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## BREEDING AND IMPROVEMENT OF QUALITY TRAITS IN ALFALFA (*Medicago sativa* ssp. *sativa* L.)

Dragan MILIĆ, Đura KARAGIĆ, Sanja VASILJEVIĆ, Aleksandar MIKIĆ, Branko MILOŠEVIĆ,  
and Slobodan KATIĆ

Institute of Field and Vegetable Crops, Novi Sad, Serbia

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An important objective in alfalfa breeding is the improvement of forage quality. The aim of this experiment was to determine the genetic control of the most important quality traits in whole plant leaves and stems of alfalfa genotypes. Quantitative genetic parameters were estimated for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) content. A 5x5 diallel design with reciprocals among parents from geographically different populations were studied. In the diallel study, effects due to general combining ability (GCA) were higher than those due to specific combining ability (SCA) for majority of the tested characters in analyses of whole plant. The F1 progeny effect was significant for almost all characters (except ADF content). The highest values of narrow sense heritability's were found for NDF ( $h = 0.19$ ) and CP ( $h = 0.17$ ) in whole plant, and in leaves (0.11 and 0.08), while in stems most heritable traits are ADF ( $h = 0.29$ ) and ADL ( $h = 0.16$ ) content. As a consequence, CP, NDF and ADF content would be more efficient selection criteria in whole plant analyses, while ADF and ADL content would be more interesting to investigate in stems, in a breeding program for improved nutritive value of alfalfa.

*Key words:* alfalfa, diallel, GCA, heritability, SCA, variance

### INTRODUCTION

Cultivated alfalfa is the most important forage source of protein for dairy cattle in temperate regions. Alfalfa is a perennial crop that provides a higher yield of proteins per unit area than any field crop, which makes it highly desirable for the production of hay and feed for domestic animals (especially ruminants). Because of this, alfalfa breeding community in the whole world aims at improvement of forage quality as an important objective (RIDAY *and* BRUMMER 2002; LAMB *et al.* 2006). An understanding of the inheritance of quality component traits would facilitate the breeding of cultivars with higher nutritive value.

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**Corresponding author:** Dragan Milić, Forage Crops Department, Institute of Field and Vegetable Crops M. Gorkog 30. 21000 Novi Sad, Serbia tel.: + 381 21 4898376; fax: + 381 21 4898377, e-mail: [dragan.milic@nsseme.com](mailto:dragan.milic@nsseme.com)

Alfalfa breeding programs in the USA in the second half of the twentieth century focused on improving forage quality and increasing disease and pest resistance, and this may have led inadvertently to alfalfa forage yield stagnation (HILL *et al.* 1988). Together with digestibility, protein content is an important nutritional component of alfalfa forage, which typically has high crude protein (CP) levels above 200 g kg<sup>-1</sup>. The decline of digestibility is the consequence of two processes: (I) the reduction of a highly digestible component (leaves) because of an increase of a less digestible component (stems) and (II) the decreasing average digestibility of the stem component, with more cell walls (NDF) and lignin (VERONESI *et al.* 2010). High levels of soluble protein typically present in alfalfa exceed the capacity of rumen microbes to utilize it efficiently (TECLE *et al.* 2006).

Lignin and cellulose are the major components of plant cell wall and provide mechanical support to the plant and a framework for the transport of water and nutrients. SHEAFFER *et al.* (1998) obtained significant differences in the contents of NDF and ADF between low, medium and high-quality alfalfa cultivars.

Previous results clearly demonstrate that variation in protein content exists among high yielding alfalfa cultivars, suggesting that genetic gain could be achieved for both yield and protein content (VERONESI *et al.* 2010). Significant differences were registered in the content of crude fiber, ADF and NDF that were caused by genetic factors (SHEAFFER *et al.* 1998; RIDAY and BRUMMER 2002; GUINES *et al.* 2002). Diallel crosses in alfalfa are used to evaluate the genetic variation of offspring, estimation of breeding value of parental populations, and to determine the mode of inheritance of agronomic and quality traits that influence biomass yield, feeding value and adaptability of the newly created hybrid combinations (GUINES *et al.* 2002; MILIĆ *et al.* 2011).

Objective of current study was to determine the genetic control of the most important quality traits (CP, NDF, ADF, Lignin) in whole plant, leaves and stems of alfalfa hybrids. Another aim was to investigate presence of additive and non-additive genetic effects, narrow-sense heritability and their potential usefulness in practical breeding program in alfalfa. Obtained information should also improve concept of semi-hybrid breeding in alfalfa.

