et al., 2014).

The complex nature of environmental conditions sometimes

- leads to increased variability in crop productivity. Therefore, the problem of enhancing the cultivar productivity in regions which are unsustainable in terms of agrometeorological conditions should be combined with elements of stabilization. In such an environment, varieties do not fully realize their genetic potential due to an insufficient level of adaptability (Goncharenko, 2005; Sapega et al., 2012).

(Goncharenko, 2005; Sapega et al., 2012).

- Modern breeding programs should be oriented towards the development of adaptive cultivars characterized by both high productivity and stability in terms of main elements of yield and quality of production.

- There is a real possibility to develop genotypes with high productive potential and stability to unfavorable environmental factors as these traits are controlled by different genetic systems (Anohina and Mazuka, 2006).

(Anohina and Mazuka, 2006).

- The analysis of the genotype response to changes in environmental conditions should be done both at the stage of studying starting material and in the final stages of the selection process.
- The ecological plasticity assessment of the cultivars is conducted by mathematical methods which allow obtaining an individual characteristic for this indicator in different years or different regions of growing (Maruhnyak et al., 2010).

(Maruhnyak et al., 2010).

The aim of the present study was a breeding assessment of stability and adaptability of vetch cultivars, as well as establishing the effect of the main quantitative traits on seed productivity.

## **MATERIAL AND METHODS**

One Bulgarian (Obrazets 666) and four Moldovan cultivars (Moldavskaya,

(*Vicia sativa* L.).

2012-2014

4 m<sup>2</sup>.

220

/m<sup>2</sup>.

1000

Shukla (1972),  
(1970)  
(1993).

“D<sub>i</sub>”

(<sup>2</sup>)  
Hanson  
Kang

( )

(1990).

PBSTAT 1.2 for Windows.

Liya, Lorina, Vilena) of common vetch (*Vicia sativa* L.). were objects of this research. The experiment was carried out during the period 2012-2014 at the Institute of Forage Crops, under organic production conditions. The randomised block design was used, with three replications and a plot size of 4 m<sup>2</sup>. The sowing was conducted at the end of March, with a rate of 220 seeds per m<sup>2</sup>. In accordance with the organic production, fertilizers and pesticides were not used during the growing season. The biometric evaluation of cultivars included the following traits: plant height, pods number per plant, pod length, seeds number per pod, seeds number per plant, seed weight per plant, 1000 seeds weight.

The phenotypic stability of the cultivars regarding seed yield was evaluated by the following parameters and methods: variance of stability (<sup>2</sup>) of Shukla (1972), parameter “D<sub>i</sub>” of Hanson (1970) and non-parametric method of Kang (1993). The total adaptability (A) of all traits of the cultivars was calculated according to Valchinkov (1990).

The data were processed statistically through the program product PBSTAT 1.2 for Windows.

## RESULTS AND DISCUSSION

High effectiveness and success in breeding re based on the use of new sources of genetic diversity with valuable traits, which at a crossing and combining hereditary factors, serve as a source of valuable (in the economic view) plant forms.

The results of the two-factor analysis of variance regarding the main quantitative traits were presented in Table 1. It was found a significant influence of the cultivar factor for number and weight of the seeds, 1000 seeds weight, and seed yield.

The vetch cultivars did not differ considerably in their genetic essence with respect to the traits of plant height, pods



per plant, pod length and seeds number per pod.

1.

**Table 1. Analysis of variance regarding main quantitative traits**

| Source of variation | df | /Mean squares |                |            |               |
|---------------------|----|---------------|----------------|------------|---------------|
|                     |    | Plant height  | Pods per plant | Pod length | Seeds per pod |
| /Year               | 2  | 39.6222ns     | 0.2889ns       | 0.0222ns   | 0.0889ns      |
| /Cultivar           | 4  | 249.5222ns    | 2.1667ns       | 0.1889     | 1.2778ns      |
| /Error              | 38 | 107.7099      | 1.4526         | 0.1977     | 0.5567        |
| /Total              | 44 |               |                |            |               |

| Source of variation | df | /Mean squares   |                       |                  |              |
|---------------------|----|-----------------|-----------------------|------------------|--------------|
|                     |    | Seeds per plant | Seed weight per plant | 1000 seed weight | Seed yield   |
| /Year               | 2  | 37.4222ns       | 0.2889ns              | 2.4889ns         | 184.0222ns   |
| /Cultivar           | 4  | 174.9222**      | 2.0778**              | 323.5556**       | 7,614.4778** |
| /Error              | 38 | 35.1942         | 0.2655                | 44.1556          | 388.0164     |
| /Total              | 44 |                 |                       |                  |              |

Significance at P = 0.05 (\*), \*\* P = 0.01(\*\*)

Zharkova (2009),

- According to Zharkova (2009), the determination of regression dependencies gives the opportunity to establish direct and indirect interrelations between particular traits and indicators. This information is also of interest to the breeding on adaptability and offers the opportunity to conduct a selection on indirect traits. The results of the regression analysis (Table 2) showed that the linear component in the seed yield regression with respect to the quantitative traits studied was considerable and significant.

(T 2)

2.

(Anova)

**Table 2. Regression analysis (Anova) of the seed yield in regard to the quantitative traits in common vetch**

|            | df | SS      | MS       | F           | Significance F |
|------------|----|---------|----------|-------------|----------------|
| Regression | 8  | 18455.2 | 2306.899 | 3.058984283 | 0.009865       |
| Residual   | 36 | 27149   | 754.139  |             |                |
| Total      | 44 | 45604.2 |          |             |                |

(1),

(1).

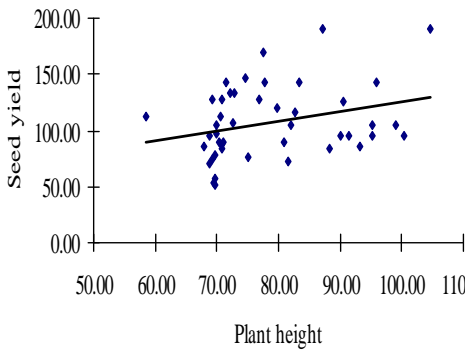
e:

$$(1) Y = -18.76 + 0.20X_1 + 5.29X_2 + 1.85X_3 + 7.69X_4 - 1.56X_5 + 27.97X_6 + 1.08X_7$$

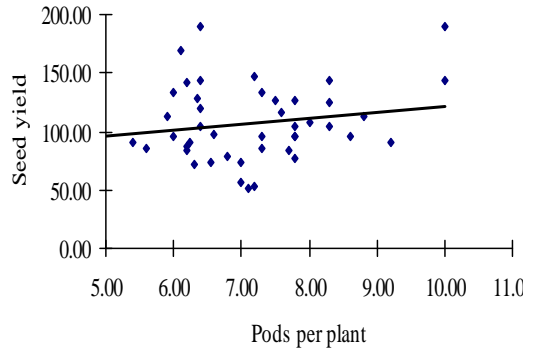
Y – ; X<sub>1</sub> – ; X<sub>2</sub> – ; X<sub>3</sub> – ; X<sub>4</sub> – ; X<sub>5</sub> – ; X<sub>6</sub> – ; X<sub>7</sub> –

From the integrated study of the traits was obtained a regression equation (1), which demonstrated the complexity of the seed yield amendment depending on the change in the other quantitative traits (Figure 1). The general appearance of the regression equation was:

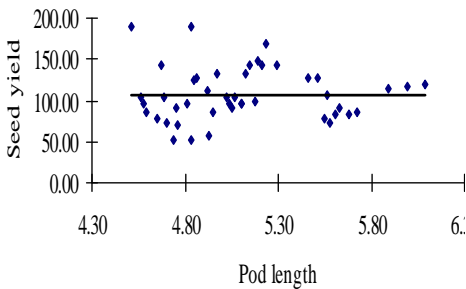
where: Y – was seed yield; X<sub>1</sub> – plant height; X<sub>2</sub> – pods per plant; X<sub>3</sub> – pod length; X<sub>4</sub> – Seeds per pod; X<sub>5</sub> – number of seeds per plant; X<sub>6</sub> – seed weight per plant; X<sub>7</sub> – 1000 seeds weight;



