

## GENOTYPES VARIATION OF *Medicago sativa* (L.) SEED YIELD COMPONENTS IN ACID SOIL UNDER CONDITIONS OF CROSS – FERTILIZATION

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Alfalfa (*Medicago sativa* L.) is the most important forage plant species in Bosnia and Herzegovina. In this study, conducted in Banja Luka, the investigated ten alfalfa genotypes originated from different regions of USA and Europe. The study objective was to determine their grain yield per plants and four morphological properties. The interactions analyses provided to select individual genotypes that are shown the highest stability and yield potential in different production conditions. Genotype with the highest yield shown lower stability level. The influence year and genotype on GYP – the grain yield per plants for investigated alfalfa genotypes was significant ( $F_{\text{exp}}=7.54^*$  and  $F_{\text{exp}}= 8.34^*$ ). The highest average value for investigated parameter in the first research year had genotypes G53 and G44 and in second research year genotype G2. The lowest value in both research years had genotype G100 (0.28 kg ha<sup>-1</sup> and 0.30 kg ha<sup>-1</sup>). Positive and strong correlations were found between the grain yield per plants and the number of pods per plants ( $r= 0.74^{**}$ ) and the grain yield per plant and the number of flower per inflorescence ( $r= 0.51^*$ ). On the basis of the obtained values, we conclude that we have excellent genotypes G2, G44, G53 and G51 for successful breeding work in order to obtain new high-yielding varieties of alfalfa.

*Key words:* *Medicago sativa*, genotypes, grain yield components, variability, correlations.

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## INTRODUCTION

The perennial, autotetraploid ( $2n = 4x = 32$ ), allogamous, seed-propagated plant alfalfa (*Medicago sativa* L.) is a member of the *Fabales* order, Fabaceae family, Trifolieae tribe, and the genus *Medicago*. Nowadays, alfalfa is considered as one of the most widely grown forage legumes in the world, and its important nutritive value makes it ideal for livestock and dairy production (VERONESI *et al.*, 2010).

Alfalfa is one of the most important perennial leguminous plants that is primarily used for animal feed production and is known for being sensitive to low pH of the soil. In the world, and even in our country, the cultivation of alfalfa is becoming a growing problem due to the increasing acidification of the land due to the application of mineral fertilizers, acid rain and other reasons.

In intensive conventional production, we have a constant acidification of soil due to the application of nitrogen fertilizers (BOLAN *et al.*, 1991). The increase in the acidity of the soil on the arable areas adversely affects the growing of leguminous plants, reduces the yield of feed and reduces the use time of such production areas. It is estimated that in the world there are 40% of agricultural land with a pH value lower than 5.5 (VONUExKULL and MUTERT, 1995). The cultivation of alfalfa on the soil in the process of acidification is of multiple importance. By establishing production areas with leguminous plants that have the ability of nitrogen fixation, the use of nitrogen fertilizers is significantly reduced, which contributes to the slowing down of soil acidification processes, reduces nitrate „flushing” and environmental pollution (GRAHAM and VANCE, 2003). On land with a lower pH, alfalfa cultivation can be handled in several ways, and one of them is a selection of varieties tolerant to increased acidity of the soil and higher aluminium content.

In the case of populations of common alfalfa, there is no significant genetic variation in terms of resistance to soil acidity in production conditions (BOUTON, 1996). The selection of new varieties of alfalfa showing tolerance to lower pH and high aluminium content is complex, but it is justified if they are grown on soils whose lower horizons are acidic (STEVOVIĆ *et al.*, 2012). The growth and development of alfalfa is largely influenced by the pH of the soil and high concentration of aluminium (RAHMAN *et al.*, 2014).

The success of the selection depends to a large extent on the divergence of the starting material. The most reliable method for collecting the initial selection material of alfalfa tolerant on acid soils is the cultivation of collected populations in field conditions on soils with a lower pH value without calcification. By examining a large number of alfalfa varieties on acidic and neutral soils, it has been found that there is a significant variability in tolerance to lower pH values (PETCU *et al.*, 2006). In the selection process, finding solutions to the problem of growing alfalfa on acid soils is primarily related to the plant itself, and not to the *Rhizobium bacteria* (BORDELEAU and PREVOST, 1994). The aim of these tests is to determine the possibility of seeding of selected alfalfa genotypes that showed tolerance towards lower pH of the soil. The achieved results of these tests should be used in the program of selection of alfalfa varieties tolerant to acid soils and in this way, it enables the spread of the area of their cultivation on soils whose pH ranges from 5.1 to 6.2.

Plant production is significantly dependent on environmental conditions. Knowledge of the interlinkages between important indicators makes it possible to improve a greater number of

signs simultaneously, especially for those parameters with low genetic variability, in which the success of the selection is achieved by indirect methods (PARIHAR *et al.*, 2015). The establishment of correlation relationships between the signs of variation lines consisting of a variety of genotypes kept under the same conditions is the basis for establishing objective criteria for the conduct of the selection (KOSEV and VASILEVA, 2021).

The main objective of this study was testing of 10 alfalfa genotypes to find their potential for grain yield per plant (GYP) and four most important parameters of alfalfa. The other objective was to determine the correlation between the examined characteristics.

## MATERIALS AND METHODS

### *Field trial management*

The collection of 68 varieties and 8 autochthonous alfalfa populations was sown in 1998 on the field of the Agricultural Institute of the Republic of Srpska on low acidity soil (pH 5.7-5.8 in H<sub>2</sub>O). During the two-year cultivation in all sown genotypes there were decay of the plants and plant assembly in a certain percentage. After two years of breeding, 41 genotypes were selected and from them the seeds from second cutting were taken under conditions of cross-fertilization. In the early spring of 2004, the selected alfalfa genotypes were sown on the Institute's experimental field on brown soil on gravel or Cambisol according to the FAO classification. The experiment was based on four repetitions, and the surface of the base parcel was 2.5 m<sup>2</sup>. According to the experiment results, the pH of the soil on the experimental plot varied from 5.33 to 5.64 in H<sub>2</sub>O, or from 4.11 to 4.41 in KCl.

