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CORRELATIONS BETWEEN MORPHO-AGRONOMIC TRAITS AND QUALITY COMPONENTS OF BIRDSFOOT TREFOIL

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Abstract: Birdsfoot trefoil is a perennial legume for the production of high-quality forage. Improving the production and quality of forage is one of the strategic objectives of breeding programs. The genotypes for this trial were selected from promising offspring collected from local populations in Bosnia and Herzegovina. A trial with eight genotypes (7 promising lines and 1 variety) was designed in a randomized block system with four replicates. In the first growth, 11 components of yield and quality of biomass were analyzed, and in the second growth, four additional parameters for seed production. In the first growth, highly significant correlations were found between plant height and the proportion of leaves (0.85**) and the yield of green matter and dry matter (0.81**), while a high negative correlation was found between the nitrogen-free extract (NFE) and the crude protein content (-0.79**). In the regrowth, statistically highly significant ($p<0.01$) positive correlations were found between the content of NFE and ash (0.77**). Statistically significant ($p<0.05$) positive relationships were found between green matter yield and dry matter yield (0.81**), green matter yield and stem diameter (0.79*), seed yield and number of pods (0.83*), and cellulose content and plant height (0.73*). The identification of positive correlations for certain productive and nutritional traits will be used in breeding programs for the creation of new varieties with improved forage quality.

Key words: birdsfoot trefoil, morphological traits, agronomic traits, correlations.

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) is a very important perennial legume in the composition of grass-clover mixtures on natural and sown meadows in Bosnia and Herzegovina. It belongs to the group of perennial small-seeded legumes of high quality for the production of protein feed. It is usually sown in mixtures and only to a very limited extent as a pure crop (Radić, 2014). It tolerates a variety of

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soils and its particular speciality is that it tolerates a wide range of pH values from 4 to 9 (Marvin, 2004).

Its particular importance in Bosnia and Herzegovina lies in the possibility of cultivation on acidic soils. In their study, Vučković et al. (2007) came to the conclusion that the local populations of birdsfoot trefoil have several positive characteristics compared to other locations, based on the morphological, chemical and nutritional values investigated. As Sareen (2004) stated, the high proportion of easily digestible proteins is of particular importance for ruminants.

Knowledge of the correlations between the analyzed traits in the selection material is very important due to their interdependence, because changing one trait in the corresponding relationship also changes the others. Correlation coefficients are determined by correlations, regardless of what the dependent and independent variables are (Hallauer and Miranda, 1988). Genetic diversity is of crucial importance to breeders in the improvement of agricultural plant species. The complexity of the red clover genome with high heterozygosity and heterogeneity has been an obstacle in genomic analyses (Li et al., 2019).

Wróbel and Zielewicz (2019) investigated the variety of red clover and birdsfoot trefoil by taking samples of the green mass 9 times a week during a period from the end of April to the end of June. They carried out chemical analyses of the plant material in which they determined crude protein (CP), water-soluble carbohydrate (VSC), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin (ADL) and dry matter digestibility (DDM). The analyses showed that the birdsfoot trefoil leaf had a higher content of crude proteins, NDF and ADF than that of red clover at all stages of development.

Phenotypic differences in morpho-agronomic traits and forage quality traits within a population are based on genetic variation, but are also influenced by environmental conditions (precipitation, temperatures) and by the frequency of cutting, the maturity stage of the plant at the time of cutting and the cutting height (Swarup et al., 2021).

If there is a positive correlation between the two analyzed characteristics, selection of one characteristic also improves the other. Progress can be made in the selection of target traits through the direct selection of selected traits. However, negative correlations often mean problems with selection (Neyhart et al., 2019; Radinović et al., 2022).

The aim of this study is to evaluate the correlation between morphological and qualitative traits in perspective genotypes of the birdsfoot trefoil during a two-year experimental work. The results obtained can be used in selection based on the interdependence in the inheritance of the individual traits as well as in the simultaneous selection of several traits of birdsfoot trefoil.

