

MORPHOLOGICAL CHARACTERISTICS OF ALFALFA GENOTYPES TOLERANT TO LOW SOIL pH

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In total 76 varieties of alfalfa were collected and sown and 41 alfalfa genotypes showed tolerances to lower soil pH. The selected alfalfa genotypes are then sown in a test field on the soil that belongs to the type of brown soil on the gravel or the cambisol with the soil pH from 5.33 to 5.64. After 5 years, 10 genotypes were selected based on the agronomic properties and the experiment was set up on the soil with the pH from 5.0 to 5.1 in H₂O. The following genotypes were used for these tests: G-2, G-11, G-19, G-34, G-39, G-44, G-48, G-51, G-53 and G-100. The following properties were observed: plant height (cm), number of stems per plant, stem thickness (mm), number of internodes per stem, leaf/stem ratio (%), yield of biomass in the I cut (g plant⁻¹). During these tests, the highest degree of tolerance towards the lower soil pH was shown by the genotype G-44. Good results were achieved with alfalfa genotypes G-11 and G-48 where the percentage of survivors was over 80%. The highest average plant height during the three-year study (36.9 cm) was recorded in the genotype G-100. The high and stable yield of dry mass per

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plant have genotypes G-44 and G-19 and they are grouped in group I and are desirable for further breeding. Genotype and year have statistically significant and highly significant influence on the tested parameters, while the significance of the interaction of the genotype x year was determined only for the thickness of the stem.

Keywords: alfalfa, genotype, environment, productivity characteristics, tolerance to acid soil

INTRODUCTION

Alfalfa is one of the oldest and most important perennial forage plants. It is especially important for feeding of domestic animals, since it may produce high quality biomass, rich in crude proteins, vitamins, carotenes and mineral substances. Alfalfa has a significant place in the crop rotation because it improves the physical properties and biogenicity of the soil, influences the microbiological processes, increases nitrogen content and generally improves soil fertility. But alfalfa growth and development are strongly affected by soil acidity and Al toxicity (KHU *et al.*, 2012; RAHMAN *et al.*, 2014). Alfalfa is a perennial leguminous plant that is very sensitive to the low soil pH (CLARCE *et al.*, 1993).

In the agro-ecological conditions of Bosnia and Herzegovina and Serbia alfalfa is grown on neutral soil, with pH ranges from 6.2-7.8. The land suitable for alfalfa cultivation is represented in a small percentage. The expansion of areas under alfalfa in our production conditions can be achieved by performing calcification and choosing tolerant varieties. On land where calcification is less economically justified as an alternative, cultivation of leguminous varieties tolerant to cultivation on low pH and high aluminium levels is proposed (SAMAC and TESFAYE, 2003). In alfalfa varieties, there is a significant variability in acid soil reactions (GREWAL and WILLIAMS, 2003). Due to the use of physiologically acidic mineral fertilizers, acid rain and some other reasons causing problems of acidification of soil (VON UEXKULL and MURTER, 1995).

A number of scientists from different regions of the world emphasized a problem of susceptibility of alfalfa to soil acidity (GREWAL and WILLIAMS, 2003; POPOVIC *et al.*, 2009; ROUSK *et al.*, 2009). Problems that limit the cultivation of alfalfa on low-pH soils can be treated in several ways. One of these methods is the selection of alfalfa varieties that show tolerance to soil acidity and high concentrations of aluminium in the soil (STEVOVIĆ *et al.*, 2012). A similar procedure for assessing the degree of tolerance to the acidity of the varieties Banat, NS median, K-28, Sinskaja and OS-66 on pseudogley with 4.79 pH in KCl was performed on the field of the Agricultural School in Kraljevo (STEVOVIĆ *et al.*, 2010). They make an effort to solve the problem by liming (BRAUER *et al.*, 2002), and breeding, that is creation of germplasm tolerant to low pH (CAMPBELL *et al.*, 1993; DALLAGNOL *et al.*, 1996; GREWAL and WILLIAMS, 2003).

The aim of this research is to investigate the production properties of selected alfalfa genotypes that were shown tolerance to lower pH values in the previous research.

MATERIAL AND METHODS

In the period 1998-2001 collection of 76 varieties of alfalfa was formed. The collected material was sown on a slightly acidic soil (pH 5.7-5.8 in H₂O). After two years of testing, 41 alfalfa genotypes were selected, showing tolerance to this soil pH. Selected alfalfa genotypes were sown in the field of the Agricultural Institute in the early spring of 2004. The experiment is set on the soil belonging to the type of brown soil on the gravel or cambisol according to the

FAO classification. The surface area was 2.5 m². The pH soil on the experimental plot varied from 5.33 to 5.64 in H₂O and from 4.11 to 4.41 in KCl. Most genotypes were started to grown up. At the end of the vegetative season of the first year up to 90% of the grown plants of some genotypes were die out. For following several years, the experimental field was mowed and the agronomic properties of all surviving alfalfa genotypes were monitored. After 5 years, on the basis of agronomic properties, 10 genotypes were selected from which the seed was taken from the second rotation under conditions of cross-fertilization. The seed was processed and prepared for sowing. Each genotype was represented with 30 plants per repetition. The distance between plants in row and between rows was 80 cm, and between repetitions was 100 cm. The sowing was carried out in 4 repetitions. Therefore, each alfalfa genotype was analysed in total on 120 plants. The following genotypes were used for these tests: G-2, G-11, G-19, G-34, G-39, G-44, G-48, G-51, G-53 and G-100. Seeds of these genotypes were taken under conditions of cross-fertilization. The origins of the selected genotypes are: G-2 from Vuka variety, G-11 from OS-66 variety, G-19 from Apica variety, G-34 from Gloria variety, G-39 from Pampeana variety, G-44 from Petrovac genotype, G -48 from genotype 1/81 exp., G-51 from the Adriana variety, G-53 from Albfa genotype and G-100 from 99 Maglajani genotype, Table 1.