#### MATERIALS AND METHODS

**Plant material:** The trial was conducted at the experimental field of Institute of Field and Vegetable Crops Novi Sad, Serbia during 2006-2008. Twenty F<sub>1</sub> alfalfa hybrids and 5 parental populations were used in this research. A 5x5 diallel design with reciprocals among parents from geographically different populations was studied (for the details see MILIĆ *et al.* 2011).

**Chemical analyses** were performed on samples taken in the third year of crop life (2008). Samples from the first cut were used for a whole plant analysis and from first and second cuts for leaf and stem analyses. Samples for the analysis were taken from of all F<sub>1</sub> hybrids and their 5 parents in three replications. The analyses were carried out using standard methods, namely the Filter Bag Technique for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The analyses were performed on an Ankom 2000 Fiber Analyzer (Ankom Technology Corp., NY, USA). The crude protein content was determined using the standard chemical analysis proposed by Kjeldahl.

**Statistical analyses:** The mode of inheritance was assessed using the program DIALLEL-SAS05 according to ZHANG *et al.* (2005). General combining ability (GCA) and specific combining ability (SCA) were calculated according to GRIFFING (1956), Method 1, Mathematical Model 2

(parents, F<sub>1</sub> and reciprocal crosses with all p<sup>2</sup> combinations). The statistical method (1) and mathematical model (2) used is a random model in which the experimental materials (genotypes, i.e. parents and hybrids) are a random sample from the reference population.

The effects of genotype, environment and all possible interactions were tested with the appropriate mean squares, as determined from the expected mean squares. Mean squares were calculated from Type III sums of squares of the program ZHANG *et al.* (2005). Variance components were calculated from diallel analysis in case the parents were not inbred according to the formula:

$$\sigma_{GCA}^2 = \frac{1}{4}\sigma_A^2 + \frac{1}{16}\sigma_D^2 + \frac{1}{8}\sigma_{SCA}^2 = \frac{1}{8}\sigma_D^2 + \frac{1}{16}\sigma_A^2 + \frac{1}{16}\sigma_D^2$$

Additive variance:  $\sigma_A^2 = 4 \sigma_{GCA}^2$ ; Dominance variance:  $\sigma_D^2 = 6 \sigma_{SCA}^2$ ; in practical breeding, the estimates of the non-additive genetic variances are very small and they were neglected according to POSSELT (2010).

Narrow sense heritability was calculated from diallel analysis using the formula of WRICKE and WEBER (1986):

$$h_n^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_D^2 + \sigma_e^2} = \frac{c_A^2}{r}$$

where  $\sigma_e^2$  is the variance of error calculated from the standard error of variance analysis of diallel analysis, and r is the number of replicates.

## RESULTS AND DISCUSSION

The means for the quality traits and the analysis of variance are shown in Table 1.

The effect of the genotype was significant for most of the characters in whole plants (except ADF content). Results clearly show differences between hybrids in CP content in leaves. In stems, significant effects of genotype were found only in ADL content.

The results of the diallel analysis indicated highly significant GCA and SCA effects (except content of ADF), and showed that additive and non-additive effects were significant in determining forage quality traits in whole plant analysis (tab. 1). Significant both GCA and SCA effects were found in CP content in leaves, while in stems they were present only in ADL content. Diallel analyses of leaves demonstrate only significant GCA effects for NDF content, while only significant GCA effects were obtained for ADF content in stems. The diallel analysis showed that GCA effects were larger than SCA effects almost for all characters, (except ADL) in whole plants, indicating mainly additive inheritance. Values of GCA mean squares were larger than SCA values mean squares in leaves for CP and NDF content, while in stems larger mean squares values of GCA effects were found for ADF and ADL content. The mean squares from the diallel crosses indicated significant reciprocal and maternal, effects for NDF and ADF content in whole plant analyses. The maternal effect was highly significant for NDF and ADF in whole plant, and no significance were found in leaves and stems for these traits. The presence of significant reciprocal effects suggests that they originated from the maternal and non-maternal effects, which are in agreement with the findings of GUINES *et al.* (2002) and MILIĆ *et al.* (2011).