After sowing, most of the genotypes were come up, but by the end of the vegetation season, some of them had extinction of up to 90% of the arisen plants. For several years, the agronomic properties of all surviving alfalfa genotypes were monitored. After 5 years seed from 10 genotypes selected on the basis of agronomic properties was taken from the second cutting under conditions of cross-fertilization. The creation of a new experiment with selected genotypes was carried out in the spring of 2009 at a location in Maglajani where pH value was from 5.0 to 5.1 in H<sub>2</sub>O, and from 3.8 to 4.0 in KCl. The experiment was based on a soil with low to medium content of easily accessible phosphorus (11.4 to 13.3 mg / 100 g of soil), while the content of easily accessible potassium was very good. The selected genotypes are sown with 30 plants per repetition. The experiment was set in 4 replications, and each alfalfa genotype was represented by 120 plants. The sowing was done manually with 2 seeds per hole at a distance of 80 cm between rows and a depth of sowing was 1.5-2 cm. During these tests genotypes with following labels were used: G-2, G-11, G-19, G-34, G-39, G-44, G-48, G-51, G-53 and G-100. The aforementioned alfalfa genotypes originally lead from the following varieties grown under conditions of cross-fertilization: G-2 from the variety Vuka, G-11 from the varieties OS-66, G-19 and Apica, G-34 from the variety Gloria, G-39 from the variety Pampeana, G-44 from genotype Petrovac, G-48 from genotype1/81 exp., G-51 from the variety Adriana, G-53 from the genotype Albfa and G-100 from the genotype Maglajani 99. During 2009 and 2010, the experiment was maintained by mowing. In the period from 2011-2012 in the second cutting, the following seed yield components were monitored: the number of flower per inflorescence, the number of pods per inflorescence, the number of pods per plant, the number of seeds per pod and grain yield per plant (kg ha<sup>-1</sup>).

### Statistical analysis

The obtained results of the research were processed by the statistical method of variance analysis (ANOVA), and the significance of mean values difference was determined by the LSD test. The dependence between individual properties of the studied alfalfa genotypes was determined by calculating the correlation coefficient ( $r$ ). The total variation of the dependent variable attributable to the variation in the independent variable is calculated using the determination coefficient ( $R^2$ ).

## RESULTS AND DISCUSSION

### Meteorological conditions

Meteorological conditions are highly variable and unpredictable and greatly affect stability and yield (POPOVIĆ *et al.*, 2012; JANKOVIĆ *et al.*, 2018; LAKIĆ *et al.*, 2019; TERZIĆ *et al.*, 2019; POPOVIĆ *et al.*, 2020a; 2020b; JOVANOVIĆ-TODOROVIĆ *et al.*, 2020; PERIĆ *et al.*, 2021). The data of the Republic Hydrometeorological Institute of the Republic of Srpska was used for the analysis of weather conditions. Temperatures during the winter period in both years of testing were higher compared to the multi-year average. Multi-year average temperature for the period from 1961-2004 was 10.9°C, and during the vegetation period was 16.4°C. The total amount of precipitation from IV-X months for the period 1961-2004 was 650.0 mm, Figure 1.

During the experiments implementation the average temperatures during the vegetation periods were higher compared to the multi-year average. During the growing seasons, the first year of the study was warmer for 2°C and the second for 2.6°C. The amount of precipitation during the growing season of the first year was 302.7 mm lower than the multi-year average. In the second year of the experiment there was 575.2 mm of precipitation, which is 74.8 mm less than the same period of multi-year average. The minimum precipitation during both years of testing was during the month of August. During these tests, the meteorological conditions for the production of alfalfa seed were more favourable in 2012. In 2011, a large amount of precipitation (112.7 mm) fell in July, which had an adverse effect on fertilization, Figure 1 and 2.

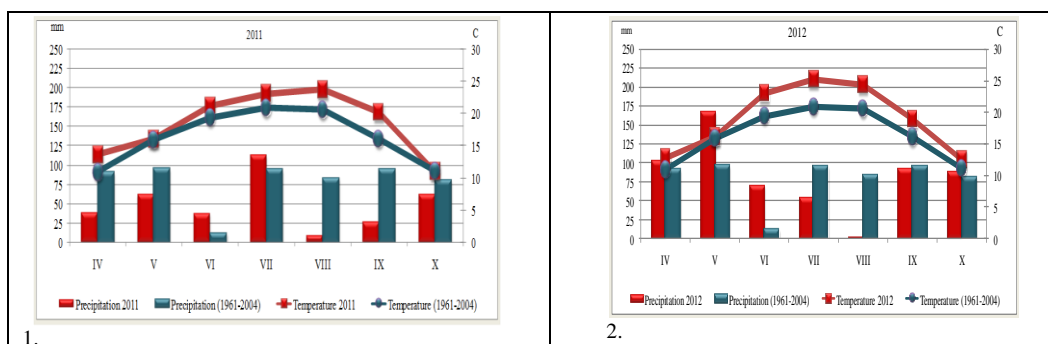


Figure 1-2. Temperatures (°C) and precipitation (mm) in 2011 (1.), and 2012 (2.), Banja Luka

Under rainfed conditions, plants with excellent adaptation are needed, hence, it would be worth focusing on perennial grass species well suited for specific sites and with low demand for inputs (GULIAS *et al.*, 2018).

*Morphological and productivity characteristics*

The influence of the year and the genotype, as well as the interaction of the same on the studied alfalfa properties, is shown in Table 1. Analysis of variance for the observed morpho-agronomic traits showed significant differences between breeding genotypes of alfalfa for all properties in tested year, except for the NPI (Tables 1-2, Figures 3-7).