Table 1. The origins of the selected genotypes from genotype

| The origins of the selected genotypes | Genotype |
|---------------------------------------|----------------|
| G-2 | Vuka |
| G-11 | OS-66 variety, |
| G-19 | Apica variety |
| G-34 | Gloria variety |
| G-39 | Pampeana |
| G-44 | Petrovac |
| G -48 | 1/81 exp |
| G-51 | Adriana |
| 53 from | Albfa |
| G-100 | 99 Maglajani |

During the pre-sowing preparation, conventional agro-technical measured were applied. The sowing was done manually in the spring 2009 by sowing 2 seeds at a depth of 1.5-2 cm. During 2009, the experiment was maintained, and only the number of emerged plants in sown populations was monitored. In the period 2010-2012 the following traits were observed in the first cut: plant height (cm), number of stems per plant, stem thickness (mm), number of internodes per stem, stem/leaf ratio (%), yield of dry matter in the first cut (g plant⁻¹). In the second growth, components of seed yield were monitored.

Agro-chemical analyses of the soil

For a new experiment, a location with soil pH ranged from 5.0 to 5.1 in H₂O, and from 3.8 to 4.0 in KCl was selected. The land selected for experiment had medium content of humus, ranged from 3.7-4.5% and a low to the medium content of easily accessible phosphorus (11.4 to 13.3 mg 100⁻¹ g of soil). The content of easily accessible potassium was very good, Table 2.

Table 2. Agro-chemical analyses of the soil

| Parameter | pH in | | Humus | P ₂ O ₅ | K ₂ O |
|-----------|------------------|---------|---------|--------------------------------|------------------|
| | H ₂ O | KCl | % | mg 100 ⁻¹ g of soil | |
| 0-30 cm | 5.0-5.1 | 3.8-4.0 | 3.7-4.5 | 11.4-13.3 | 23-25 |



Figures 1 and 2. Alfalfa on acid soil, 2010

Meteorological conditions

During the conduction of the experiment, the meteorological conditions that are important for the research were monitored, data from the Hydro-meteorological Station of Banja Luka, Bosnia & Hercegovina were used.

The obtained results were analysed by analysis of variance (ANOVA), and the significance of mean values differences was determined by the LSD test. Also, coefficients of variation (CV) of evaluated traits and coefficient of correlation (r) of the yield components of alfalfa genotypes were calculated. Estimation of the stability of alfalfa-tolerant genotypes to acidic soils was carried out using the FRANCES and KANNENBERG (1978) grouping methods. The alfalfa genotypes examined were classified into four different stability groups based on the yield of dry matter and the coefficient of variability.

Data from Hydro-meteorological Station Banja Luka were used for analysis of weather conditions (Figure 3 and 4).

The winter period during the three studied years was warmer compared to the multi-year average. The multi-year average temperature was 10.9⁰C, and during the growing period (IV-X) was 16.4⁰C. Total amount of precipitation in the vegetation period (IV-X) for the period 1961-2014 was 650.0 l/m².

The average temperatures in all three years of testing during the growing period were higher than the multi-year average. In the first year of the study, the amount of precipitation

during the vegetation period, compared to the multi-year average, was higher for 166.1 l/m². During the vegetation period in 2011, the precipitation was 347.3 l/m², that is 302.7 l/m² of precipitation less compared to the multi-year average. In 2012, the precipitation was lower for 74.8 l/m² compared to the same period of multiyear average.

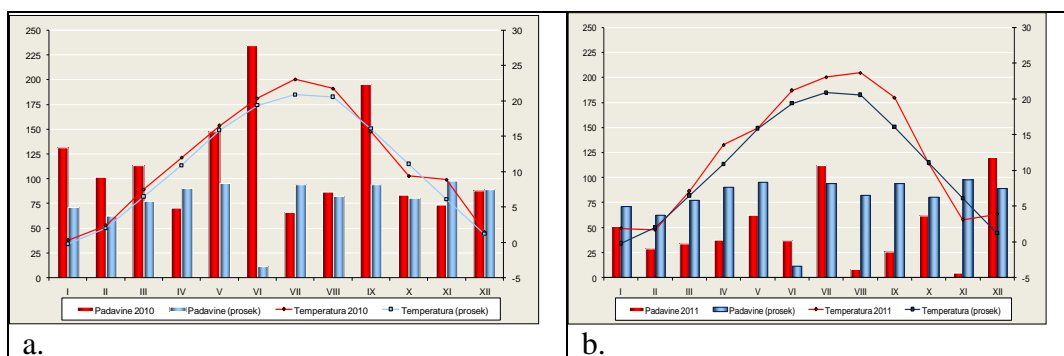


Figure 3. Precipitation and temperature in 2010 (a) and 2011 (b) Banja Luka, B&H

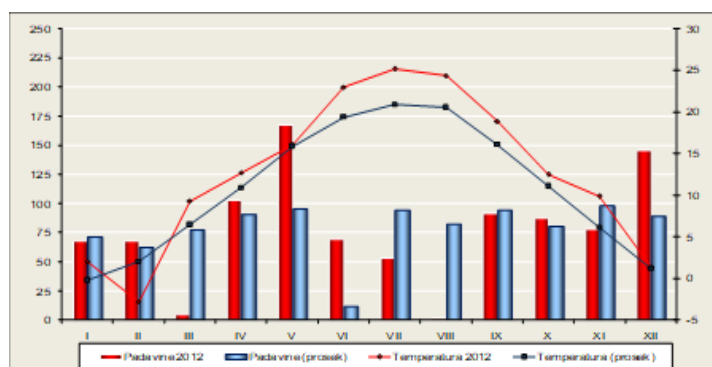


Figure 4. Precipitation and temperature in 2012, Banja Luka, B&H

RESULTS AND DISCUSSION

During the 2009 after the emergence of plants, there has been significant decreasing of number of alfalfa plants due to low soil pH. Number of survived plants was different for each alfalfa tested genotype (Table 3).