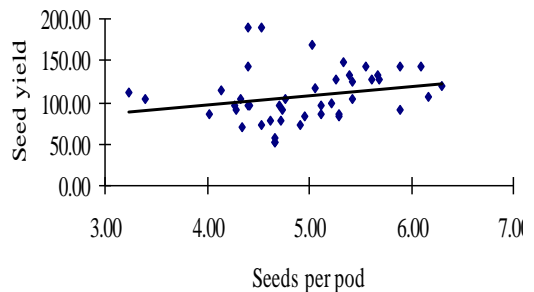
A



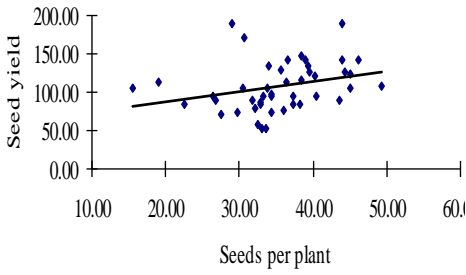
B



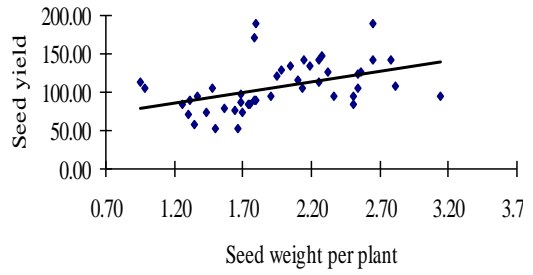
C



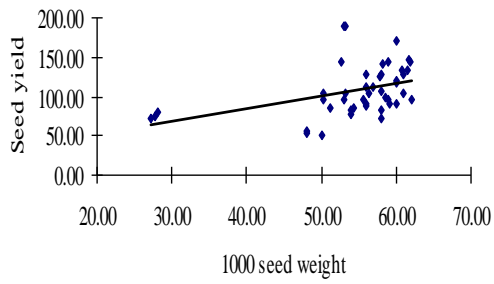
D



E



F



G

A-Plant height; B-Pods per plant; C-Pod length; D-Seeds per pod; E-Seeds per plant; F-Seed weight per plant; G-1000 seeds weight  
 A- ; B- ; C- ; D- ; E- ; F-  
 ; G- 1000

**Fig. 1. Dependences between seed yield and quantitative traits**

According to the obtained results in seed yield formation, the seed weight per plant and seeds per pod, followed by the number of pods per plant had the greatest influence (Table 3). These traits increased seed yield by 27.97 kg/da, 7.69 kg/da and 5.29 kg/da, respectively. Less, but positive was the effect of pod length (R = 1.85), followed by 1000 seeds weight (R = 1.08) and plant height (R = 0.20).

In the regression equation obtained (1), as opposed to the previous traits, the dependence between seed yield and number of seeds per plant (as a value) was negligible and negative.

According to the obtained results in seed yield formation, the seed weight per plant and seeds per pod, followed by the number of pods per plant had the greatest influence (Table 3). These traits increased seed yield by 27.97 kg/da, 7.69 kg/da and 5.29 kg/da, respectively. Less, but positive was the effect of pod length (R = 1.85), followed by 1000 seeds weight (R = 1.08) and plant height (R = 0.20).

In the regression equation obtained (1), as opposed to the previous traits, the dependence between seed yield and number of seeds per plant (as a value) was negligible and negative.

This indicates that any increment in the number of seeds above a certain limit would result in a reduction in the individual productivity per plant and hence in the yield as a complex trait.

3.

**Table 3. Regression coefficients of the seed yield in regard to the quantitative traits in common vetch**

| /Traits                                  | Coefficients | Standart error | t Stat  | P-value |
|--|--------------|----------------|---------|---------|
| /Intercept                               | -18.76       | 174.3710       | -0.1076 | 0.9149  |
| X <sub>1</sub> - / Plant height          | 0.20         | 0.5035         | 0.4019  | 0.6901  |
| X <sub>2</sub> - / Pods per plant        | 5.29         | 25.8016        | 0.2052  | 0.8386  |
| X <sub>3</sub> - / Pod length            | 1.85         | 14.3797        | 0.1287  | 0.8983  |
| X <sub>4</sub> - / Seeds per pod         | 7.69         | 36.5560        | 0.2103  | 0.8346  |
| X <sub>5</sub> - / Seeds per plant       | -1.56        | 5.6467         | -0.2770 | 0.7834  |
| X <sub>6</sub> - / Seed weight per plant | 27.97        | 19.1926        | 1.4574  | 0.1537  |
| X <sub>7</sub> - 1000 /1000 seeds weight | 1.08         | 0.6360         | 1.7014  | 0.0975  |

Y<sub>S<sub>i</sub></sub> Kang (1993)  
 (Y<sub>S<sub>i</sub></sub>=2 Y<sub>S<sub>i</sub></sub>=1),  
 (4).  
 666,  
 Hanson  
 (1970) Shukla (1972)

The yield assessment according to the parameter Y<sub>S<sub>i</sub></sub> of Kang (1993) showed that cultivars Villena and Lorina had very good ecological stability and plasticity (Y<sub>S<sub>i</sub></sub>=2 Y<sub>S<sub>i</sub></sub>=1), but Lorina was less productive than Vilena (Table 4).  
 In cultivars Moldovskaya and Obrazets 666, the values of the stability parameters according to Hanson (1970) and Shukla (1972) were the lowest. These cultivars can be defined as ecologically stable, but at the same time, they were the least productive. The remaining stability parameters also defined Moldavskaya as a relatively stable genotype. Cultivar Lorina was closest to the breeding compromise. It can be characterized as ecologically stable and was among the cultivars with seed weight per plant above the average for the group.

4.

**Table 4. Stability parameters regarding seed yield in vetch cultivars**

| /Cultivars   | D <sub>i</sub> | <sup>2</sup> | YS <sub>i</sub> | Seed yield | Seed productivity |
|--------------|----------------|--------------|-----------------|------------|-------------------|
| Liya         | 34.12          | 1707.83      | 0.00            | 149.33     | 2.50              |
| Lorina       | 24.31          | 175.79       | 1.00+           | 98.44      | 2.08              |
| Vilena       | 26.52          | 596.96       | 2.00+           | 122.56     | 2.26              |
| Moldovskaya  | 22.00          | 44.17        | 0.00            | 92.00      | 1.71              |
| Obrazets 666 | 21.86          | 19.25        | -2.00           | 74.50      | 1.44              |

D<sub>i</sub> - (Hanson, 1970); <sup>2</sup> - s (Shukla, 1972);  
 YS<sub>i</sub> - (Kang, 1993)  
 D<sub>i</sub> - genotypic stability parameter (Hanson, 1970); <sup>2</sup> - stability variance (Shukla, 1972); YS<sub>i</sub> - stability index (Kang, 1993)

-  
 Valchinkov (1990),  
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 ( 5).  
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 1000  
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 /  
 (2.5) 1000 (57.79).  
 666  
 ,  
 -  
 .

For a more objective assessment of the adaptive ability of the vetch cultivars studied, according to the method of Valchinkov (1990), the coefficient of general adaptability was calculated (Table 5). In general, cultivars were better adapted to changing environmental conditions and exhibited higher adaptability on the traits of plant height, seeds per plant and 1000 seeds weight, and low adaptability - on seed weight per plant , pod length and seed yield, respectively.

Cultivar Liya had high adaptability in terms of pods per plant, seeds per plant, seed weight per plant and seed yield. For almost all cultivars, the adaptability index had a negative value regarding the pod length.

In its possibility for adaptability, Villena was best represented in terms of the seeds weight per plant (2.5) and 1000 seeds weight (57.79). The Bulgarian cultivar Obrazets 666 gave way to the other cultivars on the tested traits, but in terms of pod length and seeds per pod, it was the most adapted to the environmental conditions for the survey period.

## 5.

(Valchinkov, 1990)

Table 5. Adaptive ability in vetch cultivars (Valchinkov, 1990)

| cultivar     | Plant height | Pods per plant | Pod length | Seeds per pod | Seeds per plant | Seed weight per plant | 1000 seed weight<br>1000 | Seed yield |
|--------------|--------------|----------------|------------|---------------|-----------------|-----------------------|--------------------------|------------|
| Liya         | 78.93        | 6.86           | -4.06      | 2.97          | 39.39           | 2.32                  | 56.32                    | 0.19       |
| Lorina       | 79.1         | 6.05           | -3.95      | 4.51          | 36.86           | -0.54                 | 56.72                    | 0.05       |
| Vilena       | 79.65        | 6.01           | -3.72      | 3.67          | 36.31           | 2.5                   | 57.79                    | -0.52      |
| Moldovskaya  | 81.14        | 5.93           | -4.40      | 3.98          | 30.10           | -0.8                  | 55.91                    | 0.13       |
| Obrazets 666 | 69.24        | 5.87           | 4.44       | 4.52          | 28.46           | 1.31                  | 41.01                    | -0.59      |

Anisinkov (2009),

Valchinkov (1990),

666. &gt;

/ (R=27.97),  
/ (R=7.69)  
/ (R=5.29).