Table 1. ANOVA for tested parameters

Effect	SS	Degr. of Freed.	MS	F	p
NFI - Number of flowers per inflorescence					
Intercept	18727.20	1	19827.20	2076.95**	0.00000
Genotype	713.05	9	79.23	8.787**	0.00000
Year	39.20	1	39.20	4.348*	0.04133
G × Y	201.55	9	22.39	2.484*	0.01753
Error	541.00	60	9.02		
NPI - Number of pods per inflorescence					
Intercept	4089.80	1	4089.80	1112.87**	0.00000
Genotype	238.95	9	26.55	7.224**	0.00000
Year	7.20	1	7.20	1.959 <sup>ns</sup>	0.16675
G × Y	51.55	9	5.73	1.559 <sup>ns</sup>	0.14877
Error	220.50	60	3.68		
NPP - Number of pods per plant					
Intercept	2493945.01	1	2493945.01	383.27**	0.00000
Genotype	165852.02	9	18429.01	2.832*	0.00762
Year	288360.02	1	288360.05	44.315**	0.00000
G × Y	113314.11	9	12590.01	1.935*	0.06382
Error	390418.30	60			
NSP - Number of seeds per plant					
Intercept	1824.05	1	1824.05	1170.51**	0.00000
Genotype	84.70	9	9.441	6.039**	0.00001
Year	11.25	1	11.250	7.219*	0.00932
G × Y	20.50	9	2.278	1.462 <sup>ns</sup>	0.18322
Error	93.50	60	1.558		
GYP – Grain yield per plant					
Intercept	45.814	1	45.814	391.16**	0.00000
Genotype	7.952	9	0.884	7.54**	0.00000
Year	0.977	1	0.977	8.34*	0.0054
G × Y	0.9940	9	0.110	0.94 <sup>ns</sup>	0.4955
Error	7.0274	60	0.117		

<sup>ns</sup> – not significant; \* and \*\* significant at 0.05% at 0.01%; G-Genotype; Y- Year;

The influence of the genotype and year on NFI - Number of flower per inflorescence on investigated alfalfa genotypes was significant ( $F_{\text{exp}}=8.787^{**}$ ,  $F_{\text{exp}}=4.348^{*}$ ). Based on the analysis

of variance, it can be concluded that the interaction of the genotype  $\times$  year significantly influenced NFI ( $F_{\text{exp}} = 2.484^*$ ) in the investigated alfalfa genotypes, Table 1.

Average values of the number of flower per inflorescence (NFI), the Number of pods per inflorescence (NPI), the Number of seeds per plant (NSP), the Number of pods per plant (NPP) and Grain yield per plant (GYP) in the investigated alfalfa genotypes are shown in Table 2.

Table 2. Average value for tested morpho-productive traits of tested genotypes, B&H

Parameter	Genotype										
	Year	G2	G11	G19	G34	G39	G44	G48	G51	G53	G100
NFI– Number of flower per inflorescence											
2011	18.3	17.3	13.5	11.8	15.8	13.0	11.5	14.8	19.0	11.3	14.6
2012	20.0	16.2	10.3	12.5	13.3	21.8	13.0	17.8	21.8	13.3	16.0
$\bar{x}$	19.2	16.8	11.9	12.1	14.5	17.4	12.3	16.3	20.4	12.3	15.3
CV, %	6.28	4.64	19.01	4.07	12.15	35.76	8.66	13.01	9.71	11.49	6.47
NPI – Number of pods per inflorescence											
2011	8.00	7.25	5.25	5.25	6.50	11.25	6.25	6.00	8.25	4.50	6.85
2012	8.87	7.50	5.00	6.75	8.00	8.25	7.50	8.25	11.0	3.50	7.45
$\bar{x}$	8.44	7.38	5.13	6.00	7.25	9.75	6.88	7.13	9.63	4.00	7.15
CV	7.29	2.39	3.45	17.67	14.63	21.75	12.86	22.33	20.20	17.68	5.93
NSP– Number of seeds per plant											
2011	6.25	4.00	3.75	3.50	2.50	5.00	4.75	4.75	5.00	4.50	4.40
2012	7.00	3.75	3.00	4.00	5.25	6.75	4.76	6.25	6.25	4.51	5.15
$\bar{x}$	6.63	3.88	3.38	3.75	3.88	5.88	4.76	5.50	5.63	4.51	4.78
CV	8.01	4.56	15.71	9.42	50.18	21.06	0.15	19.28	15.71	0.16	11.11
NPP – Number of pods per plant											
2011	151.25	100.00	121.25	145.50	93.00	126.00	120.50	99.75	128.00	80.00	116.53
2012	387.25	282.25	112.25	197.50	212.50	270.50	197.50	317.50	264.75	124.00	236.60
$\bar{x}$	269.25	191.13	116.75	171.50	152.75	198.25	159.00	208.63	196.38	102.00	176.56
CV	61.98	67.38	5.45	21.44	55.32	51.54	34.24	73.80	49.24	30.50	48.09
GYP - Grain yield per plant (kg ha <sup>-1</sup> )											
2011	0.90	0.69	0.30	0.41	0.52	0.93	0.69	0.78	0.96	0.28	0.65
2012	1.72	0.92	0.41	0.56	0.58	1.17	0.85	1.15	1.01	0.30	0.87
$\bar{x}$	1.31	0.81	0.35	0.48	0.55	1.05	0.77	0.97	0.99	0.29	0.76
CV	44.26	20.20	21.91	21.87	7.71	16.16	14.69	27.11	3.59	4.88	20.47

Parameter	NFI		NPI		NSP		NPP		GYP	
LSD	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1
Genotype	3.00	3.99	1.92	2.55	1.25	1.66	80.67	107.3	0.34	0.46
Year	1.34	1.79	0.86	1.14	0.56	0.74	36.07	47.98	0.15	0.20
G $\times$ Y	4.25	5.65	2.71	3.61	1.77	2.35	114.1	151.7	0.48	0.64

The influence of the genotype on the NPI – Number of pods per inflorescence in the investigated alfalfa varieties was significant ( $F_{\text{exp}}=7.224^{**}$ ). The tested parameter was not significantly influenced by year ( $F_{\text{exp}}=1.959$ ). Based on variance analysis, it is evident that the G $\times$ Y interaction did not have a statistically significant effect on NFI ( $F_{\text{exp}}=1.559$ ).