During the period of the study of selected alfalfa genotypes, the highest tolerance to the lower pH of the soil was shown by G-44 genotype, with 112 survived plants or 93.75% of the number of seedling. Good results were achieved with alfalfa genotypes G-11 and G-48 where the percentage of survivors was over 80%.

The lowest tolerance to low soil pH (5.0-5.1 in H₂O), shown genotype G-19 with only 37.5 % of survived plants. The study of the morphological characteristics of alfalfa genotypes that showed a certain degree of tolerance to acidity of the soil, shows that genotypes and years

have statistically significant and highly significant influence on the investigated parameters, while the significance of the interaction of the genotype x years has not been determined (Tables 4 and 5; Figure 5-9).

Table 3. Number of survived alfalfa genotypes in the period 2010-2012

| No. | Genotype | Number of survived plants | Number of survived plants compared to the number of sown (%) |
|---------|----------|---------------------------|--|
| 1. | G-2 | 60 | 50.00 |
| 2. | G-11 | 97 | 80.83 |
| 3. | G-19 | 45 | 37.50 |
| 4. | G-34 | 83 | 69.17 |
| 5. | G-39 | 61 | 50.83 |
| 6. | G-44 | 112 | 93.75 |
| 7. | G-48 | 98 | 81.67 |
| 8. | G-51 | 75 | 62.50 |
| 9. | G-53 | 67 | 55.83 |
| 10. | G-100 | 68 | 56.67 |
| Average | | 76.60 | 63.88 |

Table 4. Morphological characteristics of studied alfalfa genotypes

| No. | Genotypes | Year/average | Plants height (cm) | No of stems per plant | Stem thickness (mm) | Number of internodes per stem | Dry matter yield per plant (g) |
|-----|-----------|--------------|--------------------|-----------------------|---------------------|-------------------------------|--------------------------------|
| 1. | G-2 | 2010 | 88.5 | 6.0 | 2.7 | 8.3 | 9.5 |
| | | 2011 | 99.2 | 8.0 | 3.1 | 8.8 | 11.0 |
| | | 2012 | 98.5 | 8.5 | 2.8 | 7.5 | 19.3 |
| | | Average | 95.4 | 7.5 | 2.9 | 8.2 | 13.3 |
| 2. | G-11 | 2010 | 103.3 | 5.0 | 2.3 | 9.5 | 9.3 |
| | | 2011 | 106.8 | 9.3 | 2.9 | 10.0 | 12.4 |
| | | 2012 | 104.2 | 9.5 | 2.5 | 9.0 | 22.8 |
| | | Average | 104.8 | 7.9 | 2.6 | 9.5 | 14.8 |
| 3. | G-19 | 2010 | 96.7 | 9.0 | 2.4 | 9.5 | 11.1 |
| | | 2011 | 108.3 | 9.5 | 3.0 | 10.5 | 13.7 |
| | | 2012 | 119.7 | 11.0 | 2.8 | 9.5 | 25.6 |
| | | Average | 108.2 | 9.8 | 2.7 | 9.8 | 16.8 |
| 4. | G-34 | 2010 | 84.3 | 8.0 | 2.3 | 9.0 | 9.9 |
| | | 2011 | 94.5 | 8.8 | 2.8 | 9.3 | 15.0 |
| | | 2012 | 110.7 | 9.5 | 2.9 | 11.0 | 27.5 |
| | | Average | 96.5 | 8.8 | 2.7 | 9.8 | 17.5 |
| 5. | G-39 | 2010 | 100.3 | 9.5 | 2.9 | 8.5 | 10.4 |
| | | 2011 | 105.3 | 9.8 | 3.2 | 8.5 | 11.3 |
| | | 2012 | 114.0 | 12.0 | 3.0 | 8.0 | 21.7 |
| | | Average | 106.5 | 10.4 | 3.0 | 8.3 | 14.5 |
| 6. | G-44 | 2010 | 90.8 | 8.0 | 2.4 | 10.3 | 11.8 |
| | | 2011 | 95.3 | 10.3 | 3.4 | 11.5 | 15.1 |
| | | 2012 | 99.0 | 11.0 | 2.8 | 10.0 | 30.5 |

| | | | | | | | |
|-----|---------|---------|-------|------|-----|------|------|
| | | Average | 95.0 | 9.8 | 2.9 | 10.6 | 19.1 |
| 7. | G-48 | 2010 | 103.8 | 8.5 | 2.9 | 8.8 | 11.0 |
| | | 2011 | 109.0 | 9.5 | 3.1 | 7.5 | 12.5 |
| | | 2012 | 105.9 | 11.3 | 3.0 | 8.8 | 27.4 |
| | | Average | 106.2 | 9.8 | 3.0 | 8.4 | 17.0 |
| 8. | G-51 | 2010 | 97.8 | 5.0 | 2.9 | 9.3 | 8.7 |
| | | 2011 | 92.3 | 8.8 | 3.0 | 8.8 | 12.4 |
| | | 2012 | 106.4 | 9.3 | 3.0 | 11.5 | 24.0 |
| | | Average | 98.8 | 7.7 | 3.0 | 9.9 | 15.0 |
| 9. | G-53 | 2010 | 98.5 | 4.0 | 3.0 | 9.0 | 10.3 |
| | | 2011 | 99.5 | 9.0 | 3.1 | 10.3 | 13.6 |
| | | 2012 | 104.5 | 9.3 | 3.2 | 9.3 | 28.1 |
| | | Average | 100.8 | 7.4 | 3.1 | 9.5 | 17.3 |
| 10. | G-100 | 2010 | 99.0 | 8.5 | 2.7 | 8.8 | 10.7 |
| | | 2011 | 107.8 | 9.3 | 3.0 | 8.0 | 12.8 |
| | | 2012 | 103.7 | 10.0 | 3.1 | 9.0 | 27.5 |
| | | Average | 103.5 | 9.3 | 2.9 | 8.6 | 17.0 |
| | Average | 2010 | 96.3 | 7.2 | 2.6 | 9.1 | 10.3 |
| | | 2011 | 101.7 | 9.2 | 3.0 | 9.3 | 13.0 |
| | | 2012 | 106.7 | 10.0 | 2.9 | 9.4 | 25.4 |
| | | Average | 101.6 | 8.8 | 2.8 | 9.3 | 16.2 |