High adaptive cultivars represented a selection value only on condition that they exhibit low variability of a given trait under different growing conditions. According to Anisinkov (2009), in many-directedness in the comparison of different parameters of adaptability and stability of the studied varieties, it is necessary to use different approaches for integrated assessment of the indicator and its stability. Therefore, many researchers offer different criteria for simultaneous selection on productivity and stability of a given characteristic. According to the method of Valchinkov (1990), regarding adaptability of seed yield, the cultivars can be arranged in the following order: Liya > Moldovskaya > Lorina > Vilena > Obrazets 666. Cultivar Liya, followed by Lorina, combined high seed yield with relatively low variability and high adaptability.

**CONCLUSIONS**

- By regression analysis, the effect of  
- main quantitative traits on the seed yield  
- in common vetch cultivars grown in  
- organic production was determined.

In the yield formation, determining traits were seed weight per plant (R=27.97), number of seeds per pod (R=7.69) and pods per plant (R=5.29).

|                                    |      |   |
|------------------------------------|------|---|
| (R=1.85),<br>(R=1.08)<br>(R=0.20). | 1000 | The effect of pod length (R=1.85), 1000 seeds weight (R=1.08) and plant height (R=0.20) was weaker pronounced.  |
|                                    | -    | The vetch cultivars exhibited higher adaptability on the traits of plant height, number of seeds per plant and 1000 seeds weight, and low adaptability – regarding seed weight per plant, pod length and seed yield.  |
|                                    | 1000 |   |
|                                    |      | In terms of individual productivity, cultivar Lorina was the desired breeding compromise: with seed weight per plant above the average value for the group and an average level of adaptability. Cultivars Liya and Lorina represented a selective value due to the good combination of high seed yield with low variability and good adaptability. |

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## (*Sorghum sudanense* (piper.) Stapf) III.

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, 5800 ,

## Radiosensitivity in Sudangrass (*Sorghum sudanense* (Piper.) Stapf) III. In Field Conditions

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### SUMMARY

(*Sorghum sudanense* (Piper.) Stapf.)  
- Kazitachi ( ),  
Vercors ( ) 9 ( )  
- (Caesium  
<sup>137</sup>) 0, 200, 400, 600,  
800, 1000, 1200, 1400, 1600, 1800  
2000 Gy ( 146 Gy/h)

LD<sub>50</sub>,

Dry seeds from three varieties of sudangrass (*Sorghum sudanense* (Piper.) Stapf) with different origin, namely, Kazitachi (Japan), Vercors (USA) and Voronezhskaya 9 (Russia) were irradiated with gamma-rays (Caesium<sup>137</sup>) at different doses - 0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 Gy (dose rate 146 Gy/h) in Institute of Plant Physiology and Genetics – Sofia, Bulgaria. The influence of gamma rays on three physiological parameters (germination, survival and sterility) of M<sub>1</sub> progeny of the varieties used were investigated and data show clearly pronounced “dose-effect”; with increasing dose the values obtained for each of these biological parameters decrease and the differences are statistically proven.

The radiosensitivity of the cultivars is well expressed with the values of LD<sub>50</sub>, which were calculated on the basis of survival plants in the first mutant

(307,52 Gy),  
 Vercors 340,97 Gy  
 Kazitachi (M<sub>1</sub>)  
 9 – LD<sub>50</sub>  
 342,63 Gy.

*Sorghum sudanense* (Piper.) Stapf.,

generation (M<sub>1</sub>). This value is lowest for Kazitachi (307.52 Gy), which is defined as the most radiosensitive variety, while LD<sub>50</sub> for Vercors and Voronezhskaya 9 are 340.97 Gy and 342.63 Gy, respectively. These data provide valuable information when the optimal doses should be chosen for the purpose of mutation breeding program of sudangrass.

**Key words:** *Sorghum sudanense* (Piper.) Stapf., sudangrass, gamma-rays, radiosensitivity

## INTRODUCTION

(Maluszynski, 2001; Chopra, 2005).

(Majeed et al., 2010). (64%)

(Ahloowalia et al., 2004). (Bado et al., 2015).

(Mba et al., 2012).

Mutation breeding nowadays continues to be seen as a proven tool for creating and/or enhancing genetic variability other than that created by recombination (Maluszynski, 2001, Chopra, 2005). Unlike conventional breeding procedures which involve, the production of new genetic combinations from already existing parental genes, mutation breeding causes exclusively new gene combinations with high mutation frequency, the most commonly used in this regard being ionizing radiation (Majeed et al., 2010). Big part of mutant varieties (64%) were created using gamma rays (Ahloowalia et al., 2004).

Gamma irradiation is known to be the most effective in inducing a wide range of mutations (Bado et al., 2015). Whereas chemical mutagens are preferably used to induce point mutations, physical mutagens induce gross lesions, such as chromosomal abbreviation or rearrangements (Mba et al., 2012). Gamma-rays penetrate deeply into target tissues than other radiations and it is less destructive.

Experimental mutagenesis emerges as a promising method in the breeding program for genetically improving the sudangrass. However, studies with induced mutagenesis in this species are scarce, and the data obtained

(Kirillenko and Golovin, 1987; Lazanyi, 1987; Golovin and Kirillenko, 1989; Tabosa et al., 2007; Alvarez-Holguin et al., 2017).

(LD<sub>50</sub>).

LD<sub>50</sub>

Preobrazhenskaya (1971) LD<sub>50</sub> 250 Gy, FAO/IAEA LD<sub>50</sub> 300 Gy (Anonimus, 2002).

50 - 600 Gy (Golovin and Kirillenko, 1989; Kostina et al., 1995). Zhi et al. (1999) reported that optimal dose of *Sorghum sudanense* 243–301 Gy.

( 1 )

LD<sub>50</sub>

so far are quite controversial, especially when it comes to ionizing radiation (Kirillenko and Golovin, 1987; Lazanyi, 1987; Golovin and Kirillenko, 1989; Tabosa et al., 2007; Alvarez-Holguin et al., 2017). First step in a mutagenesis based breeding process is to determine the semi-lethal dose (LD<sub>50</sub>). The LD<sub>50</sub> is important parameter utilized for determining the optimal radiation dose to induce effective mutations in breeding improvement programs. LD<sub>50</sub> values in sudangrass after gamma irradiation are scanty and controversial. According to Preobrazhenskaya (1971), LD<sub>50</sub> in sudangrass is 250 Gy and according to FAO/IAEA data on sudangrass, the LD<sub>50</sub> is 300 Gy (Anonimus, 2002).

Some studies have reported the use of gamma radiation in sudangrass at doses of 50 to 600 Gy (Golovin and Kirillenko, 1989; Kostina et al., 1995). Zhi et al. (1999) reported that optimal dose of *Sorghum sudanense* was 243–301 Gy. Gamma radiation was found to have a significant effect on the first generation ( 1 ) of sudangrass (Krishna et al., 1983). Gamma radiation treatment on sudangrass showed some changes in stem, leaf, reproductive organ morphology and plant height.

After select the genotypes for improvement - cultivars and elite genotypes, must be determine the optimal dose of gamma-rays, as they vary between species and genotypes.

No summary results are available on the physiological response and the sensitivity of the udder after irradiation with gamma-radiation.

The aim of the current study was to determine the LD<sub>50</sub> treatments and radiosensitivity in sudangrass using gamma-irradiation for induced a genetic variability.

## MATERIAL AND METHODS

(Piper.) Stapf.)  
Kazitachi ( ), Vercors ( )  
9 ( )

(Caesium<sup>-137</sup>) – 0, 200, 400, 600, 800,  
1000, 1200, 1400, 1600, 1800 2000 Gy.

GOU-3M

Gy/h.

30

2007-2008

1977)

(Shanin,  
2 m 45/5 cm

1987).

(Dechev et al.,

(M<sub>1</sub>)

) –

M<sub>1</sub>,

**Plant materials.** Three varieties of different origin, namely, Kazitachi (originating from Japan), Vercors (USA) and Voronezhskaya 9 (Russia) that are renowned for their agronomic characteristics, including resistance to drought were used as experimental material.

**Irradiation procedures.** Air-dried seeds of the varieties mentioned above were irradiated with ten doses of gamma-rays (Caesium<sup>-137</sup>) – 0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 Gy. Radiation treatments were carried out with gamma-irradiation facility GOU-3M in Institute of Plant Physiology and Genetics – Sofia emitting gamma-rays at dose rate 146 Gy/h. The seeds were soaked in distilled water for 30 min immediately after irradiation and dried until their sowing in the field.