The influence of the year on NPP - the number of pods per plant in the alfalfa genotypes was highly significant ( $F_{\text{exp}}=44.315^{**}$ ) while the influence of the genotype was significant ( $F_{\text{exp}}=2.832^*$ ). Based on the variance analysis, it can be concluded that, on average, for the investigated genotype, the  $G \times Y$  interaction does significantly influence the grain yield ( $F_{\text{exp}}=1.935^*$ ).

The influence of the genotype and the year on NSP – the number of seeds per plant in tested alfalfa genotypes was significant ( $F_{\text{exp}}=6.039^{**}$  and  $F_{\text{exp}}=7.219^*$ ). Based on the analysis of variance, in the studied alfalfa genotypes the interactions of genotype  $\times$  year do not significantly influence NSP ( $F_{\text{exp}}=1.462$ ).

The influence of the year and the genotype on GYP - grain yield per plant in the investigated alfalfa genotypes was significant ( $F_{\text{exp}}=7.54^{**}$  and  $F_{\text{exp}}=8.34^*$ ). Based on the analysis of variance, it can be concluded that in the investigated alfalfa genotypes the interactions of genotype  $\times$  year did not have a significant impact on grain yield ( $F_{\text{exp}}=0.94$ ).

The year, the genotype and the interaction year  $\times$  genotype had statistically significant influence on the NFI,  $P \leq 0.01$ , Figure 3. In the first year of the study, the average NFI—the number of flowers per inflorescence was 14.6 and was significantly lower than in the second year of the study (16.0), Table 2, Figure 3a.

In the first year, the highest value for the tested parameter had the genotype G53 (19.0), followed by G2 (18.3) and G11 (17.3), while the lowest values had the genotypes G100, G48 and G34 (11.3, 11.5 and 11.8). In the second year of research, the highest average values for NFI had genotypes G44 and G53 (21.8), while the lowest value had a genotype G19 (10.3). In average for the investigated period, the highest average value for NFI had the genotype G53 (20.4) and the lowest value had genotype G19 (11.9), Tab. 2, Figure 3a.

The genotype had a very statistically significant influence on NPI – the number of pods per inflorescence, Figure 3b.

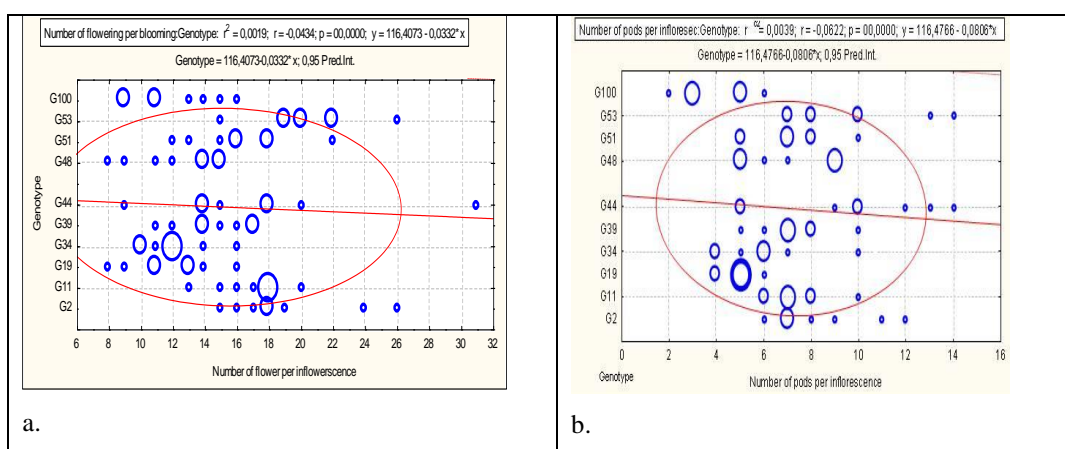


Figure 3. Effect of the genotype of NFI– number of flower per inflorescence, 3a., Effect of genotype of NPI - number of pod per inflorescence, 3b.

In the first year of the survey, the average value of NPI was 6.85. The highest average value for the investigated parameter in the first year of the study had the genotype G44 (11.25) and in the second year the genotype G53 (11.0), while the lowest value in both examined years had the genotype G100 (4.5 and 3.5). On average, during the study period, the highest average value for NPI had genotypes G44 (9.75) and G53 (9.63), and the lowest average value had genotype G19 (5.13) and G100 (4.00), Table 2, Figure 3b.

The year and the genotype had a statistically significant influence on the NPP- the number of pod per plant. In the first year of the survey, the average values for the NPP were 116.53. The highest average values for the tested parameter in both examined years had the genotype G2 (151.25 and 387.25). The lowest value in the first year of the study had the genotype G100 (80.00) and in the second year the lowest value had genotype G19 (112.25). The highest average value for NPB for the investigated period had the genotype G2 (269.25) while the lowest value had the genotype G100 (102.00), Table 2, Figure 4a.

