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Morphometric characteristics of *Lotus corniculatus* L. genotypes

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The aim of this study was to examine the degree of variability in morphological and agronomic characteristics of 20 *Lotus corniculatus* L. local genotypes, and also to set aside germplasm that will be used as a source of genetic basis for improvement of the studied properties. In poor quality soils, *L. corniculatus* L. plays an important role providing protein component in animal feeds. The importance of *L. corniculatus* L. in livestock development at hilly-mountainous areas is enormous. This study presents productive characteristics of 20 *L. corniculatus* L. genotypes selected from local populations in the previous period of selection. Significant differences were observed for most of the monitored parameters. The studied genotypes showed the greatest variability in green matter yield (CV 49, 58), number of pods per plant (CV 26, 27) and seed yield (CV 34, 31). To determine the significance of differences between the genotypes and their ranking for the level of significance, Duncan's multiple range test was used. Based on the results of Duncan's multiple range test and analysis of the significance of differences between genotypes, it was ascertained that genotypes G1, G3, G15 and G17 are the basis for the creation of synthetic variety with good production properties.

Key words: *Lotus corniculatus* L., genotype, variability.

INTRODUCTION

Birdsfoot trefoil (*Lotus corniculatus* L.) is a perennial legume, important for forage production. Genus *Lotus* is highly polymorphic and widespread (Buselinck and Grant, 1995; Balan et al., 2002). It comprises about 200 annual and perennial species (Grant and Niizeki, 1999). From the agricultural standpoint, birdsfoot trefoil is the most important species in this genus, with high nutritive value. The greatest divergence of *L. corniculatus* L. occurs in the Mediterranean region (Grant and Niizeki, 1999), and includes regions of Serbia, Bosnia and Herzegovina (Vojin and Jelačić Slavica, 2007). Large collections of variability for a great number of agronomically important traits are local populations that are well adapted to local ecological conditions. Large number of *L. corniculatus* L. populations exists in climatic and soil conditions of Republic of Srpska and possess great genetic variability (Gatarić et al., 2010). Variability for the great number of agronomically traits is very important in order to identify the most productive genotypes, and to introduce them into the further breeding process. Genetic improvement of the population cannot be achieved beyond the boundaries that are determined by genes. Therefore, the selection of germplasm that will be included in the breeding is a very sensitive stage of breeding process (Rumbaugh et al., 1988). In order to achieve the expected progress, sources of parental germplasm, population size and intensity of selection are very important. Most breeders (Prosperi et al., 2006) reported a higher breeding value of

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locally adapted populations in relation to germplasm from other geographic areas.

The aim of this study was to determine the divergence degree of morphological and agronomical characteristics among 20 *L. corniculatus* L. genotypes, and to set aside germplasm that will be used as a source of genetic basis for improvement of the studied properties. Parental combinations with the most positive features for the new cultivars creation should be allocated on the basis of morphological and biological variability.

MATERIALS AND METHODS

From different localities throughout Bosnia and Herzegovina, 20 *L. corniculatus* L. genotypes were obtained in the previous cycle of selection from natural populations. All together, 20 *L. corniculatus* L. genotypes of national origin were examined. The experiment was established during three growing seasons (2006 to 2008) at the site of Bumbara (N 44°44' 59" E, 17° 02' 52", 431 m above sea level), close to the city of Banja Luka. Into a Jiffy pots filled with substrate, sowing of seeds was carried out on 17th March 2006. A greenhouse was used for the production of transplants. The seed was planted in the substrate and after planting, containers were placed in the greenhouse. After thirty days, plants were set out in the open due to hardening. When the plants were about 10 cm high, transplantation was performed on experimental plots. Transplanting of young plants was completed on 10th May, 2006. From each genotype, 10 plants were transplanted at the distance of 50 cm between plants and 80 cm between rows. There was no isolation between the plants, and pollination was free. Morphometric analysis of genotypes was carried out during 2006 to 2007 on five randomly selected plants of each genotype. The following parameters were measured: green mass yield (g), plant height (cm), number of stems (pieces), number of pods (pieces), thickness of the stems (mm), yield of air-dried hay (g), seed yield (g), weight of 1000 seeds (g) and number of seeds per pod (pieces). These parameters were measured on 100 plants in one growing season, and at the same plants these parameters were measured in the following year.