Material and Methods

The genotypes for this trial were selected from promising offspring collected from local populations in Bosnia and Herzegovina. Seeds were collected from ten natural sites of birdsfoot trefoil in the mountains and hills and sown the following year. In the first selection cycle, seven promising accessions were selected from 28 new accessions.

The experiment was conducted southwest of Banja Luka (Fig. 1), in the village of Dobrnja on Manjača (N 44°39'57", E 17°00'24", 527 *m* above sea level). The trial was set up in four replicates in a randomized block system. The size of the trial plot was 1 x 2 *m*. The experimental units were sown manually at a row spacing of 0.2 *m*. The sowing rate was 1.5 $g\ m^{-1}$ or 15 $kg\ ha^{-1}$. No pesticides, chemical fertilizers or irrigation were used during the trial.

Figure 1. The experimental site at the “Center for Agriculture and Rural Development” Banja Luka, Bosnia and Herzegovina.

The results in 2013 showed the production characteristics of the newly created genotypes in comparison with the Tera variety and in comparison with each other. In 2013, there were two growths that were analyzed individually and together. The first growth was used for the production of green matter, and the second growth for the production of green matter and seeds. During the vegetation period, the following parameters were measured, analyzed and recalculated: green matter yield ($kg\ ha^{-1}$), dry matter yield ($kg\ ha^{-1}$), tiller diameter (*mm*), plant height (*cm*), number of tillers per plant, proportion of leaves (%), crude proteins, crude fats, crude cellulose, ash and nitrogen-free extract (NFE) in the first growth. In addition to these parameters, the following components of the seed yield were analyzed in the regeneration: number of pods per plant, number of seeds in a pod, seed yield (*kg ha*$^{-1}$) and thousand-seed weight.

The chemical analyses were carried out according to the following methodology: crude proteins by the micro-Kjeldahl method, modified according to Bremner (1960), i.e., crude proteins by multiplication by a factor of 6.25; the amount of crude fats in the plant material by the Soxhlet method; the content of crude cellulose in the plant material by the Henneberg-Stohmanov method; the crude ash content in the plant material by annealing at 550^{o}C to constant mass.

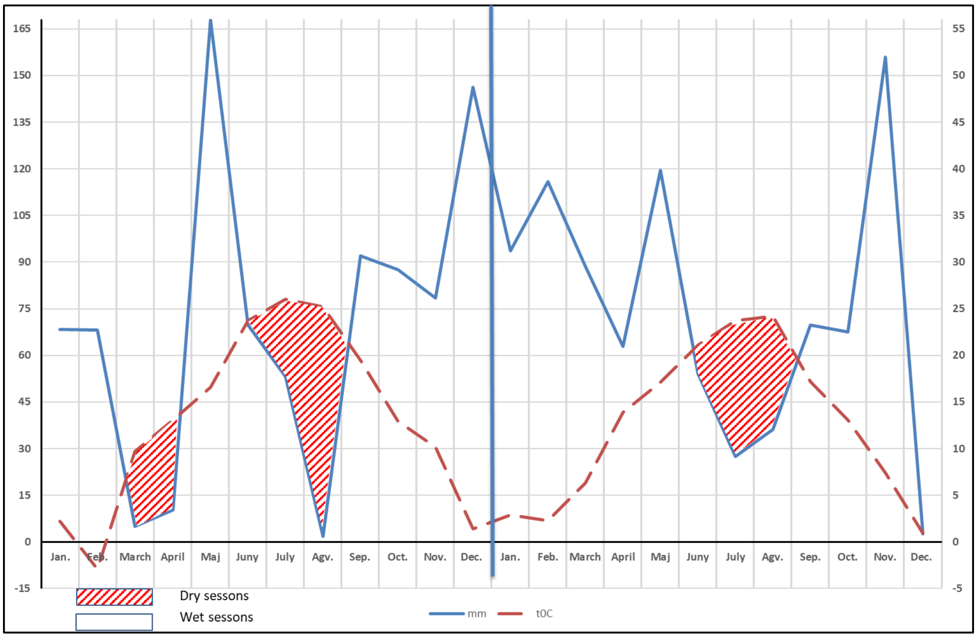

Figure 2. The relationship between precipitation and air temperature for the Banjaluka region in 2012 and 2013 (according to *H.Walther*).