Table 5. The analysis of variance

| Parameter | LSD | Genotype | Year | G x Y |
|----------------------------|------|----------|-------|-------|
| Plant height (cm) | 0.05 | 8.5* | 4.7* | 14.7 |
| | 0.01 | 11.3** | 6.2** | 19.5 |
| Number of stems/plants | 0.05 | 1.2* | 0.7* | 2.1 |
| | 0.01 | 1.6** | 0.9** | 2.8 |
| Stem thickness (mm) | 0.05 | 0.2* | 0.1* | 0.4 |
| | 0.01 | 0.3** | 0.2** | 0.5 |
| Number of internodes/stems | 0.05 | 1.3* | 0.7 | 2.3 |
| | 0.01 | 1.7** | 0.9 | 3.0 |
| Dry matter yield/plant (g) | 0.05 | 3.0* | 1.6* | 5.2 |
| | 0.01 | 4.0** | 2.2** | 6.9 |

The alfalfa improvement efforts in breeding and selection strategy is assessed by MILIC *et al.*, 2014 and concluded that yield and quality of alfalfa hay are significantly influenced by the choice of cultivar. Yield and quality of alfalfa highly depend on genetic factors (cultivar) and applied level of cultivation practices (MILIC *et al.*, 2014).

Plant height is one of the most important traits affected by cultivation and exploitation, in addition to genetic factors. This property is at the same time the most important component of the yield of green biomass and next to the quality of nutrients.

It has been observed that in alfalfa genotypes this phenotypic feature varies in the range of 29.6 cm (genotype G-2) to 36.9 cm (genotype G-100), table 4. On the height of the plants, the genotype and year had a statistically significant effect (Table 3). During 2011 and 2012, alfalfa plants were higher than in 2010. The highest plants were determined in the genotype G-44 in

2012 (41.2 cm), while the lowest plants were observed by genotype G-2 in 2010 (28.9 cm). The genotypes G-100 and G-44 have a statistically significantly higher average plant height than the genotypes G-2, G-11 and G-51, Figure 5.

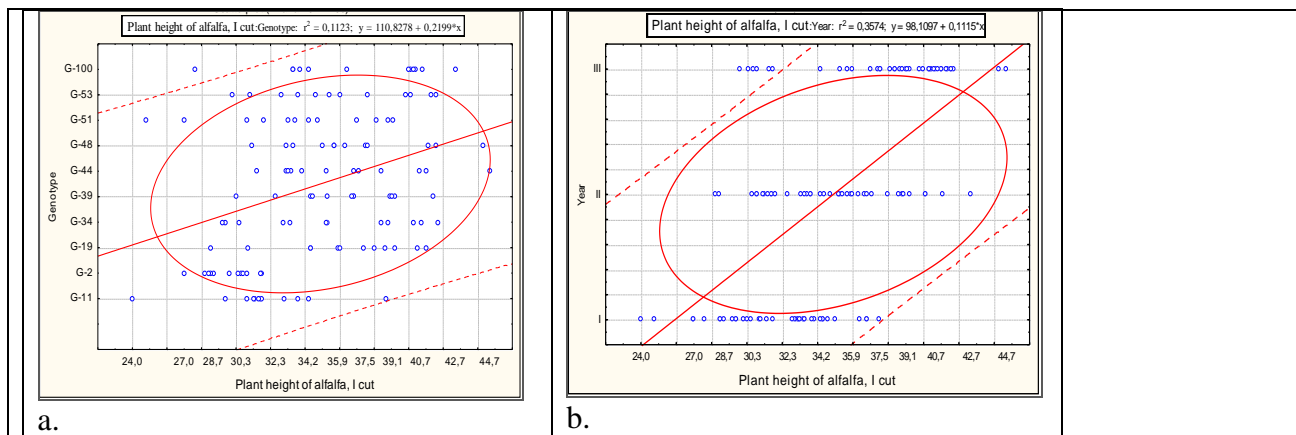


Figure 5. Influence of the genotype (a) and year (b) on the height of the plants

In alfalfa genotypes cultivated on pseudogley-type soil during 2005-2006 the average plant height was 29.8 cm (KATIĆ *et al.*, 2007). Plant height is an important yield component and it is often used as a criterion when choosing superior genotypes in an early stage of selection (TUČAK *et al.*, 2008).

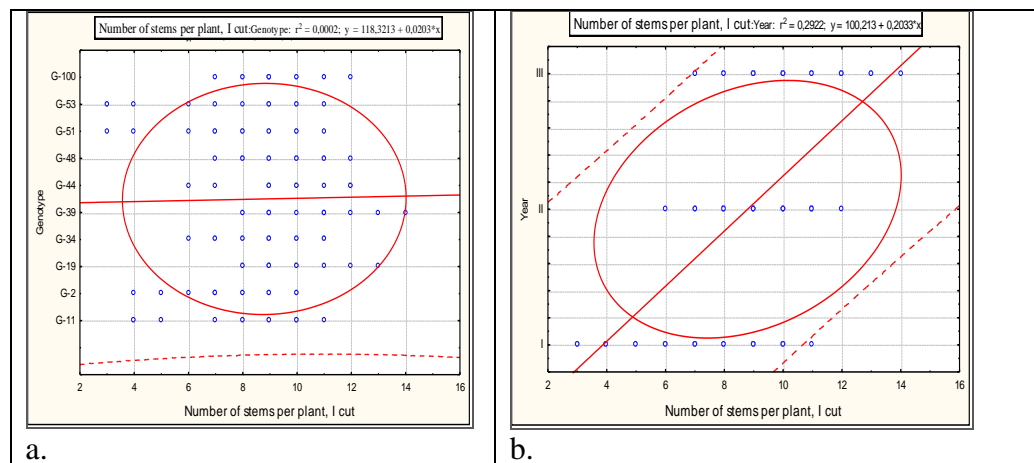


Figure 6. Influence of the genotype (a) and year (b) on the number of stems per plant

