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Variability and relationships of important alfalfa germplasm agronomic traits

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Abstract

Background and Purpose: *The objective of this paper was to establish variability as well as to estimate stability of the most important alfalfa agronomic traits, with respect to germplasms of different origin. Correlations between the investigated traits were also analysed, since data on relationships are of great importance in selection, especially in traits with low genetic variability.*

Material and Methods: *Experimental material consisted of 12 alfalfa germplasms. Investigation was carried out during a two-year period on two locations, at two different soil types. The field trial was arranged according to the randomised block design with four replications.*

Results and Conclusions: *There was a statistically significant difference of green mass yield from 59.43 t ha^{-1} (Astra) to 69.04 t ha^{-1} (PCP), of dry matter from 12.26 t ha^{-1} (Astra) to 14.10 t ha^{-1} (PCP), of proteins from 2.49 t ha^{-1} (Panonija) to 3.03 t ha^{-1} (PCP), of plant height from 54.67 cm (OS – 90) to 60.82 (Elena), of leaf ratio to green mass from 51.03% (Elena) to 53.56% (Panonija) and of protein content in dry matter from 19.86% (Posavina) to 20.96% (PCP). There were positive correlations between green mass yield, dry matter, protein level and plant height, leaf ratio in green mass was in a negative correlation to yields and plant height. High and stable green mass yields were provided by germplasm OS-88, Du Puits, Elena, Posavina, Vuka and the breeding population PCP in various environmental conditions of plant growth.*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is considered to be one of the most important fodder crops worldwide, due to its high nutritive value, growth potential and quality, different methods of utilisation, potentiality and benefits to the soil (*1*). Since it is a natural autotetraploid, characterised by high allogamy and self-incompatibility, its inheritant patterns are particularly complex (*2*). For the above reasons, alfalfa cultivars are mostly synthetic populations of wide genetic base and may be considered as heterogeneous populations of heterozygous individuals (*3*). Rotili (*4*) states that there are three parameters important for the genetic improvement of alfalfa: 1. genetic homogeneity, 2. yielding capacity, 3. biomass quality. Green mass yield indicates agronomic values of cultivars and it is the most commonly used trait in alfalfa breeding programs. Expression of variability is the result of both genotype and environmental factors, as well as of their complex interaction. Since alfalfa is one of the most important fodder crops, many breeding programs are aimed at the improvement of its quality. The quality of alfalfa includes chemical (content of proteins and fibre in dry matter), morphological (plant height, the number of internodes, leafiness) and nutritional composition (digestibility, utilisation, energy, and protein value) (*5*). Generally, there is a negative correlation between yield and quality characters because high yield implies mature, tall plants distinguished by a high fibre content and a low protein content in dry matter (*6*). Johnson *et al* (*7*) emphasise the importance of relationship between quantitative and economic traits, since selection without yield estimation may often lead to high-quality but low-yielding populations. However, the value of a germplasm depends not only on the realisation of the genetic potential in the sense of yield and quality, but also on its ability to maintain those traits in different environments *(8)*. The objective of this paper was to establish variability as well as to estimate stability of the most important alfalfa agronomic traits, with respect to germplasms of different origin. Correlations between the investigated traits were also analysed, since data on relationships are of great importance in selection, especially in traits with low genetic variability.