Statistical analysis

The results of the biometric measurements were processed using PC applications for the Statistical Package for Social Sciences and Excel. The results of the analyzed traits were processed by analysis of variance (ANOVA) with a computer program using the GLM procedure. The Duncan's Multiple Range Test (DMRT) was used to determine the significance of the differences between the genotypes and their ranking at a significance level of p=0.01. Correlations between the analyzed traits were calculated as the Pearson's correlation coefficients and the significance was determined.

Results and Discussion

The positive tendency of plant breeding involves the improvement of several traits at the same time, but this is difficult to achieve due to the genetic correlations between the different traits (Breseghello and Coelho, 2013). The correct evaluation of genetic correlations requires large sample sizes and the presence of genetic generic data, which are not always available. Therefore, phenotypic correlations are often assumed to reflect genotypic correlations (Sodini et al., 2018). The correlations are caused by pleiotropic genes, physical linkage of genes on the chromosome or by the genetic structure of a population (Breseghello and Coelho, 2013).

The descriptive statistical parameters of the analyzed morphological-agronomic and qualitative traits of eight genotypes (seven promising offspring and the variety Tera) are listed in Table 1. Based on the f-test carried out, it can be seen that there was no statistical significance for fat content. There was a very high statistical significance for all other analyzed parameters ($p<0.001$). To determine which genotypes differed in the analyzed parameters, the Duncan's post-hoc test was used. The greatest differences were found in the tiller diameter and the leaf/stem ratio. The Duncan's test showed a higher degree of differentiation for quantitative than for qualitative traits.

Table 1. Average values of the analyzed characteristics of the first growth.

Genotype	1	2	3	4	5	6	7	8	f-test
Green mass	12.56^{a}	12.44^{a}	10.13^{b}	11.27^{b}	10.38^{b}	10.26^{b}	10.96^{b}	10.77^{b}	***
Dry mass	4.33^{b}	4.96^{a}	3.52^{d}	4.17^{bc}	$4.21b^{c}$	3.88^{c}	4.04^{bc}	4.06^{bc}	***
Stem diameter	1.53^{a}	1.47^{abc}	1.49^{ab}	1.53^{a}	1.35^{d}	1.38^{cd}	1.40^{bcd}	1.25^{e}	***
Stem height	37.2^{bc}	36.4^{bcd}	38.4^{b}	35.6^{cd}	35.9^{cd}	34.6^{d}	42.8^{a}	36.3^{bc}	***
Tiller number	7.65^{b}	6.65^{c}	9.15^{a}	7.15^{bc}	6.55^{c}	6.80^{c}	9.22^{a}	7.19^{bc}	***
Leaf/stem	65.5^{a}	59.5^{bcd}	56.5^{de}	55.5^{e}	62.0^{b}	61.5^{bc}	57.5^{de}	58.5^{cde}	***
Proteins	21.55^{a}	19.14^{b}	17.75^{b}	18.20^{b}	18.01^{b}	18.22^{b}	16.16^{c}	19.10^{b}	***
Fats	2.75^{a}	2.81^{a}	2.63^{a}	2.70^{a}	2.72^{a}	2.75^{a}	2.78^{a}	2.90^{a}	ns
Cellulose	22.95^{d}	23.42^{cd}	24.68^{ab}	23.96^{bc}	23.49^{cd}	23.86^{bc}	25.01^{a}	23.52^{cd}	***
Ash	9.55^{b}	10.44^{b}	10.64^{b}	11.61^{a}	11.46^{a}	10.44^{b}	10.23^{b}	11.44^{a}	***
NFE	43.2^{b}	44.19^{bc}	44.30^{bc}	43.53^{b}	44.32^{bc}	44.73^{bc}	45.82^{a}	43.04^{b}	**

**** ($p<0.001$), *** ($p<0.01$), * ($p<0.05$), ns – no significant, $^{a,b,c,d...}$ Values denoted by the same letter are not significantly different at the $p<0.01$ level of probability (Duncan's Multiple Range Test), NFE – nitrogen-free extract.