The number of stems per plant is statistically significantly affected by the genotype and year. In year 2010, the average number of stems per plant was 7.2. During year 2011, number of stems was 9.2 in average and in year 2012 there were 10 stems per plant. The highest number of stems had the genotype G-39 (12 stems) in 2012, and the lowest had the genotype G-53 (4

stems) in 2010. The highest average number of stems per plant had the genotype G-19 (11 stems), Figure 6 a and b.

The stem diameter of the alfalfa genotypes has a significant impact on the yield of biomass, but increased stem thickness may have an adverse effect on the quality of biomass. Statistically significant differences in stem thickness were determined between genotype G-53 and genotypes G-11, G-19 and G-34. The highest average thickness of the stem is observed at the genotype G-53 (3.1 mm), and the smallest thickness of the stem at the genotype G-11 (2.6 mm), Figure 7a and b.

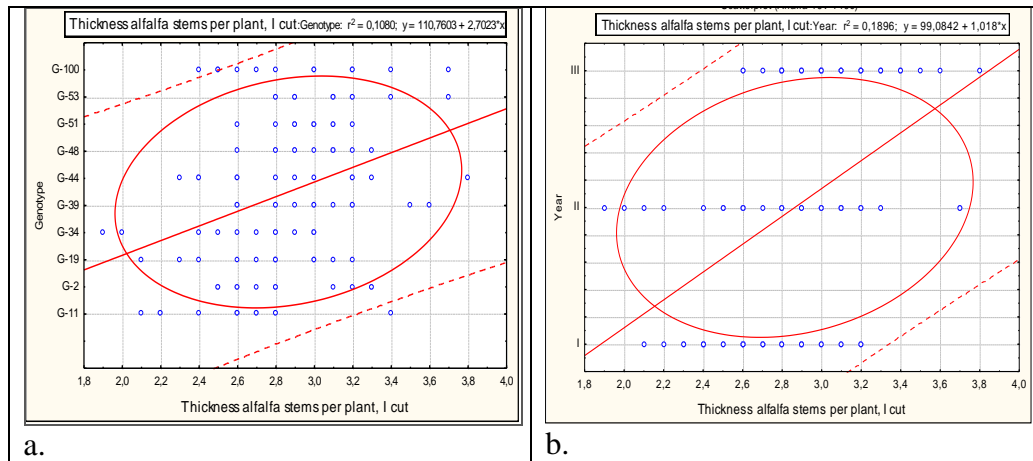


Figure 7. Influence of the genotype (a) and year (b) on the stem thickness

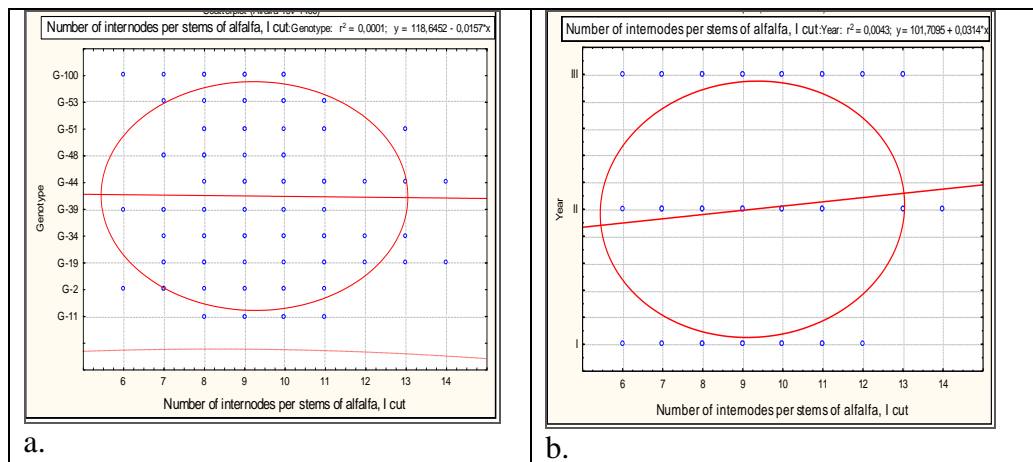


Figure 8. Influence of genotype (a) and year (b) on the number of internodes per stem

The number of internodes per stem was statistically significantly dependent on the alfalfa genotype (Table 5; Figure 8). Observed by years of research, the average number of internodes per stem was uniform and ranged from 9.1 (2010) to 9.3 (2012). The highest number of internodes per stem was found at the genotype G-51 (11.5 internodes) in year 2012, and the lowest (7.5 internodes) at genotypes G-2 (2010) and G-48 (2011). The average number of internodes per stem was found at genotype G-44 (10.6 internodes). During these studies, statistically significantly higher number of internodes per stem had genotype G-44 compared to genotypes G-2, G-39, G-48 and G-100. The average number of internodes at alfalfa grown on acid soil was 10 (KATIĆ *et al.*, 2007).

The dry matter yield per plant is a quantitative property that, with quality, is the most important indicator of value of productivity. Research shows that the genotype and year had a statistically significant effect on the yield of dry matter per plant. The highest average yield was achieved in year 2012 (25.4 g/plant), that was also the most favourable for production. The highest average yield of dry matter was achieved with the genotype G-44 (19.1 g plant⁻¹), and the lowest with the genotype G-2 (13.2 g/plant), so the differences among them are statistically significant. The G-44 alfalfa genotype had a significantly higher dry matter yield than the genotypes G-2, G-11, G-39, and G-51, Table 3, Figure 9.