In the first year of the survey, the average values for NSPs were 4.40 and were significantly lower in comparison to the second year (5.15). The highest average value for the tested parameter in both examined years had the genotype G2 (6.25 and 7.00). The lowest value in the first year of the study had the genotype G39 (2.50) and in the second year had the genotype G19 (3.00). For the investigated period, the highest average value for NPI had the genotype G2 (6.63), while the lowest value had the genotype G19 (3.38), Table 2, Figure 4b.

The year and the genotype had a statistically significant impact on the NSP- the number of seed per plant, Figure 4b.

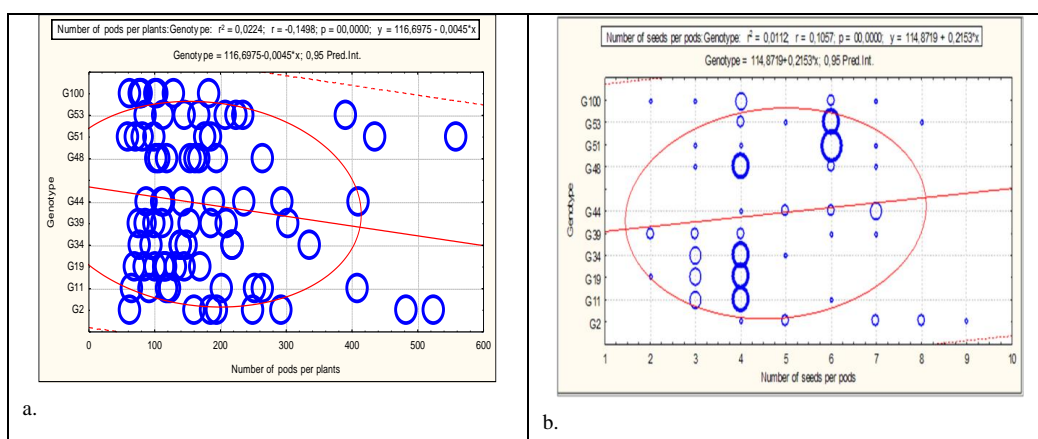


Figure 4. Influence of the genotype of NPP – the number of pod per plants, a, Effect of the genotype of NSP – number of seed per plants, b.

The year and the genotype had a statistically significant effect on the GYP- grain yield per plant. In the first year of the study, the average GYP values were 0.65 kg ha<sup>-1</sup> and were significantly lower than in the second year (0.87 kg ha<sup>-1</sup>). The highest average value for the tested parameter in the first year of the study had the genotype G53 (0.96 kg ha<sup>-1</sup>) and in the second year the genotype G2 (1.72 kg ha<sup>-1</sup>). The lowest value in both years of testing had the



genotype G100 (0.28 kg ha<sup>-1</sup> and 0.30 kg ha<sup>-1</sup>). On average for the entire study period, the highest average GYP value had the genotype G2 (1.31) while the lowest value had the genotype G100 (0.29 kg ha<sup>-1</sup>), Table 2, Figure 5.

The variability of the average values of NFI - the number of flowers per inflorescence of the studied alfalfa genotypes is expressed by the coefficient of variation. Data analysis showed a low and medium variability for all genotypes other than genotype G44 with the highest variability (CV=35.76%). The lowest variability was observed in genotypes G34, G11 and G2 (CV=4.07%, CV=4.64%, CV=6.28%).

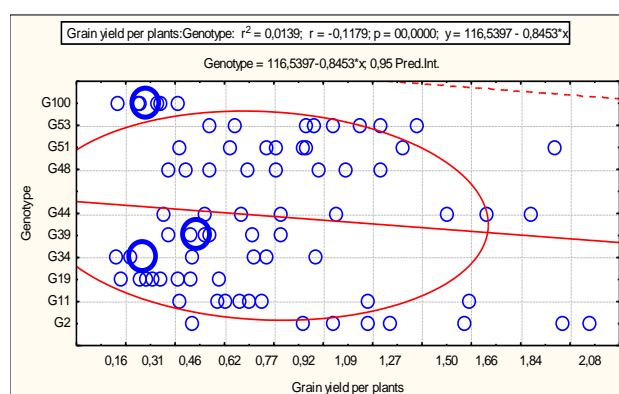


Figure 5. Effect of the genotype on GYP – grain yield per plant

The coefficient of variation, as a measure of dispersion, with indicator NPI -Number of pod per inflorescence ranged  $2.39\% < Cv < 22.33\%$  which leads to the conclusion that it is a relatively low and medium level of variation of this indicator within the genotype (Table 2).

The variability of the average values of NSP - the number of seeds per plant of the studied alfalfa genotypes expressed by the variation coefficient was low and medium, and was  $0.15\% < Cv < 21.06\%$  for all genotypes other than the genotype G48 with the highest variability (CV = 50.18%).

The average values for NPP - The number of pods per plants of the studied alfalfa genotypes shows a great variation during the examined years. The coefficient of variation, as a measure of dispersion, with indicator NPP-Number of pods per plants ranged  $5.45\% < Cv < 73.80\%$  which leads to the conclusion that it is a relatively high level of variation of this indicator within the genotype (Table 2) except the genotype G19 that had low and stable values during research period (CV=5.45%).

The average values for GYP - grain yield per plant for all investigated alfalfa genotypes are on average recorded by the mean variation during the examined years (CV=20.47%). Data analysis showed a low and medium variation of the GYP value for all genotypes other than the G2 genotype, with a large variation in the value of the tested parameter (CV=44.26%). The highest stability for GYP value was observed in genotypes G53, G100 and G39 (CV=3.59%, CV=4.88%, CV=7.71%), Table 2.

The genotype G2 could be recommended for further breeding program and large-scale production due to its high yielding performance. Grain yield being a complex trait routinely exhibit genotype  $\times$  environment interactions (GEI) thus requires genotype evaluation in multi-location trials (MET) in the advanced stages of selection (FAN *et al.*, 2007). Therefore, selection of suitable genotypes and testing sites is crucial to the success of a plant breeding program.