MATERIAL AND METHODS

Materials. Experimental material consisted of 10 cultivars of various geographic origin and two breeding populations. The following cultivars were included in the experiment: Vuka, OS-90, OS-88, Stela (all created within the frames of the alfalfa breeding program at Agricultural Institute Osijek, East Croatia), Posavina, Mirna, Astra, Panonija (all created at the Institute for Plant Breeding and Production Zagreb, West Croatia), Elena (Italian cultivar) and De Puits (French cultivar). The breeding populations (marks PCO and PCP) are the result of multi-year selection of superior genotypes on acid soil within frames at the Agricultural Institute Osijek. Our investigation was carried out during a two-year period (1999 and 2000) on two locations, i.e. on two different types of soil: 1. euthric cambisol of neutral reaction (pH in H_2O = 7.00; pH in 1n KCl = 6.41; AL-soluble P_2O_5 and K_2O = 39.70 and 37.70 mg 100 g^{-1}, respectively; humus content 2.01%) at the field of the Agricultural Institute Osijek, 2. pseudogley soil type of acid reaction (pH in H_2O = 4.72; pH in 1n KCl = 3.88; AL-soluble P_2O_5 and K_2O = 39.22 and 30.85 mg 100 g $^{-1}$, respectively; humus content 1.53%) at the location Petrijevci. The field trials were not fertilized at either the Osijek or Petrijevci location. On both locations sowing was carried out in April (06/04, 14/04) 1999.

Methods. The trial was carried out in a randomised block design with four replications using 10 m^2 plot and a sowing rate of 15 kg/ha^{-1} of seed. Alfalfa was harvested at 10–15% flowering stage. There were four cuts in the first year of the investigation (June, July, August and October), and five cuts in the second year of the investigation (May, June, July, August and October) on both locations. Each cut was characterised by analysis of the following traits: green mass yield (t ha^{-1}), dry matter yield (t ha^{-1}), protein yield (t ha^{-1}), plant height (cm), ratio of leaf mass to green mass (%) and ratio of protein content to dry matter content (%). Plant height was determined from 10 randomly selected plants from the middle row from each plot, prior to the cutting of each swath. In order to establish dry matter content in green mass, and for the purpose of chemical analysis, an average sample of 1 kg green mass was picked (collected), placed in a paper bag and immediately taken to the laboratory. Half of the sample was dried at 105 °C for 24 hours to establish dry matter content. The other half of the sample was used to determine leaf ratio to green mass (leaves, petioles, and

TABLE 1

Summary of ANOVA for variables examined.

		Means of squares					
Source of variation	df	Green mass yield	Dry matter yield	Protein yield	Plant height	Leaf ratio	Protein content
Location (L)	1	28644**	1096**	70685**	5756**	360**	297.80**
Replication	6	961.07	40.92	1968	165	2.85	0.78
Cultivars (C)	11	167.68**	6.99**	361**	49.05**	7.78**	1.65**
C x L	11	74.98	3.34	224**	9.55	2.02**	2.74**
Error 1	66	51.44	2.02	82	8.16	0.29	0.19
Year (Y)	1	60554**	2005**	10919**	6470**	1475**	2.37**
L x Y	1	5181**	344**	18007**	59.39*	45.45**	83.84**
C x Y	11	43.02	1.58	186**	3.83	2.89**	2.85**
C x L x Y	11	25.91	1.50	112	8.28	4.65**	1.98**
Error 2	72	38.98	1.45	61	9.53	0.46	0.15

df = degrees of freedom

**, * statistically significant differences between the levels of a factor at P ≤ 0.01 and P ≤ 0.05, respectively

TABLE 2

Mean values of investigated agronomic traits of alfalfa germplasm.