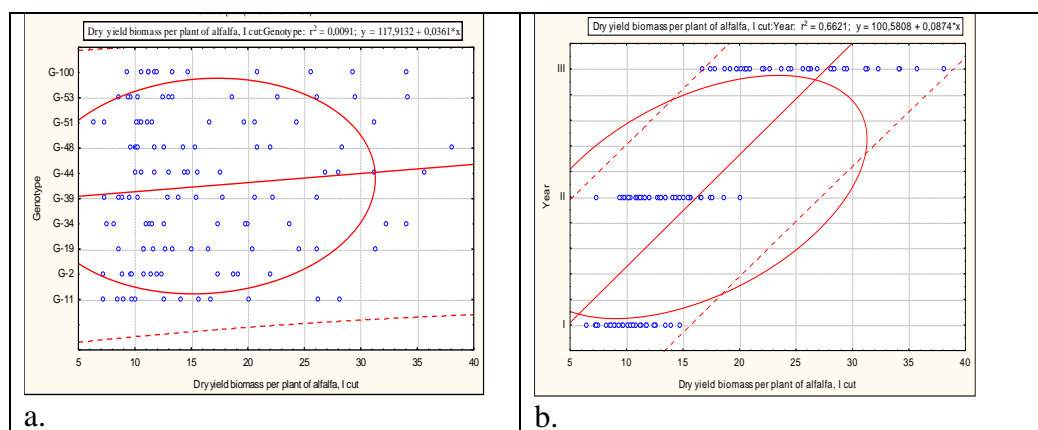


Figure 9. Influence of the genotype (a) and year (b) on the yield of dry matter per plant

The leaf stem ratio

The alfalfa leaf contains a higher percentage of proteins than stem. If in the yield structure we have a higher proportion of leaf in relation to the stem, the quality of the feed is better. In the selection process the plants with a larger share of the leaf are better variants. The percentage share of the leaf in the overall yield structure depends on morphological properties of the plant, climatic conditions, genetic factors and plant maturity.

The variation in the share of leaf in total green biomass in alfalfa genotypes was quite expressed, ranging from 31.28% for G-39 genotype in year 2010 to 47.14% for G-44 genotype in year 2011.

Table 6. The leaf/stem ratio in alfalfa green matter of the I cut

| Parameter | Share of leaf/stem in the green matter (%) | | | | | | | | | |
|-----------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Leaf | | | | | Stem | | | | |
| Year | 2010 | 2011 | 2012 | X | CV | 2010 | 2011 | 2012 | X | CV |
| Genotypes | | | | | | | | | | |
| G-2 | 35.74 | 43.27 | 38.76 | 39.26 | 9.46 | 64.26 | 56.73 | 61.24 | 60.74 | 5.62 |
| G-11 | 38.98 | 46.78 | 40.35 | 42.04 | 14.55 | 61.02 | 53.22 | 59.65 | 57.96 | 9.18 |
| G-19 | 34.09 | 44.25 | 40.36 | 39.57 | 17.00 | 65.91 | 55.75 | 69.64 | 63.77 | 9.23 |
| G-34 | 33.76 | 41.76 | 41.88 | 39.13 | 14.20 | 66.24 | 58.24 | 58.12 | 60.87 | 7.89 |
| G-39 | 31.28 | 40.91 | 39.75 | 37.31 | 17.66 | 68.72 | 59.09 | 60.25 | 62.69 | 8.98 |
| G-44 | 35.91 | 47.14 | 42.91 | 41.99 | 17.02 | 64.09 | 52.86 | 57.09 | 58.01 | 10.07 |
| G-48 | 34.08 | 45.18 | 39.79 | 39.68 | 15.27 | 65.92 | 54.82 | 60.21 | 60.32 | 8.73 |
| G-51 | 35.84 | 41.23 | 38.84 | 38.64 | 10.50 | 64.16 | 58.77 | 61.16 | 61.36 | 5.99 |
| G-53 | 37.74 | 46.20 | 40.53 | 41.49 | 13.39 | 62.26 | 53.80 | 59.47 | 58.51 | 8.40 |
| G-100 | 40.53 | 41.60 | 41.96 | 41.36 | 7.98 | 59.47 | 58.40 | 58.04 | 58.64 | 5.28 |
| Average | 35.80 | 43.83 | 40.51 | 40.05 | - | 64.20 | 56.17 | 59.49 | 59.95 | - |

The coefficient of variation, as a measure of dispersion, for leaf, between years, ranged from 9.46% to 17.66% which leads to the conclusion that it is a relatively medium level of variation of this indicator within the genotype (Table 6). The coefficient of variation, as a measure of dispersion, for stems, between years, ranged 5.28% < CV < 10.07% which leads to the conclusion that it is a relatively low level of variation of this indicator within the genotype (Table 6). The lowest coefficient of variation (CV) for the leaf had genotype G-100 (7.98%), and also the lowest coefficient of variation (CV) of the stem share in the green mass of alfalfa genotypes had genotype G-100 (5.28%) (Table 6).

The genotype G-11 had the highest average leaf content in total green mass, which amounted to 42.04%, while the share of the stem was 57.96%. In most alfalfa genotypes, the largest proportion of leaf mass in green mass was determined in year 2011. The exception were the genotypes G-34 and G-100, with the largest share of leaf in the green mass recorded in year 2012, that was dry year. During these trials, the genotype G-100 had the lowest coefficient of variation of the leaf and stem stock in the green mass. During the tests carried out on the field of the Agricultural School in Kraljevo, on pseudogley-type soil, the share of leaves in the total green mass in 2006 was 39%, and the average for 2005-2006 was 31% (KATIĆ *et al.*, 2007).