Analyses were performed on first cut plants, and the second year the same parameters were analyzed on second cut plants. In the first year of the research, manual harvesting of individual plants was done when 65% of the pods got dark brown color (the technological maturity of the seeds). The harvesting was done at that stage of maturity in order to avoid dispersal and the loss of seed. In the second year of the research, the first mowing was conducted when 50% of flowers were in bloom. The second mowing was conducted in August 2007 when 65% of pods got golden brown color, that is, when the shooting of the most mature pods began. The green mass yield was measured on an electronic scale after manual harvesting, directly at the experimental plot. The yield of air-dried hay was determined from the samples that were dried in the greenhouse, and then weighed on electronic scale. Plant height was measured immediately prior to mowing, on the five stems of each genotype and expressed as the average plant height (cm). Stem thickness was measured immediately after harvest (between first and second nodes above the cut), on the five stems and expressed as the average stem thickness (mm). Number of stems and number of pods per plant was analyzed by counting on each plant in the first and second year after harvest. Separation of seeds was done manually from pods on each plant. After cleaning, seed yield per plant was determined by weighing (g). Mass of 1000 seeds (g) was determined in the laboratory. The number of seeds per pod was calculated as the average from the ten pods from one plant.

The results of biometric measurements were processed by PC applications for Windows (SAS Institute Inc., 2003). The results of the studied traits were analyzed by analysis of variance (ANOVA) using SAS 9.1. program with the GLM procedure. Duncan's multiple range test (DMRT) was used to determine the significance of differences between genotypes and plants within genotypes and their ranking for the level of significance P = 0.05.

RESULTS A ND DISCUSSION

The success of selection depends on the existence of characteristics variability. Coefficient of variation is a very usable and useful measure in the selection work. It allows a direct comparison of the properties expressed in different units of measurement.

For the studied genotypes, high coefficients of variability were determined for most measured traits in both years of research. The coefficient of variation ranged from 6.51 for the mass of 1000 seeds (2006) up to 49.58 for the green mass yield (2006). The obtained values indicate high level of differences between analyzed traits. In 2006, the green matter yield had the highest coefficient of variation (CV 49.58), and in 2007, it was lower (CV 24.77) (Table 1). The coefficient of variation of air-dried hay yield was in the proportion of green mass coefficient. Similar results were obtained by other researchers (Radovic et al., 2007). Examined genotypes had higher coefficient of variation for the number of stems per plant, number of pods per plant and seed yield per plant than for plant height, stem thickness, mass of 1000 seeds and number of seeds per pod. Number of stems per plant had a great coefficient of variation, but it can be explained by the fact that observed genotypes had a large number of stems. Based on the maximum and minimum values, it can be concluded that there is wide amplitude of examined characteristics values in the studied material. Observed plants had wide variation within the ecological valence, and the aim was to identify and isolate individuals with more positive features for the further selection (Garcia de Santos et al., 2001). Based on the results of Duncan's test, it can be concluded that within observed genotypes, there are three significantly different groups in green mass yield, number of stems per plant and plant height.

The first group comprises G1, G3 and G17; although, between them, there was statistically significant difference. Thus, genotype G1, had the highest green mass yield and differed from all other genotypes, while between the genotypes G3 and G17, there was no statistically significant difference. In the second group that comprises genotypes G2, G4, G7, G9, G11 and G13, there were no statistically significant differences. Genotypes of this group had more or less uniform green mass yield and they were the closest to the average values of all investigated genotypes. The third group included the G6, G8 and G16 and those were the genotypes that had modest yield. Genotype G8 was statistically different from all other genotypes, and between G6 and G16, there was no statistically significant difference. Statistically, other genotypes did not differ from second group (Table 2). Regarding

Table 1. Descriptive statistical parameters of the tested genotype properties.