**Field experiments.** The experiment was carried out in two consecutive years in the period 2007-2008. One thousand seeds were sowed under field condition for each treatment from the experiment. The field trials were set according to the standard method with consecutive arrangement of the variants (Shanin, 1977) in 2 m wide beds at 45/5 cm row spacing. The preparation of the soil, the control of diseases, pests and weeds is based on the technology for growing sorghum for grain and silage (Dechev et al., 1987).

Three criteria in first mutant progeny (M<sub>1</sub>) were scored as percent of controls (non-irradiated seeds) – germination, sterility and survival. The sterility was scored in percent of control variants on the basis of the number of M<sub>1</sub> plants with complete or partial reduction of seed setting. “Surviving plants” were counted at the time of harvest of M<sub>1</sub> generation and survivors were defined as those plants that produce at least one

(Meier, 2001).

LD<sub>50</sub>

( )

(Plockinsky, 1967).

LD<sub>50</sub> e  
TRIMED SPEARMAN KARBER METOD,  
VERSION 1.5 (Hamilton et al., 1978).

Microsoft Office Excel 2010.

1.

(BBCH 10) 800 Gy 4,0  
38,84 %

77,78 84,97 %  
2000 Gy (Golubinova and Serafimov,  
2015).

( 800 Gy),  
LD<sub>50</sub> 410,27 420,30  
Gy.

- inflorescence regardless of whether seeds are produced.

- The growth stages of development sudnggrass is determined by a system of uniform coding of growth stages of development for mono- and dicotyledonous plant species (Meier, 2001).

Data on survival and central stems height were measured at defined growth stages. The LD<sub>50</sub> is determined by growth stage for each variant of the trial depending on the irradiation doses of seeds. In the end of vegetation period the number of tillers was counted.

The statistical processing of the germination and survival at the end of the vegetation period under field condition was performed using Fisher's -criterion (Plockinsky, 1967); for the determination of LD<sub>50</sub>, the program product TRIMED SPEARMAN KARBER METOD, VERSION 1.5 was used (Hamilton et al., 1978); correlations are calculated with Microsoft Office Excel 2010.

## RESULTS AND DISCUSSION

Data on field germination in the sudangrass varieties is shown in Table. 1. Plant emergence (BBCH 10) under field conditions, was observed at doses up to 800 Gy from 4.0 to 38.84% of the control variants. The radiosensitivity in field conditions of the sudangrass varieties is higher than in controlled conditions of cultivation.

Under greenhouse conditions, germination of sudangrass ranged from 77.78 to 84.97% at 2000 Gy (Golubinova and Serafimov, 2015). The presence of emerging plants at significantly lower doses (to 800 Gy) determines LD<sub>50</sub> values of 410.27 to 420.30 Gy in field conditions. The weather conditions in the period from sowing to germination may enhance the effects of gamma-radiation. Therefore, field germination depends on the doses of

|  |  |
|--|--|
| -  | - seed irradiation and on the conditions of the environment - quantity and distribution of rainfall and average daily air temperatures.  |
| -  | - Similar results for enhancing the inhibitory effect of mutagenic doses on seed germination under field conditions reported Harding et al. (2012), Gaur et al. (2018). In the following growth stages, the percentage of dead plants compared in all varieties with increasing irradiation doses from 200 to 2000 Gy and the LD <sub>50</sub> values decreased.   |
| Harding et al. (2012), Gaur et al. (2018).   |  |
| 200 LD <sub>50</sub> 2000 Gy,  |  |
| (BBCH-47),   | - The effect of gamma-rays increases as vegetation progresses in all varieties. In the beginning of boot stage (BBCH-47), doses above 400 Gy cause complete lethality. At the end of the vegetation, surviving plants were recorded at doses of 200 Gy (from 63.7 to 74.0%) and 400 Gy (from 44.2 to 45.1%), which were shown to decrease over control variants in all varieties.  |
| 400 Gy   |  |
| 74,0%) 400 Gy (  |  |
| 200 Gy ( 63,7 44,2 45,1%),   |  |
| LD <sub>50</sub>   | - The LD <sub>50</sub> values decrease depending on the dose of irradiation. The strongest radioactivity is the Kazitachi variety (LD <sub>50</sub> =316.2 Gy), while Voronezhskaya 9 (LD <sub>50</sub> =333.8 Gy) and Vercors (LD <sub>50</sub> =339.8 Gy) are more radio-resistant. These data provide valuable information when choosing an optimal dose for irradiation of dry seeds for the purpose of mutation breeding specifically for these varieties. The variation in the radio-sensitivity between species of the same species and between varieties (Pathirana and Subasingbe, 1993), as well as the wide range of doses used to induce mutations in sudangrass (Lazanyi, 1987; Kirillenko and Golovin, 1987; Govovin and Kirilenko, 1989, Kostina et al., 1995, Kostina, 2000, Tabosa et al., 2007) is a prerequisite for the need for preliminary experimental determination of the working doses of gamma rays in the mutation breeding. |
| Kazitachi (LD <sub>50</sub> =316.2 Gy), 9 (LD <sub>50</sub> =333.8 Gy) Vercors (LD <sub>50</sub> =339.8 Gy)                            |  |
| -  |  |
| (Pathirana and Subasingbe, 1993),  |  |
| (Kirillenko and Golovin, 1987; Lazanyi, 1987; Golovin and Kirilenko, 1989; Kostina et al., 1995; Kostina, 2000; Tabosa et al., 2007) e |  |
| -  |  |
| -  |  |

1.

LD<sub>50</sub>Table 1. Field germination, survival and LD<sub>50</sub> in sudangrass

| BBC scale  | Gy       | /Varieties |      |                     |         |      |                     |                 |      |                     |        |
|------------|----------|------------|------|---------------------|---------|------|---------------------|-----------------|------|---------------------|--------|
|            |          | Kazitachi  |      |                     | Vercors |      |                     | Voronezhskaya 9 |      |                     |        |
|            |          | %          | F    | LD <sub>50</sub> Gy | %       | F    | LD <sub>50</sub> Gy | %               | F    | LD <sub>50</sub> Gy |        |
| BBCH-10    | 0        | 804        | 100  | 0,00                | 827     | 100  | 0,00                | 832             | 100  | 0,00                | 414,89 |
|            | 200      | 673        | 83,7 | 13,22**             | 696     | 84,2 | 13,00**             | 656             | 78,8 | 17,54**             |        |
|            | 400      | 495        | 61,6 | 31,27***            | 430     | 52,0 | 39,41***            | 485             | 58,3 | 34,50***            |        |
|            | 600      | 189        | 23,5 | 56,09***            | 271     | 32,8 | 52,78***            | 234             | 28,1 | 55,37***            |        |
|            | 800      | 26         | 3,23 | 38,84***            | 110     | 13,3 | 56,52***            | 33              | 4,0  | 42,26***            |        |
|            | -2000    | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
| BBCH-13    | 0        | 767        | 100  | 0,00                | 815     | 100  | 0,00                | 806             | 100  | 0,00                | 376,5  |
|            | 200      | 607        | 79,1 | 16,60**             | 664     | 81,5 | 15,12***            | 618             | 76,7 | 18,98***            |        |
|            | 400      | 454        | 59,2 | 32,43***            | 405     | 49,7 | 40,90***            | 428             | 53,1 | 38,06***            |        |
|            | 600      | 126        | 16,4 | 55,40***            | 137     | 16,8 | 57,14***            | 135             | 16,7 | 56,86***            |        |
|            | 800-2000 | 0          | 0,00 | 0,00***             | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
| BBCH-15    |          | 749        | 100  | 0,00                | 800     | 100  | 0,00                | 792             | 100  | 0,00                | 355,0  |
|            | 200      | 534        | 71,3 | 22,57***            | 648     | 81,0 | 15,40***            | 596             | 75,3 | 19,96***            |        |
|            | 400      | 421        | 56,2 | 34,35***            | 394     | 49,3 | 40,81***            | 387             | 48,9 | 40,91***            |        |
|            | 600      | 79         | 10,5 | 52,06***            | 71      | 8,9  | 51,92***            | 88              | 11,1 | 53,96***            |        |
|            | 800-2000 | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
| BBCH-17    |          | 739        | 100  | 0,00                | 796     | 100  | 0,00                | 785             | 100  | 0,00                | 343,5  |
|            | 200      | 501        | 67,8 | 25,16***            | 600     | 75,4 | 19,92***            | 583             | 74,3 | 20,68***            |        |
|            | 400      | 355        | 48,0 | 40,18***            | 380     | 47,7 | 41,92***            | 369             | 47,0 | 42,13***            |        |
|            | 600      | 45         | 6,1  | 45,48***            | 24      | 3,0  | 37,66***            | 18              | 2,3  | 33,76***            |        |
|            | 800-2000 | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
| BBCH-43-49 |          | 739        | 100  | 0,00                | 796     | 100  | 0,00                | 785             | 100  | 0,00                | 333,2  |
|            | 200      | 471        | 63,7 | 28,36***            | 589     | 74,0 | 21,07***            | 564             | 71,8 | 22,71***            |        |
|            | 400      | 327        | 44,2 | 42,85***            | 368     | 46,2 | 43,03***            | 354             | 45,1 | 43,51***            |        |
|            | 600      | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
|            | 800-2000 | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
| BBCH-56-65 |          | 739        | 100  | 0,00                | 796     | 100  | 0,00                | 785             | 100  | 0,00                | 333,2  |
|            | 200      | 471        | 63,7 | 28,36***            | 589     | 74,0 | 21,07***            | 564             | 71,8 | 22,71***            |        |
|            | 400      | 327        | 44,2 | 42,85***            | 368     | 46,2 | 43,03***            | 354             | 45,1 | 43,51***            |        |
|            | 600      | 0          | 0,00 | 0,00                | 0       | 0    | 0,00                | 0               | 0,00 | 0,00                |        |
|            | 800-2000 | 0          | 0,00 | 0,00                | 0       | 0    | 0,00                | 0               | 0,00 | 0,00                |        |
| BBCH-89    |          | 739        | 100  | 0,00                | 796     | 100  | 0,00                | 785             | 100  | 0,00                | 333,2  |
|            | 200      | 471        | 63,7 | 28,36***            | 589     | 74,0 | 21,07***            | 564             | 71,8 | 22,71***            |        |
|            | 400      | 327        | 44,2 | 42,85***            | 368     | 46,2 | 43,03***            | 354             | 45,1 | 43,51***            |        |
|            | 600      | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |
|            | 800-2000 | 0          | 0,00 | 0,00                | 0       | 0,00 | 0,00                | 0               | 0,00 | 0,00                |        |