The results of the simple correlation analysis of the tested parameters are given in Table 7. For the production-morphological properties, the correlation analysis shows very different values of the results of correlation of the production-morphological characteristics.

Table 7. Coefficient of correlation between 10 populations

| Parameter | Plant height | Stem thickness | No of stems per plant | No of internodes per stem |
|-----------------------|--------------|---------------------|-----------------------|---------------------------|
| Yield of dry matter | 0.793* | 0.561 ^{ns} | 0.626* | 0.622* |
| Plant height | - | 0.460 ^{ns} | 0.736* | 0.257 ^{ns} |
| Stem thickness | - | - | 0.347 ^{ns} | 0.065 ^{ns} |
| No of stems per plant | - | - | - | 0.074 ^{ns} |

*significant at P < 0.05

Significant positive correlation was recorded between yield of dry matter with plant height ($r = 0.793^*$), then between yield of dry matter and number of stems per plant ($r = 0.626^*$), and between yield of dry matter and number of internodes per stem ($r = 0.622^*$). Also, significant positive correlation was identified between plant height and number of stems per ($r = 0.736^*$). A positive, non-significant correlation was identified among the other investigated traits, Table 7.

Based on the estimation of the stability of the alfalfa genotypes tolerant on acid soil according to the Frances and Kannenberg methods, they are found to differ from one another in the yield of dry matter and stability, and are therefore grouped into four groups (Figure 10).

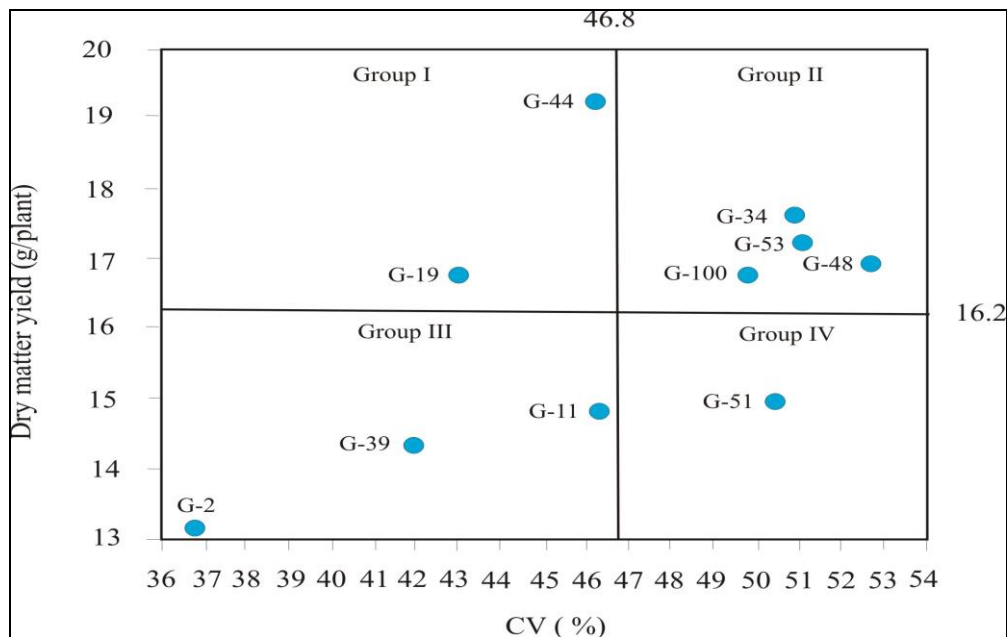


Figure 10. Frances and Kannenberg stability diagram

The alfalfa genotypes grouped into group I have a high and stable yield of dry matter per plant. In this group there are genotypes G-44 and G-19. The II group includes the highest alfalfa genotypes. They have a high yield, but yield variation is more expressed in relation to group one. These genotypes are also desirable for further work. In this group are genotypes G-34, G-48, G-53 and G-100. Group III includes alfalfa genotypes which according to Frances and Kannenberg methods are considered unstable because they have a low yield of dry matter per plant in agroecological conditions in which the tests were carried out. Group III includes G-2, G-11 and G-39 genotypes. The G-51 alfalfa genotype is in group IV. This group is characterized by very high variability, and a low and unstable yield of dry mass.

Agronomic and quality traits in alfalfa are very important to forage industry. In alfalfa industry, biomass yield and forage quality are the key traits for genetic improvement. Other than

the direct traits such as plant height, biomass yield, and dry matter that can deflect the biomass yield of alfalfa, some phenology-related agronomic traits such as plant height in fall can also affect the biomass yield. In this study, it is therefore investigated the possibility of genomic prediction applied to alfalfa germplasm resources and genomic selection applied to 10 important agronomic traits and 15 forage quality traits of alfalfa production (JIA *et al.*, 2018).

The degree of survival of alfalfa plants on acid soil over 80% was determined for genotypes G-44, G-48 and G-11. Alfalfa achieves maximum productivity when pH is 6.2-7.5. Soil pH levels near 7 are optimal for overall nutrient availability, crop tolerance, and soil microorganism activity. Soil microorganism activity is greatest near neutral conditions and microbial activity is considerably reduced at pH 5 and below. A purely physiological approach is not sufficient to identify resistance mechanisms in alfalfa and this will require an interdisciplinary approach integrating genetic, molecular, and physiological investigations.