For regrowth, the components of yield and quality of green matter and the components of seed yield were analyzed (Table 2). The F-test shows that there was no statistical significance for thousand-seed weight, protein content and NFE. A low significance ($p<0.05$) was found for the number of tillers per plant. A very high difference ($p<0.01$) was found between green matter yield, plant height and cellulose and ash content. A very high ($p<0.001$) difference was found for all other analyzed characteristics.

The Duncan's test was used to determine the difference between the genotypes within each trait. The greatest difference was noted in the number of pods per plant and the seed yield. There is a very high degree of differentiation in the ratio of leaf to stem among the genotypes tested.

Table 2. Average values of the investigated characteristics of regrowth.

	1	2	3	4	5	6	7	8	F- test
Green matter	10.37^{a}	9.42^{b}	9.73^{ab}	9.56^{ab}	9.34^{b}	9.2^{b}	9.82^{ab}	9.26^{b}	**
Dry mass	2.77^{a}	2.46^{bc}	2.47^{bc}	2.41^{bc}	2.59^{abc}	2.43^{bc}	2.63^{ab}	2.38^{c}	***
Stem diameter	1.47^{a}	1.35^{ab}	1.41^{a}	1.42^{a}	1.11^{c}	1.14^{c}	1.38^{ab}	1.22^{bc}	***
Plant height	31^{ab}	28.2^{ab}	28.4^{ab}	25^{b}	24.6^{b}	27.6^{b}	34.8^{a}	25^{b}	**
Tiller number	8.24^{ab}	7.23^{ab}	9.21^{ab}	7.65^{ab}	7.13^{ab}	6.98^{b}	9.57^{a}	8.11^{ab}	*
Seeds per pod	10.8^{b}	11.2^{b}	18.8^{a}	20.2^{a}	12.8^{ab}	11.0^{b}	14.6^{b}	14.8^{b}	***
Pods per plant	12.8^{e}	21.3^{bc}	22.5^{b}	18.8^{cd}	25.3^{a}	26.5^{a}	17.8^{d}	20.4^{bcd}	***
Seed weight	1.15^{a}	1.15^{a}	1.15^{a}	1.25^{a}	1.20^{a}	1.30^{a}	1.25^{a}	1.30^{a}	ns
Grain yield	195^{e}	236.5^{d}	245.0^{c}	268.5^{b}	280.5^{a}	27.05^{a}	243.0^{cd}	241.5^{cd}	***
% leaf	58.5^{a}	48.2^{d}	42.7^{e}	49.9^{cd}	52.4^{bc}	51.1^{cd}	55.1^{ab}	53.2^{bc}	***
Proteins	17.5^{a}	18.51^{a}	18.86^{a}	16.59^{a}	16.83^{a}	17.06^{a}	16.48^{a}	18.55^{a}	ns
Fat	2.85^{a}	2.89^{a}	2.74^{ab}	2.52^{aba}	2.90^{a}	2.68^{ab}	2.56^{b}	2.65^{ab}	***
Cellulose	28.96^{ab}	27.40^{b}	28.55^{ab}	28.6^{ab}	28.2^{ab}	29.4^{ab}	30.5^{a}	27.05^{b}	**
Ash	11.23^{ab}	9.71^{b}	12.1^{a}	11.68^{a}	11.4^{ab}	11.2^{ab}	12.5^{a}	12.47^{a}	**
NFE	39.46^{a}	41.49^{a}	37.75^{a}	40.61^{a}	40.67^{a}	39.66^{a}	37.96^{a}	39.28^{a}	ns

**** ($p<0.001$), *** ($p<0.01$), * ($p<0.05$), ns – no significant, $^{a,b,c,d...}$ Values denoted by the same letter are not significantly different at the $p<0.01$ level of probability (*Duncan's Multiple Range Test*), NFE – nitrogen-free extract.