Variable	N	μ	MIN	MAX	STD	CV (%)
GMY*	20	70.45	34.72	181.02	34.93	49.58
DMY*	20	26.85	12.91	64.62	12.45	46.37
PH*	20	32.83	28.08	40.48	3.87	11.80
SW*	20	1.78	1.31	2.40	0.27	15.18
NSP*	20	33.20	24.60	56.20	7.95	23.96
NPP*	20	344.24	257.80	566.60	90.44	26.27
SYP*	20	3.28	1.92	5.77	1.13	34.31
TSW*	20	1.12	1.01	1.26	0.07	6.51
NSPo*	20	17.53	12.43	20.30	1.64	9.35
GMY**	20	189.18	107.68	276.98	46.86	24.77
DMY**	20	68.08	45.72	97.07	14.37	21.11
PH**	20	36.18	29.22	40.66	2.88	7.96
SW**	20	1.46	1.18	1.80	0.17	11.61
NSP**	20	105.08	65.40	164.00	19.84	18.88
NPP**	20	837.88	520.00	1113.20	179.08	21.37
SYP**	20	8.12	3.97	12.50	2.03	24.98
TSW**	20	1.03	0.88	1.14	0.07	6.92
NSPo**	20	12.87	9.83	16.30	1.84	14.26

*2006th year, **2007th year; The SAS system, the means procedure; GMY, Green mass yield (g); DMY, air-dry hay yield (g); PH, plant height (cm); SW, stem thickness (mm); NSP, number of stems per plant; NPP, number of pods / plant, SYP, seed yield per plant (g); TSW, mass of 1000 seeds (g); NSPo, the number of seeds per pod; N, repetition; μ, average; MIN, minimum; MAX, maximum; STD, standard deviation; CV, coeficient of variation; Values of examined morphological and agronomic traits, obtained by analysis of variance, illustrate statistically significant differences between both genotypes and years of use. Interaction effects were not determined, which means that conclusions can be based on the values of basic parameters. Duncan's multiple range test was used to determine the significance of differences between genotypes.

the number of pods per plant, it can be concluded that there were three groups of genotypes which are significantly different. The first group comprised G1, G3 and G15; although, between them, there was a statistically significant difference. Genotypes G1 and G3 had the highest number of pods per plant and they were significantly different from all other genotypes in the number of pods per plant and seed yield. In subsequent selection cycles, G1, G3 and G15 will represent a significant source of germplasm for the characteristics number of pods per plant and seeds production (Sindhu, 2004; Vuckovic et al., 2005). Based on the Duncan's test, results and analysis of the significance of differences, it can be concluded that genotype G7 had the highest, while the genotypes G1 and G3 had the lowest number of seeds per pod.

In subsequent selection cycles, G7 could be important source of germplasm for the characteristic number of seeds per pod. Thus, the final assessment should be made based on correlations and not on the basis of partial properties (Radic et al., 2011). Crossing between genotypes that showed good results can give synthetic variety with high yield components (Mc Graw et al., 1986; Smith et al., 2009).

Conclusions

Research of biological, morphological and agronomic characteristics of 20 *L*. *corniculatus* L. genotypes showed a number of variable traits in the studied genotypes. High coefficients of variability for most measured traits were determined on the studied genotypes in both years of research. The highest variability was found for the green matter yield (CV 49, 58). In subsequent selection cycles, genotypes G1, G3 and G17 represent a significant germplasm source for high green mass yield, while the genotypes G1, G3 and G15 had the highest production of pods per plant and seed yield. The results obtained in this study can be used for a variety of selection processes, and represent a good base for the selection activities of the domestic *L. corniculatus* L. cultivars with good production properties. The erosion of diversity of most cultivated plant species indicates the need for collection, study and evaluation of genetic resources, and for creation of new germplasm as a genetic resource in future breeding programs.

Table 2. Average values of examined traits (DMRT).