Soil organic matter is an essential component of soil, contributing to soil biological, chemical, and physical properties. Soil pH is a measure of a soil solution's acidity and alkalinity that affects nutrient solubility and availability in the soil. Factors influencing soil pH include organic matter decomposition, nitrogen fertilizer source, weathering of minerals and parent material, climate, and land management practices. Availability of nutrients for plant uptake varies depending on soil pH. The availability of cation nutrients is often hindered by decreased solubility in highly basic soils and increased susceptibility to leaching or erosion losses in acidic soils. For anion nutrients, availability is generally the opposite. Soil pH levels near 7 are optimal for overall nutrient availability, crop tolerance, and soil microorganism activity. Soil pH can be modified by using chemical amendments; however these treatments may only be effective for a relatively short amount of time and are generally not economically viable (MCCAULEY *et al.*, 2017). In general, nitrogen (N), potassium, calcium, magnesium and sulfur are more available within soil pH 6.5 to 8, while boron (B), copper, iron, manganese, nickel (Ni), and zinc are more available within soil pH 5 to 7. Phosphorus is most available within soil pH 5.5 to 7.5. At pH less than 5.5, high concentrations of H⁺, aluminum and manganese in soil solution can reach toxic levels and limit crop production (KIDD and PROCTOR, 2001). Neutral conditions are the best for crop growth. However, optimum pH conditions for individual crops vary: alfalfa 6.2-7.5; barley 5.5-7.0; dry bean 6.0-7.0; maize 5.5-7.0; oats 5.5-7.0; pea 6.0-7.0; potato 5.0-5.5 (HAVLIN *et al.*, 1999). Some crop varieties are being developed to tolerate lower pH and higher aluminum levels. Soil microorganism activity is greatest near neutral conditions, but optimal pH ranges vary for each type of microorganism. Microbial activity is considerably reduced at pH 5 and below (ROUSK *et al.*, 2009). Moreover, certain 'specialized' microorganisms, such as nitrifying bacteria (convert ammonium [NH₄] to nitrate [NO₃ -]) and nitrogen-fixing bacteria associated with many legumes, generally perform poorly when soil pH falls below 6 (MUNNS, 1986). For example, alfalfa (a legume) grows best in soils with pH levels greater than 6.2, conditions in which its associated nitrogen-fixing bacteria grow well too. In contrast, fungi generally thrive at low pH, so fungal diseases are more common in acidic soils. Finally, pesticide effectiveness and residual (carry-over) is an issue in acidic soils (READER *et al.*, 2015). When soil pH is extremely acidic or basic, pH modifications may be needed to obtain optimal growing conditions for specific crops.

Genetic resources are the basis for the development of modern high-yielding varieties worldwide (RADINOVIĆ *et al.*, 2018; JANKOVIĆ *et al.*, 2018). A lot of breeding aims for alfalfa are similar to those in other crops-increasing yield, enhancing nutritive value and improving

tolerance to abiotic and biotic stresses are all important. Most used methods for the development of alfalfa cultivars is recurrent phenotypic selection (OTEN *et al.*, 2018).

Alfalfa is the most important forage plant species. There are numerous alfalfa varieties in the world with improved yield potential and forage quality (STRBANOVIC *et al.*, 2017).

The high stability of high-yielded alfalfa genotypes is the most desirable feature of a genotype. When genotypes are tested for stability, often genotypes with the highest yield show a low level of stability and vice versa.

CONCLUSION

The degree of survival of alfalfa plants on acid soil over 80% was determined for genotypes G-44, G-48 and G-11. During three-year trials, the alfalfa genotype G-100 had the highest average plant height (36.9 cm). The highest average dry matter yield per plant during the three-year trial was achieved with the genotype G-44.

The analysis of the interaction allowed the isolation of individual genotypes that showed the greatest stability and potential for the yield of alfalfa in different production conditions. The high and stable yield of dry matter per plant have genotypes G-44 and G-19 and they are grouped in group I and are desirable for further breeding.

The highest yield have alfalfa genotypes G-34, G-48, G-53 and G-100, classified into group II, characterized by expressed yield variation, and these genotypes are desirable for further work.

The alfalfa genotypes G-2, G-11 and G-39 are classified into group III, with low and unstable yields of dry matter per plant and are not desirable for further selection process. The group IV includes genotype G-51, characterized by very high variability, low and unstable yield of dry matter, and it is not important for further selection in terms of the trait, because expression of the most important selection trait is below the average.

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MORFOLOŠKE KARAKTERISTIKE GENOTIPOVA LUCERKE TOLERANTNIH NA NISKE pH VREDNOSTI ZEMLJIŠTA

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Izvod

Od prikupljenih i zasijanih 68 sorti i 8 autohtonih populacija lucerke izdvojen je 41 genotip lucerke koji je pokazao tolerantnost prema zemljištu niže pH vrednosti. Odabrani genotipovi lucerke su zatim zasijani na oglednom polju na zemljištu koje pripada tipu smeđeg zemljišta na šljunku ili cambisolu, čija je pH vrijednost u vodi bila od 5,33 do 5,64. Nakon 5 godina na osnovu agronomskih svojstava odabrano je 10 genotipova sa kojima je postavljen ogled na zemljištu čija je pH vrednost bila od 5,0 do 5,1 u H₂O. Za ova ispitivanja korišćeni su genotipovi: G-2, G-11, G-19, G-34, G-39, G-44, G-48, G-51, G-53 i G-100. Praćena su sledeća svojstva: visina biljaka (cm), broj stabljika po biljci, debljina stabljike (mm), broj internodija po stabljici, odnos stablo/list (%), prinos biomase u I otkosu (g/biljci). Tokom ovih ispitivanja najveći stepen tolerantnosti prema nižim pH vrijednostima zemljišta ispoljio je genotip G-44. Dobri rezultati postignuti su i sa genotipovima lucerke G-11 i G-48 kod kojih je procenat preživjelih biljaka bio preko 80%. Najveću prosječnu visinu biljke tokom trogodišnjeg istraživanja imao je genotip G-100 (36,9 cm). Visok i stabilan prinos suve mase po biljci ostvarili su genotipovi G-44 i G-19, grupisani u I grupu i poželjni su za dalje oplemenjivanje. Na ispitivane parametre statistički značajan i visoko značajan uticaj imali su genotip i godina, dok je značaj interakcije genotip x godina utvrđen samo za debljinu stabljike.

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