Table 3 shows the correlation coefficients between the 11 characteristics analyzed in the first cut. Highly significant correlations were found between height and leaf percentage (0.85**) and green and dry matter yield (0.81**). Vasiljević et al. (2006) found high correlations between the yield of green matter and dry matter and plant height in their studies on red clover, assuming that we can increase the production of green matter by selecting taller plants.

The coefficients of the qualitative traits showed a high significance level of the relationship between NFE and leaf content, while a high negative correlation was found between NFE and crude protein content (-0.79**). The proportion of crude ash was highly correlated with the number of tillers (0.78**), while a negative correlation was found with the proportion of leaves (-0.71*) and the crude protein content.

Table 3. Correlation coefficients between the analyzed properties of the first cut.

	11	10	9	8	7	6	5	4	3	2
10	-0.25									
9	**0.73***	0.04								
8	-0.21	0.05	-0.35							
7	**-0.79***	-0.34	**-0.87****	0.21						
6	-0.21	-0.53	**-0.71***	0.15	0.68					
5	0.44	-0.36	**0.78***	-0.33	-0.42	-0.39				
4	0.61	-0.38	0.69	-0.27	-0.48	-0.27	**0.85****			
3	-0.06	-0.41	0.02	-0.64	0.26	-0.17	0.22	0.06		
2	-0.18	-0.13	-0.59	0.45	0.40	0.30	-0.56	-0.18	0.15	
1	-0.35	-0.49	-0.55	0.30	0.66	0.37	-0.20	0.01	0.50	**0.81****

p<0.05*; p<0.01**; N=8; Properties: 1– green matter, 2 – dry matter yield, 3 – stem diameter, 4 – plant height, 5 – number of tillers, 6 – leaf share, 7 – crude protein, 8 – crude fat, 9 – crude cellulose, 10 – ash, 11 – NFE.

The correlation coefficients of the analyzed traits in the regrowth between 15 traits, based on the average values of all traits, are shown in Table 4. The obtained values of the correlation coefficients show that most of the qualitative traits had a positive relationship with each other, except for the proportion of leaves, which had a negative relationship with most of the measured parameters. The relationships between the qualitative characteristics were mostly negative.

Table 4. Correlation coefficients between the analyzed properties of the second cut.

	15	14	13	12	11	10	9	8	7	6	5	4	3	2
14	**0.77***													
13	-0.52	0.23												
12	0.40	-0.65	-0.41											
11	-0.12	-0.16	-0.67	0.37										
10	0.05	0.13	0.29	-0.004	-0.51									
9	0.23	0.08	0.02	-0.25	-0.38	-0.39								
8	-0.09	0.47	0.14	-0.68	-0.37	0.23	0.50							
7	0.18	-0.13	-0.18	0.13	0.06	-0.55	**0.83***	0.30						
6	-0.31	0.54	0.00	-0.62	0.01	-0.52	0.27	0.09	-0.04					
5	**-0.87****	0.66	0.44	-0.39	0.11	-0.01	-0.43	-0.15	-0.47	0.42				
4	-0.55	0.13	**0.73***	-0.12	-0.18	0.33	-0.54	-0.22	-0.51	-0.23	0.67			
3	-0.26	0.02	0.21	-0.19	0.13	-0.02	-0.68	-0.52	**-0.80***	0.36	0.58	0.51		
2	-0.19	-0.02	0.48	0.38	-0.33	0.64	-0.57	-0.48	-0.58	-0.42	0.30	0.62	0.33	
1	-0.36	0.09	0.41	0.10	-0.08	0.40	**-0.79***	-0.57	-0.84	-0.02	0.55	0.64	**0.79***	**0.81***

p<0.05*; p<0.01**; N=8; Properties: 1– green matter, 2 – dry matter yield, 3 – stem diameter, 4 – plant height, 5 – number of stems, 6 – number of seeds per pod, 7 – number of pods, 8 – seed weight, 9 – grain yield,10 – leaf share, 11 – crude protein, 12 – crude fat, 13 – crude cellulose, 14 – ash, 15 – NFE.