The analysis of the interaction allowed the identification of individual genotypes that showed the highest stability and yield potential in different production conditions. The high stability of high-yield genotypes is the most desirable feature of a hybrid. When genotypes are tested for stability, often genotypes with the highest yield show a low level of stability and vice versa. What is in agreement with the research of BOŽOVIĆ *et al.* (2018; 2020).

Based on this analysis, it can be concluded that genotype alfalfa achieve different yields of grains in different environments, and that the identification of genotypes with higher phenotypic stability can contribute to the improvement of the overall alfalfa production. Technology which will achieve the yield level almost the same as its genetic potential should be used and in that way it would be easier to avoid expensive mistake in production process and achieve profitable production.

#### *Correlations of tested parameters*

In plant breeding, it is important to know the correlations between the various traits, because intense selection on one trait can affect other traits in both the desired (by improving the properties of interest) and in the undesired direction. Knowledge about the strength and direction of the association between the traits is particularly important for the traits of low genetic variability, where the genetic progress can be achieved by indirect selection. In table 3 shows the linear correlation by Spearman coefficient and the level of significance for the analyzed traits.

Positive and strong correlations were found between the grain yield per plants and number of pods per plants ( $r=0.74^{**}$ ) and yield per plant and number of flowers per inflorescence ( $r=0.51^*$ ), Table 3.

*Table 3. The Spearman correlation coefficients between tested traits of alfalfa genotypes*

Variable	NFI	NPI	NSP	NPP	GYP
NFB	1.00	0.36 <sup>ns</sup>	0.47 <sup>*</sup>	0.40 <sup>ns</sup>	0.51 <sup>*</sup>
NPB	0.36 <sup>ns</sup>	1.00	0.37 <sup>ns</sup>	0.28 <sup>ns</sup>	0.42 <sup>ns</sup>
NSP	0.47 <sup>ns</sup>	0.37 <sup>ns</sup>	1.00	0.30 <sup>ns</sup>	0.37 <sup>ns</sup>
NPP	0.40 <sup>ns</sup>	0.28 <sup>ns</sup>	0.30 <sup>ns</sup>	1.00	0.74 <sup>**</sup>
GYP	0.51 <sup>*</sup>	0.42 <sup>ns</sup>	0.37 <sup>ns</sup>	0.74 <sup>**</sup>	1.00

<sup>ns</sup> – not significant; \* and \*\* significant at 0.05% at 0.01%; \* NFI–number of flowers per inflorescence; NPI - number of pods per inflorescence; NPP– number of pods per plant; NSP–number of seeds per plant; GYP – grain yield per plant;

Positive correlations were found between the number of seed per pods and number of flowers per inflorescence ( $r=0.47$ ), number of pods per inflorescence ( $r=0.37$ ) and number of pods per plant ( $r=0.30$ ), Table 3.

According to TUCAK *et al.* (2008), there was not a significant correlation between dry matter yield (DMY) and crude protein (CP) content ( $r=0.04$ ) as there was between DMY and CP yield ( $r=0.91$ ,  $P\leq 0.001$ ). Positive correlations were found between the yield of green mass and dry matter yield ( $r=0.93^{**}$ ), yield of green mass and dry matter with the plant height ( $r=0.85^{**}$ ,  $r=0.81^{**}$ ), number of internodes ( $r=0.74^{**}$ ,  $r=0.65^{**}$ ) and width and length of central leaflet ( $r=0.72^{**}$ ,  $r=0.54^*$ ,  $r=0.79^{**}$ ,  $r=0.62^{**}$ ). Plant height was in a significant positive correlation with the number of internodes ( $r=0.79^{**}$ ) and with the width and length of central leaflet ( $r=0.60^{**}$ ,  $r=0.84^{**}$ ) (TUCAK *et al.*, 2013).

Genetic resources are the basis for the development of modern high-yielding varieties worldwide (RADINOVIĆ *et al.*, 2018; JANKOVIĆ *et al.*, 2018; POPOVIĆ *et al.*, 2020). The genotype factor had significant effect on alfalfa productivity parameters ( $P\leq 0.05$ ) which were not significantly affected by the other factors or their interactions. There are many reports on the potentialities of breeding in improving the alfalfa forage quality (RIDY and BRUMMER, 2002; LAMB *et al.*, 2006; STANISAVLJEVIĆ, 2006; ŠTRBANOVIĆ, 2010; MILIĆ *et al.*, 2014; RADINOVIĆ *et al.*, 2018).

### CONCLUSION

In this study, conducted in Banja Luka, the investigated ten genotypes originated from different regions of USA and Europe. Year and genotype had a statistically significant impact on the NSP-number of seed per plant. In the first year of the survey, the average values for NSP were significantly lower than in the second year. On average, for the investigated period, the highest average value for NPI had the G2 genotype (6.63) while the lowest value had the G19 genotype (3.38).

The influence of the year and the genotype on GYP - grain yield per plant in the investigated alfalfa varieties was significant ( $F_{exp}=7.54^*$  and  $F_{exp}=8.34^*$ ). In the first year of the study, the average GYP values were significantly lower than in the second year. The highest average value for the tested parameter in the first year of the study had the genotype G53, and in the second year the genotype G2. The lowest value in both years of testing had the genotype G100 (0.28 and 0.30). The genotype G2 could be recommended for further breeding program and large-scale production due to its high yielding performance.

Positive and strong correlations were found between the grain yield per plants and number of pods per plants ( $r=0.74^{**}$ ) and yield per plant and number of flowers per inflorescence ( $r=0.51^*$ ).

The obtained results could be helpful when choosing alfalfa varieties, in this or similar region, for conventional growing and/or for selection and breeding of genotypes with good potential for high grain yield per plant.