Cultivar	Green mass yield (t ha^{-1})	Dry matter yield (t ha^{-1})	Protein yield (t ha^{-1})	Plant height (cm)	Leaf ratio (%)	Protein content (%)
Vuka	65.36 ab	13.27 abc	2.77 bcd	56.31 bcde	52.85 b	20.46 bc
Astra	59.43 c	12.26 c	2.63 de	55.60 cde	52.64 bc	20.38 bc
OS – 90	62.68 bc	12.67 bc	2.69 cde	54.67 e	52.65 bc	20.47 bc
Posavina	67.42 ab	13.74 ab	2.77 bcd	57.93 b	51.37 cde	19.86 d
Stela	66.62 ab	13.44 ab	2.87 abc	55.20 de	52.68 bc	20.68 ab
Mirna	63.75 abc	12.99 abc	2.66 cde	56.63 bcde	51.99 f	19.95 d
OS – 88	68.26 ab	14.03 a	2.86 abc	58.29 b	52.18 de	20.13 cd
Elena	67.12 ab	14.09 a	2.99 ab	60.82 a	51.03 f	20.58 b
PCO	63.88 abc	13.03 abc	2.75 cd	57.14 bcd	52.43 bcd	20.55 b
PCP	69.04 a	14.10 a	3.03 a	56.74 bcde	52.45 bcd	20.96 a
Panonija	59.65 c	12.27 c	2.49 e	54.70 e	53.56 a	20.64 ab
Du Puits	67.75 ab	13.61 ab	2.80 bcd	57.66 bc	51.14 f	20.16 cd
Mean	65.08	13.29	2.78	56.81	52.25	20.40
CV (%)	9.59	9.05	8.97	5.43	1.30	1.95

CV = coefficients of variation
[a,b]Means followed by the same letter are not significantly different at the $P \leq 0.05$

stipules were removed by hand). The same sample was dried at 60 °C for 48 hours to determine protein content (N x 6.25) according to the Kjeldahl procedure (*9*). Green mass yield was calculated on the basis of the mass from the experimental plots cut and weighed by a Hege 212 combine equipped with an electronic scale.

Data analysis. The data were analysed by the analysis of variance (ANOVA) method. Significance of differences was tested by Duncan's multiple range test – DMRT for the significance level P≤0.05. Each of the nine cuts at one location was considered as one environment (18 environments in total). To establish correlation between the analysed traits, both phenotypic correlation coefficients and significance of r were determined (*10*). Significance of correlations was interpreted according to Roemer – Orphal's table (*11*). To estimate stability of alfalfa green mass yield in different environments the germplasms were divided into various stability groups (Group I – high yield, small variation; Group II – high yield, large variation; Group III – low yield, small variation; Group IV – low yield, large variation) based on their coefficient of variation and mean yields (*12*). Statistical data processing was conducted by SAS version 8.0 software (*13*).

RESULTS

The results from analysis of variance are presented in Table 1. The data showed that there were significant (P≤0.01) differences in phenotypic expression of all investigated traits with regard to locations, cultivars and years. In addition, some significant interactions of cultivar x location, location x year, cultivar x year and cultivar x location x year were obtained. Table 2 shows significant variations in mean green mass yield from 59.43 t ha^{-1} (Astra) to 69.04 t ha^{-1} (PCP), in dry matter yield from 12.26 t ha^{-1} (Astra) to 14.10 t ha^{-1} (PCP), in protein yield from 2.49 t ha^{-1} (Panonija) to 3.03 t ha^{-1} (PCP); in plant height from 54.67 cm (OS – 90) to 60.82 cm (Elena); leaf ratio in green mass from 51.03% (Elena) to 53.56% (Panonija) as well as protein content in dry matter from 19.86% (Posavina) to 20.96% (PCP), which points to genetic diversity of the investigated alfalfa germplasm.

In our investigation the lowest coefficient of variation was established for leaf: green mass ratio (1.30%) and protein content (1.95%), and the highest coefficient for green mass yield (9.59%).

Phenotypic correlation coefficients between the analysed traits are shown in Table 3. The data show that green mass yield was positively correlated with dry matter yield (0.94**) and proteins (0.90**). Positive correlation between dry matter and proteins (0.91**) was also established. Furthermore, a weak positive correlation was determined between yield and protein content (0.32**). Plant height had a very strong positive correlation with yields (0.85**, 0.87**, 0.79**). Leaf ratio was very strongly and negatively correlated with green mass yield (–0.78**), dry matter (–0.65**), proteins (–0.62**) and plant height (–0.68**).

The objective of most commercial alfalfa breeding programs is to create high quality cultivars and stable yield in as many various environments as possible (*14*).