\* (*t* crit. 2.0) = 0.05; \*\* (*t* crit. 2.6) = 0.01; \*\*\* (*t* crit. 3.3) = 0.001;LSD = 95 %/ The values of LD<sub>50</sub> at LSD = 95 % was calculatedLD<sub>50</sub>

Gamma-rays also negatively affect the fertility of sudangrass, which is a sensitive and stable criterion for determining the mutagenic effect of

2). „ - ”, . . .  
 ( 1).  
 Kazitachi, 15,83%  
 Vercors – 15,06%,  
 9 13,50%  
 (Parry, 2009).

gamma-rays ( able 2). In all varieties, a „dose-effect“ linear relationship is established, i.e., with increasing doses of irradiation, the percentage of sterile plants has been shown to increase relative to the respective control variants (Figure 2). According to this biological criterion, the varieties can be ranked in the following order: Kazitachi with 15.83% sterility, followed by Vercors with 15.06% and relatively more resistant to irradiation is Voronezhskaya 9 with 13.50% sterility.

In diploid species such as sudangrass, higher doses of gamma-rays lead to a significant decrease in fertility, which is essential in optimizing experimental doses for work and determining the volume of M<sub>1</sub> generation (Parry et al., 2009).

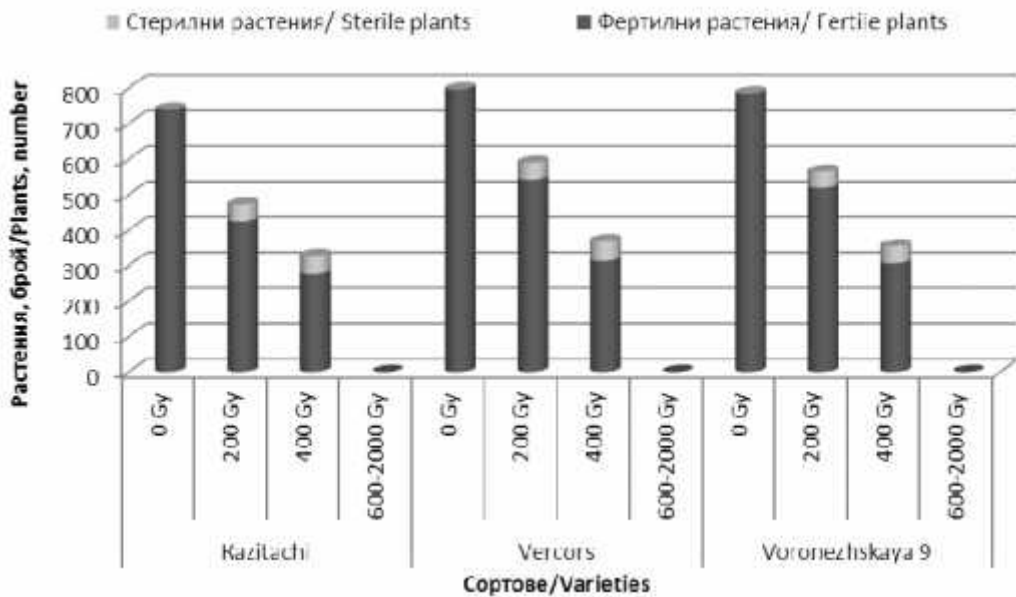
2.

( 1)

**Table 2. Influence of different doses of -rays on sterility in sudangrass in the M<sub>1</sub> generation**

| / Varieties     | Doses of irradiation, Gy | Parameters/                             |              |       |
|-----------------|--------------------------|---|--------------|-------|
|                 |                          | Plants at the end of vegetation, number | Sterility, % | F     |
| Kazitachi       | 0                        | 739                                     | 0            |       |
|                 | 200                      | 471                                     | 8,39         | 5,87  |
|                 | 400                      | 327                                     | 15,83        | 10,06 |
|                 | 600-2000                 | 0                                       | -            | -     |
| Vercors         | 0                        | 796                                     |              |       |
|                 | 200                      | 589                                     | 12,06        | 9,29  |
|                 | 400                      | 368                                     | 15,06        | 10,10 |
|                 | 600-2000                 | 0                                       | -            | -     |
| Voronezhskaya 9 | 0                        | 785                                     |              |       |
|                 | 200                      | 564                                     | 8,03         | 5,96  |
|                 | 400                      | 354                                     | 13,50        | 8,84  |
|                 | 600-2000                 | 0                                       | -            | -     |





. 1.

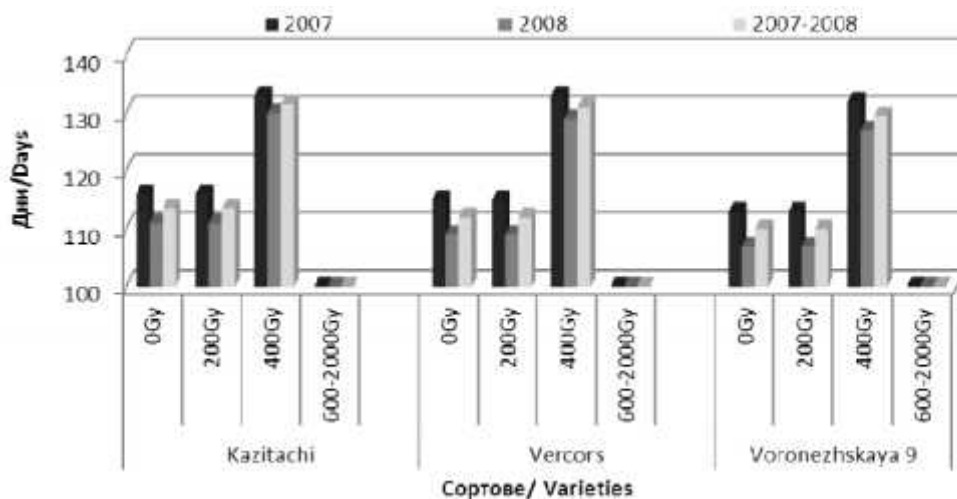
**Fig. 1. Quantitative ratio between fertile and sterile plants in Sudangrass depending on the mutagenic effect of gamma-rays**

200 Gy

17 20

( 2).

In the three tested varieties with an increase in radiation doses above 200 Gy, there was a tendency to prolong the vegetation period of 17 to 20 days. Obviously, irradiation of gamma-rays has a negative effect on plant growth and development, resulting in a delay in the onset of phenophases through ontogenesis, and hence prolongation of the vegetation period (Figure 2).