Table 1. Means and ANOVA mean squares for quality traits of alfalfa in whole plant, leaves and stems from diallel crosses

Source <sup>1</sup>	df	Crude protein (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )	ADF (g kg <sup>-1</sup> )	ADL (g kg <sup>-1</sup> )
<b>Whole plant</b>					
Mean		195.13	446.64	413.5	99.15
Rep	2	676.05**	104.16 <sup>ns</sup>	660.49 <sup>ns</sup>	169.01**
Genotype	24	292.71**	883.55**	580.14 <sup>ns</sup>	85.42**
GCA	4	601.627**	1394.37**	733.89 <sup>ns</sup>	115.29**
SCA	10	286.965*	633.80**	395.42 <sup>ns</sup>	148.36**
Reciprocal	10	174.883 <sup>ns</sup>	928.98**	703.35*	10.53 <sup>ns</sup>
Maternal	4	297.267 <sup>ns</sup>	1606.03**	914.57*	17.57 <sup>ns</sup>
Nonmaternal	6	93.294 <sup>ns</sup>	477.62*	562.54 <sup>ns</sup>	5.84 <sup>ns</sup>
Error	48	132.54	200.29	381.22	15.94
<b>Leaves</b>					
Mean		283.57	182.71	151.27	38.88
Rep	2	10333.47**	1001.73**	1183.89 <sup>ns</sup>	2237.81**
Genotype	24	217.51*	114.56 <sup>ns</sup>	123.52 <sup>ns</sup>	84.74 <sup>ns</sup>
GCA	4	380.17**	176.68*	116.39 <sup>ns</sup>	36.58 <sup>ns</sup>
SCA	10	308.34**	106.59 <sup>ns</sup>	205.20 <sup>ns</sup>	75.14 <sup>ns</sup>
Reciprocal	10	61.62 <sup>ns</sup>	97.69 <sup>ns</sup>	44.68 <sup>ns</sup>	113.60 <sup>ns</sup>
Maternal	4	98.15 <sup>ns</sup>	110.96 <sup>ns</sup>	75.88 <sup>ns</sup>	80.16 <sup>ns</sup>
Nonmaternal	6	37.26 <sup>ns</sup>	88.85 <sup>ns</sup>	23.88 <sup>ns</sup>	135.89 <sup>ns</sup>
Error	48	92.85	67.43	182.21	82.43
<b>Stems</b>					
Mean		105.02	600.58	511.29	113.80
Rep	4	789.63**	21924.18**	2707.01*	400.45**
Genotype	24	34.19 <sup>ns</sup>	311.57 <sup>ns</sup>	560.67 <sup>ns</sup>	41.92**
GCA	4	34.57 <sup>ns</sup>	676.48 <sup>ns</sup>	1286.77*	82,56**
SCA	10	41.41 <sup>ns</sup>	314.96 <sup>ns</sup>	292,98 <sup>ns</sup>	40,66*
Reciprocal	10	26.82 <sup>ns</sup>	162.22 <sup>ns</sup>	537,91 <sup>ns</sup>	26,91 <sup>ns</sup>
Maternal	4	39.93 <sup>ns</sup>	241.09 <sup>ns</sup>	540,95 <sup>ns</sup>	24,64 <sup>ns</sup>
Nonmaternal	6	18.08 <sup>ns</sup>	109.64 <sup>ns</sup>	535,89 <sup>ns</sup>	28,42 <sup>ns</sup>
Error	48	24.21	334.60	501.95	13.45

\*, \*\*, \*\*\* Significant at P = 0.05, and P = 0.01, respectively; ns, not significant.

The highest narrow-sense heritabilities were found for NDF (0.19), CP (0.17) and ADF (0.13) content in whole plant (Table 2.).

Table 2. The variance components and narrow-sense heritabilities in the diallel analyses for quality traits of alfalfa

Estimates	Crude protein (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )	ADF (g kg <sup>-1</sup> )	ADL (g kg <sup>-1</sup> )
<b>Whole plant</b>				
Variance additive ( $\delta^2_A$ )	870.7	4644.8	1323.0	47.3
Variance dominance ( $\delta^2_D$ )	4063.1	19371.4	8816.7	1042.2
Narrow- sense heritability ( $h^2_n$ )	0.17	0.19	0.13	0.04
<b>Leaves</b>				
Variance additive ( $\delta^2_A$ )	895.2	176.7	144.2	16.9
Variance dominance ( $\delta^2_D$ )	10487.5	1409.0	6181.4	901.1
Narrow- sense heritability ( $h^2_n$ )	0.08	0.11	0.02	0.02
<b>Stems</b>				
Variance additive ( $\delta^2_A$ )	9.1	2468.3	8435.0	37.1
Variance dominance ( $\delta^2_D$ )	208.2	15503.0	20282.5	183.8
Narrow- sense heritability ( $h^2_n$ )	0.04	0.14	0.29	0.16

In leaves highest values of heritability were found in NDF (0.11) and CP (~ 0.10) content. As a result from diallel analyses values of narrow sense heritability in stems show highest values for ADF (0.29) ADL (0.16), and NDF (0.14). Preliminary results obtained in our study show also presence of non-additive effects in genetic control of quality traits in alfalfa, because the dominance variance for all examined quality traits was higher than additive variance.