TABLE 3

Phenotypic correlation coefficients between analysed traits, calculated with 12 alfalfa germplasms over 18 environments (location x cuts).

Variable	Green mass yield	Dry matter yield	Protein yield	Plant height	Leaf ratio	Protein content
Green mass yield	1.00	0.94**	0.90**	0.85**	–0.78**	0.03
Dry matter yield		1.00	0.91**	0.87**	–0.65**	-0.04
Protein yield			1.00	0.79**	-0.62**	0.32**
Height plant				1.00	-0.68**	-0.11
Leaf ratio					1.00	0.03
Protein content						1.00

**, * significant differences between the levels of a factor at $P \leq 0.01$ and $P \leq 0.05$, respectively

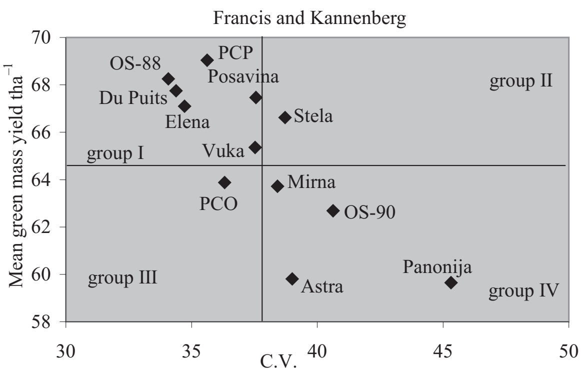

Figure 1. *Mean green mass yield plotted against coefficient of variations on 12 germplasm alfalfa in 18 environments.*

In order to estimate green mass yield stability of the investigated cultivars and newly created breeding populations of alfalfa described in our paper, we used a simple method of germplasm division into different stability groups (Figure 1). The group of germplasm of high and stable green mass yield in various agroecological growth conditions includes cultivars OS–88, Du Puits, Elena, Posavina, Vuka and breeding population PCP. Cultivars Astra, Mirna, Panonija and OS–90 are grouped as low yielding and unstable germplasm. The group of high yielding unstable germplasm is presented by the cultivar Stela, whereas breeding population PCO is defined as low yielding and stable.

DISCUSSION

The obtained results on variability of yield height confirm the well-known fact that it is a complex trait caused by a large number of genes whose phenotypic expression is modified under the influence of environmental factors (*15*, *16*). The morphological trait of 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. Namely, research conducted by numerous authors confirm that there is a positive correlation between yield and plant height (*17–19*). Because of its high protein content and easy digestibility, alfalfa leaf ratio to green mass is an important trait in alfalfa breeding regarding its quality. The selection of plants with a high leaf: green mass ratio would probably indirectly increase protein content, i.e. improve its quality, but, on the other hand, it would decrease total green mass yield (*20*). Therefore, selection on quality traits alone without yield is not recommended.

Coefficient of variation is a useful expression of variability (*21*). This low coefficient of variation for protein content is in accordance with the results published by Rotili *et al* (*22*). They point out that successful improvement in alfalfa protein content may be achieved indirectly by selection of the following traits: high tolerance of early cutting (green bud), resistance to diseases and insects, delay of leaf senescence, modification of stem morphology (a larger number of shorter internodes).

Similar results of phenotypic correlation coefficients between the analysed traits were obtained by many authors *(3, 17, 23, 24*). The recognition of correlations between traits is an important issue in plant breeding programs. When choosing superior individuals for a certain trait according to phenotypic expression, other traits may be changed at the same time. Recognition of correlation provides the possibility of improvement of a larger number of traits at the same time. Recognition of correlation is also important for traits of low genetic variability, in cases of which the progress in selection is achieved by indirect methods.

Differences in green mass yield stability among germplasms were probably caused by cultivar – environmental interaction whose intensity depends on genetic composition of cultivar and on intensity of effects of particular environmental factor.

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