2. ( 1)

Fig. 2. Vegetation period in sudangrass varieties in first mutant generation ( 1) depending on the irradiation dose by year and average for the period

3).

BBCH-13 BBCH-47,

200 Gy. (400 Gy)

Gamma rays also affect the growth of the central stem (Table 3). Increasing the dose of radiation has shown a tendency to reduce the length of the central stem with weak genotypic differences in the varieties of sudangrass. Growth in the early stages of culture development from BBCH-13 to BBCH-47 is most strongly suppressed, and differences are statistically proven. Therefore, gamma-irradiation has a negative effect on the generative development of the plants. At the end of the vegetation the differences in the height of the central stem are negligible and statistically unproven reduced at 200 Gy. At the highest dose (400 Gy), the differences were statistically proven to be reduced, despite the significant growth-related compensation processes.

3.

( 1)

**Table 3. Influence of different doses of gamma-rays on height of the central stem in sudangrass in the first mutant generation ( 1)**

| Varieties /     | Gy        | / Plant height |        |        |           |           |         |
|-----------------|-----------|----------------|--------|--------|-----------|-----------|---------|
|                 |           | BBCH13         | BBCH15 | BBCH17 | BBCH43-49 | BBCH55-65 | BBCH89  |
| Kazitachi       | Control   | 8,67d          | 18,65d | 91.50c | 138.85c   | 185.25b   | 216.83b |
|                 | 200       | 7,53c          | 16,64c | 80.58b | 132.20b   | 178.53b   | 208.38a |
|                 | 400       | 4,28b          | 7,82b  | 36.30a | 102.38a   | 167.60a   | 201.18a |
|                 | 600       | 1,36a          | 2,35a  | -      | -         | -         | -       |
|                 | 800       | 1.02a          | -      | -      | -         | -         | -       |
|                 | 1000-2000 | -              | -      | -      | -         | -         | -       |
| Vercors         | Control   | 8.84c          | 19.91d | 91.50a | 132.90c   | 183.53b   | 205.60b |
|                 | 200       | 9.19c          | 16.70c | 80.58c | 124.28b   | 183.63b   | 208.00b |
|                 | 400       | 4.73b          | 7.59b  | 40.95b | 95.18a    | 161.40a   | 184.35a |
|                 | 600       | 1.14a          | 2.99a  | 5.80a  | -         | -         | -       |
|                 | 800       | 0.62a          | -      | -      | -         | -         | -       |
|                 | 1000-2000 | -              | -      | -      | -         | -         | -       |
| Voronezhskaya 9 | Control   | 11.02c         | 22.04d | 86.30b | 141.65c   | 205.38b   | 224.45b |
|                 | 200       | 10.61c         | 17.51c | 79.75b | 135.05b   | 178.28a   | 220.13b |
|                 | 400       | 5.22b          | 9.86b  | 51.18a | 110.35a   | 173.98a   | 201.90a |
|                 | 600       | 1.69a          | 3.32a  | -      | -         | -         | -       |
|                 | 800       | 1.16a          | -      | -      | -         | -         | -       |
|                 | 1000-2000 | -              | -      | -      | -         | -         | -       |

a, b, c, d, e -  
at P=0.01

P=0.01 / a, b, c, d, e - statistically proven differences

- Analyzing the results obtained with  
 - regard to reduced field germination and  
 - survival can be summarized as being a  
 - more sensitive criterion for assessing the  
 - sensitivity of the sudangrass. In order to  
 - increase the frequency of mutations in  
 - the next generation, it is important to  
 - optimize the dose of radiation (P rry,  
 (P rry,  
 2009).

### CONCLUSIONS

- The biological effect of gamma-rays  
 - in the range 200, 400, 600, 800, 1000,  
 200, 400, 600, 800, 1000,  
 1000, 1200, 1400, 1600, 1800 2000 Gy  
 3  
 ( 1)  
 - in three  
 - varieties sudangrass is expressed in a  
 - proven reduction in the number of  
 - emerging, fertile and surviving plants  
 - under field conditions.

- It was found that survival decreases  
 - as the gamma-rays increase and the  
 - duration of the vegetation period in field  
 - conditions. The highest radiosensitivity by  
 - this indicator shows the Kazitachi variety  
 Kazitachi (LD50=316,2 Gy), (LD50=316.2 Gy), followed by

|                   |                          |   |
|-------------------|--------------------------|---|
| 9 (LD50=333,2 Gy) | Vercors (LD50=339,8 Gy). | Voronezhskaya 9 (LD50=333.2 Gy) and Vercors (LD50=339.8 Gy).  |
| -                 | 400 Gy                   | Doses above 400 Gy cause complete lethality in all varieties. These ranges of LD50 values determined for the different varieties may be useful in selecting optimal doses of irradiation of dry seeds for the purposes of experimental mutagenesis in sudangrass. |
| LD50,             |                          |   |

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**1/2017 –**

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## **Synthetic Population 1/2017 – New Starting Material for Maize Selection. Phenotypic description**

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### **SUMMARY**

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In 2017 at Maize Research Institute (MRI) - town of Knezha was generated new synthetic population "1/2017" that unites 16 self-bred lines from the Institute's work collection. Lines originate from local populations (dent and hard dent) with high adaptive abilities and such ones that were established as a result of experimental mutagenesis in hybrids with proven qualities. They were selected after analysis of selective ability estimated in three testers belonging to different genetic groups. In an isolation field the lines demonstrating a high total combinative ability were re-bred with equal seed number to establish the synthetic population. In 2018 this synthetic was included in a program for periodical selection in order to increase its combinative ability. An improvement selection with two lines - PAU 1617 and B 113 began. The necessary number of breedings' and their respective self-bred ears were achieved. The hybrids are to be tested in Preliminary Varietal Experiments

(PVE) and an improved synthetic population should be formed.

- The aim of this research is to establish and improve a new starting material for maize selection. After the execution of periodic selection with this synthetic, perspective lines will be produced from it and then will be created high-yield, adaptively valuable maize hybrids.

**Key words:** synthetic maize population, self-bred maize lines, combinative ability, periodical selection

## INTRODUCTION

- Contemporary seed production requires high-yield maize hybrids that are resistant to biotic and abiotic stress (Carena, 2011). Successful programs for generating such hybrids depend to a great extent on the starting material as a basis for effective work, for its variability and direction for its production.

(Carena, 2011).

- The increase of desired genes in starting material increases directly the effectiveness of selection process as well because thus the production of genotypes with desired qualities is facilitated (Mustyatsa, 2009; Suprunov, Chuprina, 2010; Hallauer and Carena, 2012; Suprunov et al., 2013).

(Mustyatsa, 2009; Suprunov and Chuprina, 2010; Hallauer and Carena, 2012; Suprunov et al., 2013).

- Taking in consideration the relatively small number of lines that are used worldwide and the growing danger of genetic erosion in maize, the work for gene pool extension becomes more important. This is done by production and improvement of new starting material and appears to be a long process. In the same time practice demands fast results from selection and commercial hybrids for immediate application. This poses the question of necessity to apply not only the traditional selection methods and but also some rare and effective programs and their modifications. One of these is the

(Novoselov, 2008).

periodic selection that applies to the maximum the genetic mechanisms in their full specter and whose effectiveness is not doubted today (Novoselov, 2008).

Synthetic populations previously generated in the needed direction of selection are dynamic depot of germ plasma and favourable genes' concentration and this allows long-term and effective improvement work.

The aim of this research is to establish and produce a new starting material for maize selection.

## MATERIAL AND METHODS

For the research target were used 16 medium late and late self-bred maize lines received after classic selection in local maize populations (Dent and Hard dent synthetic) and lines that were established as a result of experimental mutagenesis in heterozygous material with proven qualities. In 2017 in an isolation field of MRI - town of Knezha these lines were re-bred with equal seed number to achieve synthetic population on broad gene basis. In the synthetic was applied massive selection.

The study of synthetic's phenotypic features, its morphologic and biologic character is based on the executed phenologic observations in the following stages: "germination", "tasseling", "ear flowering" and "ripening".

In 2018 this newly established synthetic was included in a program for periodic selection (Hull, 1945) with two combinatively valuable self-bred lines - PAU 1617 and B 113 as recurrent parents; the target was to improve the population's combinative ability. In the selection field were seeded 2000 plants from the synthetic population and opposite them - 1000 plants from each line-improver. As a result of the breeding

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(Hull, 1945)

PAU 1617 113

2000

1000



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 (Sotchenko et al, 2016).  
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 (Sevov  
 and Sevov., 2011; Pencheva and  
 Vulchinkov, 2013; Pencheva, 2017/  
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 (Valkova, 2005).

between them, under isolator were produced 300 experimental hybrids – 162 with line PAU 1617 and 138 with line B 113. In the synthetic were produced respective self-bred ears.