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## REFERENCES

- BOLAN, N.S., M.J. HEDLEY, R.E. WHITE (1991): Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant and Soil*, 134: 53-63.
- BORDELEAU, L.M., D. PREVOST (1994): Nodulation and nitrogen fixation in extreme environments. *Plant and Soil*, 161: 115-125.
- BOUTON, J.H. (1996): Screening the alfalfa core collection for acid soil tolerance. *Crop Science*, 36: 198-200.
- BOŽOVIĆ, D., T. ŽIVANOVIĆ, V. POPOVIĆ, M. TATIĆ, Z. GOSPAVIĆ, Z. MILORADOVIĆ, G. STANKOVIĆ, M. ĐOKIĆ (2018): Assessment stability of maize lines yield by GGE- biplot analysis. *Genetika*, 50(3): 755-770.
- BOŽOVIĆ, D., V. POPOVIĆ, V. RAJIČIĆ, M. KOSTIĆ, V. FILIPOVIĆ, LJ. KOLARIĆ, V. UGRENOVIĆ, V. SPALEVIĆ (2020): Stability of the expression of the maize productivity parameters by AMMI models and GGE-biplot analysis. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 48(3): 1387-1397.
- GULÍAS, J., R. MELIS, D. SCORDIA, J. CIFRE, G. TESTA, L.S. COSENTINO, C. PORQUEDDU (2018): Exploring the potential of wild perennial grasses as a biomass source in semi-arid Mediterranean environments. *Italian Journal of Agronomy*, 13: 103-111.
- FAN, X.M., M.S. KANG, H. CHEN, Y. ZHANG, J. TAN, C. XU (2007): Yield stability of maize hybrids evaluated in multi-environment trials in Yunnan, China. *Agron. J.*, 99: 220-228.
- GRAHAM, P.H., C.P. VANCE (2003): Legume importance and constraints to greater. *Plant Physiology*, 131: 872-877. DOI:
- JANKOVIĆ, V., S. VUČKOVIĆ, V. MIHAILOVIĆ, V. POPOVIĆ, LJ. ŽIVANOVIĆ, D. SIMIĆ, A. VUJOŠEVIĆ, P. STEVANOVIĆ (2018): Assessment of some parameters productivity and quality of populations *Phleum pratense* (L.) grown in conditions of Serbia. *Genetika*, 50(1): 1-10.
- JOVANOVIĆ-TODOROVIĆ, D., V. POPOVIĆ, S. VUČKOVIĆ, S. JANKOVIĆ, A. MIHAILOVIĆ, M. IGNJATOV, V. STRUGAR, V. LONČAREVIĆ (2020): Impact of row spacing and seed rate on the production characteristics of the perennial ryegrass (*Lolium perenne* L.) and their valorization. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 48(3): 1495-1503. DOI: 10.15835/nbha48312057
- KOSEV, V., V. VASILEVA (2021): Correlation dependences on quantitative signs in grass pea (*Lathyrus sativus* L.) accessions. *Genetika*, 53(3): 1031-1042.
- LAMB, J.F.S., C.C. SHEAFFER, L.H. RHODES, R.M. SULC, D.J. UNDERSANDER, E.C. BRUMMER (2006): Five decades of alfalfa cultivar improvement: Impact on forage yield, persistence, and nutritive value. *Crop Science*, 46: 902-909.
- LAKIĆ, Ž., S. STANKOVIĆ, S. PAVLOVIĆ, S. KRNJAJIĆ, V. POPOVIĆ (2019): Genetic variability in quantitative traits of field pea (*Pisum sativum* L.) genotypes. *Czech J. Genet. Plant Breed*, 54(3): 1-7.
- MILIĆ, D., Đ. KARAGIĆ, S. VASILJEVIĆ, A. MIKIĆ, B. MILOŠEVIĆ, S. KATIĆ (2014): Breeding and improvement of quality traits in alfalfa (*Medicago sativa* ssp. *sativa* L.). *Genetika*, 46(1): 11-18.
- PERIĆ, S., M. STEVANOVIĆ, S. PRODANOVIĆ, S. MLADENOVIĆ DRINIĆ, N. GRČIĆ, V. KANDIĆ, J. PAVLOV (2021): Genetic distance of maize inbreds for prediction of heterosis and combining ability. *Genetika*, 53(3): 1219-1228.
- POPOVIĆ, D., J. VITOMIR, S. TOMAŠ-MISKIN, T. DAVIDOV, S. POPOVIĆ, M. JOVANOVIĆ, M. AĆIMIĆ REMIKOVIĆ, S. JOVANOVIĆ, (2021): Implementation of internal control with reference to the application of "IT" in companies operating on the principles of the green economy. *Agriculture and Forestry*, 67 (2): 257-266. DOI: 10.17707/AgricultForest.67.2.19
- POPOVIĆ, V., M. VIDIĆ, DJ. JOCKOVIĆ, J. IKANOVIĆ, S. JAKŠIĆ, G. CVIJANOVIĆ (2012): Variability and correlations between yield components of soybean [*Glycine max* (L.) Merr.]. *Genetika*, 44(1): 33-45.
- POPOVIĆ, V., S. VUČKOVIĆ, Z. JOVOVIĆ, N. RAKAŠČAN, M. KOSTIĆ, N. LJUBIČIĆ, M. MLADENOVIĆ, GLAMOČLIJA, J. IKANOVIĆ (2020a): Genotype by year interaction effects on soybean morpho-productive traits and biogas production. *Genetika*, 52(3): 1055-1073.