Genotype	GMY	DMY	PH	SW	NSP	NPP	SYP	TSW	NSPo
G1	221.76^{A}	80.85^{A}	39.92^{A}	1.94^{AB}	89.00^{AB}	839.90^{A}	8.29^{AB}	1.013^{DE}	11.90^{C}
G2	116.13^{BCDEF}	43.02^{BCD}	33.42^{ABC}	1.44^{CD}	68.90^{ABC}	542.90^{CDEF}	5.72^{ABCD}	1.003^{DE}	14.80^{ABC}
G3	175.12^{AB}	66.05^{AB}	38.35^{AB}	1.63^{BC}	78.20^{ABC}	818.00^{AB}	9.12^{A}	1.005^{E}	14.00^{BC}
G4	135.11^{BCDEF}	50.31^{BCD}	37.85^{AB}	1.69^{BC}	71.70^{ABC}	639.60^{ABCDE}	6.74^{ABCD}	1.157^{ABCD}	16.40^{AB}
G5	107.01^{CDEF}	44.18^{BCD}	32.81^{ABC}	1.50^{CD}	65.60^{ABC}	685.60^{ABCD}	6.60^{ABCD}	1.131^{ABCDE}	15.70^{ABC}
G6	83.38^{EF}	33.85^{CD}	29.57^{C}	1.45^{CD}	45.00^{C}	393.40^{F}	3.25^{D}	1.028^{E}	14.40^{ABC}
G7	124.20^{BCDEF}	49.68^{BCD}	36.00^{ABC}	1.76^{BC}	62.00^{ABC}	598.20^{CDEF}	6.64^{ABCD}	1.189^{AB}	17.80^{A}
G8	71.20^{F}	29.32^{D}	29.86^{C}	1.26^{D}	59.70^{BC}	453.00^{EF}	4.00^{CD}	1.025^{CDE}	14.50^{ABC}
G9	110.47^{BCDEF}	42.93^{BCD}	35.12^{ABC}	1.57^{CD}	61.70^{ABC}	503.50^{CDEF}	5.03^{BCD}	1.196^{A}	17.10^{AB}
G10	107.41^{CDEF}	40.93^{CD}	34.09^{ABC}	1.44^{CD}	62.00^{ABC}	476.40^{DEF}	5.50^{BCD}	1.034^{BCDE}	17.90^{AB}
G11	115.80^{BCDEF}	44.27^{BCD}	35.05^{ABC}	1.67^{BC}	82.60^{AB}	685.10^{ABCD}	5.51^{BCD}	1.126^{ABCDE}	14.50^{ABC}
G12	140.03^{BCDE}	50.02^{BCD}	36.10^{ABC}	1.70^{BC}	73.10^{ABC}	553.20^{CDEF}	5.52^{BCD}	1.133^{ABCDE}	15.70^{ABC}
G13	118.05^{BCDEF}	43.52^{BCD}	35.86^{ABC}	1.75^{BC}	58.70^{BC}	607.70^{BCDEF}	5.36^{BCD}	1.153^{ABCDE}	15.00^{ABC}
G14	99.29^{DEF}	35.40^{CD}	32.27^{BC}	1.64^{BC}	56.90^{BC}	413.20^{F}	4.27^{CD}	1.093^{ABCDE}	16.20^{AB}
G15	142.01^{BCDE}	52.08^{BCD}	34.08^{ABC}	1.66^{BC}	71.30^{ABC}	706.40^{ABC}	7.19^{ABC}	1.104^{ABCDE}	14.80^{ABC}
G16	92.28^{EF}	32.23^{CD}	33.66^{ABC}	1.61^{BCD}	71.10^{ABC}	529.10^{CDEF}	4.14^{CD}	1.055^{ABCDE}	13.80^{BC}
G17	169.34^{ABC}	55.50^{BC}	34.16^{ABC}	1.48^{CD}	96.40^{A}	655.20^{ABCDE}	5.73^{ABCD}	1.134^{ABCDE}	15.20^{ABC}
G18	147.10^{BCDE}	49.72^{BCD}	31.31^{BC}	1.48^{CD}	72.10^{ABC}	546.70^{CDEF}	5.66^{ABCD}	1.102^{ABCDE}	15.40^{ABC}
G19	158.35^{BCD}	53.36^{BC}	32.69^{ABC}	1.55^{CD}	65.20^{ABC}	568.00^{CDEF}	5.28^{BCD}	1.093^{ABCDE}	14.80^{ABC}
G20	162.24^{BCD}	52.14^{BCD}	37.94^{AB}	2.10^{A}	71.60^{ABC}	606.10^{BCDEF}	4.52^{CD}	1.206^{ABC}	15.70^{ABC}

The SAS system, The GLM procedure. Values marked with same letter are not significantly different at the level of r = 0.05 (DMRT); GMY, Green mass yield (g); DMY, air-dry hay yield (g); PH, plant height (cm); SW, stem thickness (mm); NSP, number of stems per plant; NPP, number of pods/plant; SYP, seed yield per plant (g); TSW, mass of 1000 seeds (g); NSPo, the number of seeds per pod.

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