In 2019 these experimental hybrids will be tested in PSO and then the best seeds will be selected as improvers. They will form the improved population for next selection cycle.

## RESULTS AND DISCUSSION

- Maize selection requires constant  
 - extension of the gene pool with self-bred  
 - lines, synthetic populations, local and  
 - introduced forms that possess valuable  
 economic characteristics. When included  
 in selection programs of heterosis  
 selection they guarantee the production of  
 high-yield hybrids from different maturity  
 groups and ways for application  
 (Sotchenko et al., 2016). Several authors  
 surveyed the potential of local maize  
 populations but it has not been completely  
 exploited (Sevov and Sevov, 2011;  
 Pencheva and Vulchinkov, 2013;  
 Pencheva, 2017).

Synthetic “1/2017” (Figure 1) is generated on a broad genetic basis and it unites 16 stabilized, self-bred lines received after application of classic selection methods in local forms and chemical mutagenesis in heterozygous material. The lines included in this synthetic were tested with three testers to analyze their combinative ability (Valkova, 2005). The ones demonstrating high general combinative ability and complying with the direction for synthetic production were combined with equal number of seeds and then were re-bred in an isolation field.



Fig.1. Syntetic „1/2017“

„per se“.

30

50

1000

625 (

600.

130

625.

The syntetic was studied and described after a “per se” estimation. Biometric measurements of 30 normally grown plants have been done for the following features: “plant height”, “height to panicle”, “number of above ground nods”, “length and width of ear leaf”, “number of panicle lateral branches”. In full maturity stage, 50 ears from the average sample were measured for the following indices: “ear length”, “number of ear rows”, “grain length”, “hectoliter weight”, “1000 grain weight”, “yield” and “grain yield”.

In the comparative analysis hybrid Knezha M 625 (group pursuant FAO above 600) was used as control variant (standard). The executed description of this syntetic and the complex estimation of its indices give ground for its more effective application in particular selection programs and for a comparative estimation once the improvement work with it ends.

This syntetic is late, group pursuant FAO above 600. The period from germination to physiological maturity is 130 days. It develops floss at the same time as the group’s standard – Knezha M

- 492 kg/da,  
 17.6%.  
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 ,  
 80-95 cm. -  
 -  
 - 12.8-12.9 mm.  
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 ,  
 22-24 cm,  
 - 12-14  
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 290 g,  
 - 83,7 %.  
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625. The average yield is comparatively high - 492 kg/da with grain moisture content of 17.6%. The plant are high, with two cobs, the main cob is at 80-95 cm. This synthetic is resistant to lodging and bending. Plants have wide leaf blade of the cob leaf – 12.8-12.9 mm. Cobs are slightly conical and cylindrical with average length of 22-24 cm with small number of rows – 12-14 with big grain. Spindle is heterogenous – white, red and small percentage rose-coloured. Grain colour is yellow, yellow-orange to orange-red, its type is horse dents. Panicle is with prevailing average number of lateral branches and small number of plants are with multiple panicle. Synthetic population has 1000 grains weight of 290 g, the yield is comparatively high – 83.7%. Its protein and fat content is higher than the group standard, while the data for its starch content are contradictory.

All characteristics are with averagely high variation coefficient; for the characteristics “plant’s height” and “spindle colour” variation ranked more than 20%. The high variability in this synthetic allows long-term work without any danger its genetic basis to be limited.

In 2018 this synthetic was included in a periodic selection program to increase its combinative ability, to generate new perspective lines and to produce high-yielded, adaptively valuable maize hybrids. Improvement selection with two self-bred lines - PAU 1617 and B 113 started. As a result of the breeding between them, under isolator were produced 300 experimental hybrids – 162 with line PAU 1617 and 138 with line B 113. Respective self-breedings were done in synthetic population.

The hybrids are to be tested in preliminary varietal trials, evaluation and analysis of results to form improved synthetic population.

## CONCLUSIONS

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( ) - <https://www.mon.bg/bg/58>

- Synthetic maize population “1/2017” was established in the late maturity group, FAO above 600, and thus the collection of MRI - town of Knezha was broadened with new starting material.
- To form this population were used 16 combinatively valuable lines which guarantees variability presence for sustained screening and selection.
- The synthetic was included in an improvement program with selected recurrent parents - PAU 1617 and B 113 in order to achieve progress in combinative ability of the lines produced by it.

## ACKNOWLEDGEMENTS

- This synthetic and the improvement work with it are being included in National Scientific Program (NSP) “Healthy Food for Strong Bio-economics and Life Quality” of Ministry of Education and Science (MES) - <https://www.mon.bg/bg/58>

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## Ecological Remote Testing the Productive Opportunities for Feed Grain of the New Yielding Bulgarian Hybrid Maize Kneja 561 in the Conditions of the Republic of Romania and the Republic of Serbia

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Original scientific paper

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### SUMMARY

- The article presents the results of  
- ecological remote testing productive  
- opportunities for feed grain of the new  
yielding Bulgarian hybrid maize Kneja 561  
in the conditions of the Republic of  
Romania and Serbia. As a result of the  
study, the following conclusions are  
drawn:

The high-yielding Bulgarian hybrid  
maize Kneja 561 tested in the conditions  
of the Republic of Romania and Republic  
of Serbia for the production of forage  
grain has exceeded unambiguously the  
corn hybrids used for standards in these  
countries.

The hybrid Corn Kneja 561 is a  
high yielding hybrid, with moisture on the  
nipple while harvesting close to the  
standard and with high performances  
index - Pi.

- As a result of the ecological remote

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(Russel, 1986; Hallauer et al., 1988).

(Vulchinkov et al., 2003; Genov and Genova, 2005; Yordanov, 2006, 2010, 2014; Glogova, 2010; Valkova and Petrovska, 2018).

testing of the productive opportunities for feed grain of the new yielding Bulgarian hybrid maize Kneja 561 under the conditions of the Republic of Romania and the Republic of Serbia, a scientifically justified conclusion can be drawn that it is competitively capable and High yielding hybrid maize suitable for exporting seeds in these countries. Hybrid maize Kneja 561 combines the best high yield of feed grain with low moisture of the grain.

**Key words:** maize, new hybrid Kneja 561, ecological testing, Romania, Serbia

## INTRODUCTION

The selection and seed production of new, more productive varieties is the most important practical result of the purposeful, difficult and long-lasting scientific research on maize and other crops. And the most in-depth research on plants, the use of various new scientific methods and techniques would not be particularly relevant if they do not lead to the creation of new, higher yielding, better and more sustainable varieties than previously existing if they do not lead to real selection progress (Russel, 1986; Hallauer et al., 1988).

Fortunately, in Bulgaria, new highly productive corn hybrids are still being created, despite the humiliating funding and attitude towards science in Bulgaria, and thanks to the self-denial and patriotism of most Bulgarian scholars (Vulchinkov et al., 2003; Genov and Genova, 2005; Yordanov, 2006, 2010, 2014; Glogova, 2010; Valkova and Petrovska, 2018 and others).

In order to assess the real qualities of each new variety, it must be tested under the widest possible range of ecologically geographic conditions and compare its productive or other qualities to the best standard varieties used in those areas. Failure to comply with these basic rules leads to a misleading

- assessment of the true qualities of the variety. Sooner or later, such a variety will be rejected by farmers, which are the most accurate and objective criterion for the qualities of the new variety.

- The aim of this study was to

- evaluate the most objectively productive qualities for the production of feed grain of the new Bulgarian high-yielding corn Kneja 561 hybrid, with the help of ecologically remote randomized variety experiments on the territory of R. Serbia, R. Romania and Bulgaria as possible hybrid hybrid destination.

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- assessment of the true qualities of the variety. Sooner or later, such a variety will be rejected by farmers, which are the most accurate and objective criterion for the qualities of the new variety.

The aim of this study was to

- evaluate the most objectively productive qualities for the production of feed grain of the new Bulgarian high-yielding corn Kneja 561 hybrid, with the help of ecologically remote randomized variety experiments on the territory of R. Serbia, R. Romania and Bulgaria as possible hybrid hybrid destination.

**MATERIAL AND METHODS**

Hybrid corn Kneja 561 is a medium late grain maize hybrid, FAO group 500. There is a Patent Certificate No. 11132 P2 of 12.12.2017. It was created at the Maize Research Institute - Kneja by Assoc. Prof. Georgi Yordanov by the method of the two-loop hybridization between genetically remarkable selection materials.