Negative correlations were found between protein content and yield of green matter, dry matter, plant height, cellulose content, ash and NFE. Crude cellulose content was negatively correlated with protein, fat and NFE content, while it was positively correlated with ash content.

Statistically highly significant positive correlations with a significance level of $p<0.01$ were observed between NFE content and ash (0.77**). Statistically significant ($p<0.05$) positive correlations were found between green matter yield and dry matter yield (0.81**), green matter yield and stem diameter (0.79*), seed yield and number of pods (0.83*), cellulose content and plant height (0.73*) and the proportion of NE and ash (0.77*).

A highly significant negative correlation was found between NFE and the number of stems (-0.87**) and a significantly negative correlation between green matter yield and seed yield (-0.79*), stem diameter and number of pods and NFE content and number of tillers (-0.87**). Genotypes with higher forage production had a lower seed yield (Radić et al., 2011).

Conclusion

Positive correlations were found between yield and the yield components, green matter and seeds. These values indicate the possibility of improving yield by using individual components such as selection criteria in the breeding process.

In the newly selected synthetics, a significant correlation was found between the number of pods and seed yield (0.83*) and between plant height and the percentage of crude cellulose (0.73*). A negative correlation was found between green matter yield and seed yield (-0.79*) and between the number of tillers and NFE (-0.87**).

Potential genotypes such as 1, 2 and 4 have been identified as useful for improved production of productive and nutritional traits of birdsfoot trefoil, while genotypes 5 and 6 are of particular importance for future breeding programs due to their good generative properties.

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KORELACIJE MORFOLOŠKO-AGRONOMSKIH OSOBINA I KOMPONENTI KVALITETA SMILJKITE

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R e z i m e

Smiljkita je višegodišnja mahunarka za proizvodnju visokokvalitetne kabaste stočne hrane. Poboljšanje produkcije i kvaliteta stočne hrane jedan je od strateških ciljeva u oplemenjivačkim programima. Genotipovi za ovaj ogled odabrani su od perspektivnih potomstava koji su prikupljeni iz lokalnih populacija na području Bosne i Hercegovine. Ogled sa osam genotipova (7 perspektivnih linija i 1 sorta) postavljen je po slučajnom blok sistemu u četiri ponavljanja. U prvom porastu analizirano je 11 komponenti prinosa i kvaliteta zelene mase, a u drugom još četiri parametra za produkciju sjemena. Utvrđeni su Pirsonovi koeficijenti korelacija. U prvom porastu utvrđene su visokoznačajne korelativne veze između visine biljke i udjela lista (0,85**) kao i prinosa zelene mase i suve materije (0,81**), dok je visoka negativna korelativna veza utvrđena između *BEM*-a i sadržaja sirovih proteina (-0,79**). U drugom porastu uočene su statistički visoko značajne ($p<0,01$) pozitivne korelativne veze između sadržaja *BEM*-a i pepela (0,77**). Značajne statistički ($p<0,05$) pozitivne veze su konstatovane između prinosa zelene mase i prinosa suve materije (0,81**), prinosa zelene mase i debljine stabljike (0,79*), prinosa sjemena i broja mahuna (0,83*) i sadržaja celuloze i visine biljke (0,73*). Identifikacija pozitivnih korelativnih veza za pojedine produktivne osobine i nutritativna svojstva imaće primjenu u oplemenjivačkim programima za stvaranje novih sorti sa poboljšanim kvalitetom stočne hrane.

Ključne riječi: smiljkita, morfološke karakteristike, agronomska svojstva, korelativne veze.

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