- POPOVIĆ, V., N. LJUBIČIĆ, M. KOSTIĆ, M. RADULOVIĆ, D. BLAGOJEVIĆ, V. UGRENOVIĆ, D. POPOVIĆ, B. IVOŠEVIĆ (2020b): Genotype × Environment Interaction for Wheat Yield Traits Suitable for Selection in Different Seed Priming Conditions. *Plants–Basel*, 9(12): 1804.
- PARIHAR, A.K., G.P. DIXIT, D. SINGH (2015): Genetic variability analysis for quantitative traits in a germplasm set of grasspea (*Lathyrus* spp.). *Legume Research*, 38(4): 461-464.
- PETCU, E., M. SCHITEA, D. BADEA (2006): The response of some Romanian alfalfa genotypes to soil acidity. *Rom. Agric. Res.*, 23: 25-27.
- RAHMAN, MA., YG. KIM, BH. LEE (2014): Proteomic response of alfalfa subjected to aluminum (Al) stress at low pH soil. *Jour. of the Korean Society of Grassland and Forage Science*, 34(4): 262-268. DOI:
- RADINOVIĆ, I., S. VASILJEVIĆ, M. ZORIĆ, G. BRANKOVIĆ, T. ŽIVANOVIĆ, S. PRODANOVIĆ (2018): Variability of red clover genotypes on the basis of morphological markers. *Genetika*, 50(3): 895-906.
- RIDY, H., EC., MRUMMER (2002): Heterosis of forage quality in alfalfa. *Crop Science*, 42: 1088-1093.
- STEVOVIĆ, V., D. ĐUROVIĆ, D. ĐUKIĆ, M. JARAK (2012): Application of lime materials and bacterization to improve the production of quality animal feed on acidic soils. In "Breeding of fodder plants and production of fodder on arable land". Eds: Đukić D., Stevović V., University of Kragujevac, Faculty of Agriculture in Čačak. 189-227.
- STANISAVLJEVIĆ, R. (2006): The effect of crop density on yield and quality of fodder and alfalfa seed (*Medicago sativa* L.). PhD thesis. University of Novi Sad (Unpublished), Serbia
- STANISAVLJEVIĆ, R., D. BEKOVIĆ, D. ĐUKIĆ, V. STEVOVIĆ, D. TERZIĆ, J. MILENKOVIĆ, D. ĐOKIĆ (2012): Influence of plant density on yield components, yield and quality of seed and forage yields of alfalfa varieties. *Rom. Agric. Res.*, 29: 245-254.
- TERZIĆ, D., V. POPOVIĆ, N. MALIĆ, J. IKANOVIĆ, V. RAJIČIĆ, M. LONČAR, S. POPOVIĆ, V. LONČAREVIĆ (2018): Effects of long-term fertilization on yield of siderates and organic matter content of soil in the process of recultivation. *Journal Animal and Plant Science*, 29(3): 1-10.
- TUCAK, M., S. POPOVIĆ, S. GRLJUŠIĆ, T. ČUPIĆ, V. KOZUMPLIK, B. ŠIMIĆ (2008): Variability and relationships of important alfalfa germplasm agronomic traits. *Periodicum Biologorum*, 110(4): 311-315.
- TUCAK, M., S. POPOVIĆ, T. ČUPIĆ, G. SIMIĆ, R. GANTNER, V. MEGLIČ (2009): Evaluation of alfalfa germplasm collection by multivariate analysis based on phenotypic traits. *Rom. Agric. Res.*, 26: 47-52.
- ŠTRBANOVIĆ, R. (2010): Genetic variability for agronomical traits of different alfalfa (*Medicago sativa* L.) genotypes. MSc thesis. University of Belgrade. Serbia.
- VERONESI, F., E.C. BRUMMER, C. HUYGHE (2010): Alfalfa. (eds.) Boller, B., Posselt, U.K., Veronesi, F. In *Handbook of plant breeding: Fodder crops and amenity grasses*. Springer, New York, USA. p. 395-437.
- VONUENKULL, HR., E. MUTERT (1995): Global extent, development and economic impact of acid soils. In „Plant soil interactions at low pH: principles and management“. Eds: Date R. A., Grundan N. J., Rayment G. E., Probert M. E., Kluwer: Dordrecht, the Netherlands: 1-19.

**GENOTIPSKA VARIRANJA KOMPONENTI PRINOSA SEMENA *Medicago sativa* (L.)  
NA KISELIM ZEMLJIŠTIMA U USLOVIMA UNAKRSNE OPLODNJE**

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Izvod

Lucerka (*Medicago sativa* L.) je najvažnija krmna biljna vrsta u Bosni i Hercegovini. U ovom istraživanju, izvedenom u Banja Luci, ispitano je deset genotipova lucerke poreklom iz različitih regija SAD-a i Evrope. Cilj ove studije je bio da se odredi njihov prinos zrna po biljkama i četiri morfološke osobine. Analize interakcija pružene su za odabir pojedinačnih genotipova i treba da pokažu najveću stabilnost i potencijal prinosa u različitim proizvodnim uslovima. Genotip s najvećim prinosom pokazao je niži nivo stabilnosti. Uticaj godine i genotipa na GYP - prinos zrna po biljkama za ispitivane genotipove lucerke bio je značajan ( $F_{exp} = 7,54 *$  i  $F_{exp} = 8,34 *$ ). Najveću prosečnu vrednost ispitivanog parametra u prvoj godini istraživanja imali su genotipovi G53 i G44, a u drugoj godini istraživanja genotip G2. Najmanju vrednost u obe istraživačke godine imao je genotip G100 ( $0,28 \text{ kg ha}^{-1}$  i  $0,30 \text{ kg ha}^{-1}$ ). Utvrđene su pozitivne i jake korelacije između prinosa zrna po biljci i broja mahuna po biljci ( $0,74 **$ ) i prinosa zrna po biljci i broja cvetova po cvasti ( $0,51 *$ ). Na osnovu dobijenih vrednosti zaključujemo da imamo izvrsne genotipove G2, G44, G53 i G51 za uspešan oplemenjivački rad u cilju dobijanja novih visokoprinosa sorti lucerke.

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