- The selection process for the establishment and initial testing of the hybrid covers the period 2003-2012. In 2013, the hybrid is presented for a test on Biological and Economic Characteristics (BIA) and Distinct, Homogeneity and Stability (RHS) in the State Agency of Bulgaria - IASAS.

In 2015 from Hybrid Kneja 561 maize were sent hybrid seeds for testing in randomized block trials in three replicates in R. Serbia and in Romania, and for comparison with their high yielding maize standards.

The yields of standard grain per hectare, grain moisture during harvesting and performance index - Pi were investigated as the ratio of grain yield to grain moisture during harvest.



The experiments were performed using the method and technology adopted for the Republic of Serbia and the Republic of Romania and the results of these experiments were provided to us in an authentic manner according to their way of reporting the results obtained.

At the same time, the hybrid test was continued at IASAS and the Maize Reserch Institute - Kneja, Bulgaria. An ecologically remote test of Hybrid corn Kneja 561 was carried out at four geographic distant points to ensure the most objective evaluation of the productive qualities of the grain yield of the new high-yielding Bulgarian hybrid corn Kneja 561.

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## RESULTS AND DISCUSSION

As a result of the testing of the Kneja 561 corn hybrid production capabilities in Preliminary and Contest Variety Tests, it was found that the hybrid produced high and stable yields of grain exceeding those of most of the hybrid maize standards used.

This gave us reason to continue further testing the hybrid at further, higher and environmentally remote test levels. For this purpose hybrid hybrids Kneja 561 hybrids were sent for testing in R. Serbia and in Romania, and for comparison with their high yielding maize standards.

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The trials were conducted under non-irrigation conditions on the accepted maize growing technology in these countries, in randomized block trials in three replicates.

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Figure 1 shows the cobs in standard technological maturity, a cob and grain cut from the new high-yielding Bulgarian corn Kneja 561. As can be seen from the figure, the cobs are well shaped, garnished to the top with thin cocoa and high grain yield – up to 90% of cob weight.

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90%



Fig. 1. Hybrid Kneja 561 (Patent Certificate Reg. 11132 P2 from 12.12.2017)

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Table 1 presents the results of comparative testing between the Bulgarian corn hybrid Kneja 561 and highly productive Romanian hybrids under the conditions of Fundulia, Romania in 2015 /without irrigation/. As can be seen from the table, the Bulgarian Kneja 561 corn hybrid has performed very well against the other Romanian hybrids used in the experiment. With an average yield of 9422.0 kg/ha of standard

|       |          |
|-------|----------|
| kg/ha | 9422,0   |
|       | -        |
|       | 124,8%,  |
|       | -        |
|       | -        |
|       | - 16.1%, |
| 2%    | -        |
|       | -        |
|       | 561      |
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| Pi",  | "        |
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grain, it consistently exceeded Romanian corn standard by 124.8% and also the other hybrids used in the experiment with different but also higher percentages.

The high yield standard grain derived from the hybrid is also combined with a low moisture grain at harvest - 16.1%, which is close to the standard maize grain moisture and was 2% lower than that of the Romanian hybrid standard.

This combination of high yield grain with low grain moisture is characteristic of the Bulgarian corn Kneja 561 hybrid and is particularly appreciated by the farmers. This dependence is best expressed by the so-called "performance index - Pi", which is presented in the last column of the table. As we can see in this index, our hybrid is the highest indicator against other hybrids in the experience.

As a result of the testing of the Kneja 561 corn hybrid production capacity in the Republic of Romania, we can scientifically conclude that it is a competitive and high-yielding corn hybrid suitable for exporting the seeds in that country.

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**Table 1. Results of a comparative test between the Bulgarian Kneja 561 maize hybrid and highly productive Romanian hybrids under the conditions of Fundulia, Romania in 2015 / without irrigation/**

| Name of Test hybrids   | kg/ha<br>14%<br>Grain yield<br>kg/ha<br>14% moisture | %<br>The yields to<br>the standard | %<br>Grain moisture<br>at harvest<br>% | ±<br>%<br>±to the humidity<br>of the standard<br>% | Pi<br>Performance<br>index<br>Pi |
|------------------------|--|------------------------------------|--|--|----------------------------------|
| <b>561/Kneja 561</b>   | <b>9422,0</b>  | <b>124,8</b>                       | <b>16,1</b>                            | <b>-2,0</b>  | <b>5,8</b>                       |
| EX 563                 | 9009,0   | 119,3                              | 15.8                                   | -2,3   | 5,7                              |
| NP 14-64               | 8528,0   | 113,0                              | 16.2                                   | -1,9   | 5,3                              |
| XML 14                 | 7306,0   | 96,8                               | 19.1                                   | +1,0   | 3,8                              |
| EC 501                 | 6649,0   | 88,0                               | 17.6                                   | -0,5   | 3,7                              |
| <b>Olt – /standard</b> | <b>7549,0</b>  | <b>100,0</b>                       | <b>18.1</b>                            | <b>0,0</b>   | <b>4,2</b>                       |

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Table 2 presents the results of a comparative test between the Bulgarian hybrid corn Kneja 561 and highly

561  
 / /  
 561  
 11392.0 kg/ha  
 ZP 666 107.7%,  
 - 15.9%,  
 2,8 %  
 561  
 - Pi",

productive Serbian hybrids in the conditions of Zemun Pole - Belgrade, R. Serbia in 2015 /without irrigation/. As can be seen from the table, the Bulgarian Kneja 561 corn hybrid has performed well with other Serbian hybrids used in the experiment.

With an average yield of 11392.0 kg/ha, it has convincingly exceeded the well-known Serbian standard ZP 666 maize with 107.7%, and the other well-known hybrids with different but also higher percentages. The high yield standard grain obtained from the hybrid is also combined with a low moisture grain at harvest - 15.9%, which is close to the standard maize grain moisture and it was 2.8% lower than that of the Serbian hybrid standard. This combination of high yield grain with low grain moisture is characteristic of the Bulgarian corn Kneja 561 hybrid and is particularly appreciated by the farmers. This dependence is best expressed by the so-called "performance index - Pi", which is presented in the last column of the table. As we can see in this index, our hybrid is the highest indicator against other hybrids in the experience.

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**Table 2. Results of a comparative test between the Bulgarian Kneja 561 maize hybrid and highly productive Serbian hybrids in the conditions of Zemun Pole - Belgrade, R. Serbia in 2015 / without irrigation/**

| Name of Test hybrids         | kg/ha<br>14%<br>Grain yield<br>kg/ha<br>14%<br>moisture | %<br>The yields to the<br>standard | %<br>Grain moisture at<br>harvest<br>% | ±<br>%<br>±to the humidity<br>of the standard<br>% | Pi, %<br>Performance<br>index<br>Pi,% to<br>standard |
|------------------------------|---|------------------------------------|--|--|--|
| <b>561/Kneja 561</b>         | <b>11392.0</b>  | <b>107.7</b>                       | <b>15.9</b>                            | <b>-2,8</b>  | <b>126.9</b>   |
| NS 6030                      | 9602.0  | 90.8                               | 18.9                                   | +0,2   | 89.8   |
| B-15                         | 9812.0  | 92.8                               | 17.1                                   | -1,6   | 101.3  |
| NP 14-70                     | 9703.0  | 91.7                               | 16.9                                   | -1,8   | 101.7  |
| NP 14-64                     | 9632.0  | 91.1                               | 15.7                                   | -3,0   | 108.6  |
| <b>ZP 666 –<br/>standard</b> | <b>10577.0</b>  | <b>100,0</b>                       | <b>18.7</b>                            | <b>0,0</b>   | <b>100.0</b>   |

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As a result of the testing of the Kneja 561 corn hybrid production capacity in RS, we can scientifically conclude that it is a competitive and high-yielding hybrid of corn suitable for exporting seeds to that country.

## CONCLUSIONS

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The high-yielding Bulgarian hybrid corn Kneja 561, tested in the conditions of R. Romania and R. Serbia for the production of forage grain, exceeded the unmistakably used hybrid maize hybrids in these countries.

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Hybrid corn Kneja 561 is a high-yielding hybrid with grain moisture at harvest close to the standard and high performance index Pi.

- Pi.

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As a result of the ecologically remote study of the productive possibilities for feed grain of the new high-yielding Bulgarian Kneja 561 corn hybrid in the conditions of Romania and R. Serbia, it can be scientifically concluded that it is a competitive and high yield hybrid corn suitable for the export of seeds to these countries.

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Hybrid Kneja 561 corn combines the best grain yield with low grain moisture.

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