Our results confirm previous studies (GUINES *et al.* 2002; RIDAY and BRUMMER 2002; VERONESI *et al.* 2010), which suggests that forage quality traits behave additively, but also demonstrate significance of crossings even for quality traits such as CP content and portion of NDF, ADF and ADL in fiber content. Results obtained in our study regarding heritability of forage quality traits are in agreement with GUINES *et al.* (2002), who in factorial analyses of divergent alfalfa populations observe  $h^2_n$  of CP content 0.18.

The same authors (GUINES *et al.* 2002) received significant GCA and SCA effects and also reported significantly higher GCA values as compared to SCA ones for crude protein content. They confirm that significant reciprocal effects from diallel analysis originated from maternal effects.

Better understanding of genetic control of quality traits in alfalfa (CP, NDF, ADF, ADL) would contribute to improved feeding value of alfalfa. Forage quality traits are most likely polygenic and additive, but appearance of non-additive gene action is present. Existence of non-additive gene action in alfalfa quality traits can be explained by the fact that cumulative effects of many different dominant alleles might allow to appear in inter-population level (RIDAY and BRUMMER 2002).

**Breeding consequence:** The nutritive value of alfalfa forage is a complex issue that is influenced not only by protein content but by fiber content as well. Genetic differences in forage quality traits exist (together with impact of environmental and management effects). Our preliminary results (originated from a one year and two harvest), lead to the conclusion that more

efficient selection criteria breeding programs for improved quality of alfalfa would be to improve feeding value of stems, which means to decrease ADF and ADL content in this part of alfalfa plant. ADL content is very important in creation of alfalfa cultivars with better digestibility and higher tolerance to lodging. Results obtained in our study also show possibility for decreasing lignin content using conventional breeding methods, for forage or biofuel production of alfalfa which demands lower lignin content (SAKIROGLU *et al.* 2011).

Since alfalfa quality is a complex, polygenic trait controlled by a number of additive genes, non-additive gene action is not expected. The presence of the non-additive mode of inheritance can be explained by the cumulative effects of many different dominant alleles that act together and cause the appearance of non-additive gene expression (dominant inheritance), which manifests itself when individual crosses are made among geographically and genetically divergent populations of alfalfa (RIDAY and BRUMMER 2002). General observation of this paper should be that even for the quality traits crossing of genetically and geographically different alfalfa germplasms for the breeding purposes is very important, and can lead to creation of semi-hybrid alfalfa populations with higher feeding value, but use of hybridization in breeding for quality should be viewed in a broader context.

The next step in using this material is to develop semi-hybrid populations following a semi-hybrid breeding scheme (BRUMMER, 1999; RIDAY and BRUMMER 2002; MILIĆ *et al.* 2011). Further work must include selection at the population level plants with positive agronomic performance (making sure to maintain high yield level), and then multiplying them in space isolation (2-3 generations) for seed production for the market. Phenotypic selection within heterotic population with reduced genetic load is possible and desirable (RIDAY and BRUMMER 2002), in order to fix desirable alleles for improvement of alfalfa either in the context of forage quality or biofuel potential.

This research should contribute to better understanding of genetic control of quality traits of alfalfa, and creation of more precise breeding schemes for improving nutritive value, using conventional methods, bearing in mind not to reduce alfalfa forage yield.

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**OPLEMENJIVANJE OSOBINA KVALITETA LUCERKE (*Medicago sativa* ssp. *sativa* L.)**

Dragan MILIĆ, Đura KARAGIĆ, Sanja VASILJEVIĆ, Aleksandar MIKIĆ, Branko, MILOŠEVIĆ  
i Slobodan KATIĆ

Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija

**Izvod**

Važan cilj oplemenjivanja lucerke je poboljšanje kvaliteta krme. Cilj rada je bio da se odredi genetička kontrola najvažnijih osobina kvaliteta na nivou cele biljke, u lišću i stabljikama genotipova lucerke. Praćeni su kvantitativni genetički parametri za sadržaj sirovih proteina sadržaj neutralnih deterdžent vlakana (NDF), sadržaj kiselih deterdžent vlakana (ADF) i sadržaj kiselih vlakana lignina (ADL). Analizirano je  $F_1$  potomstvo nastalo dialelnim ukrštanjem (uključujući reciproke), između 5 neinbredovanih roditeljskih populacija različitog geografskog porekla. Dialelna analiza je ukazala na značajno veće efekte opštih kombinacionih sposobnosti (OKS), u odnosu na efekte posebnih kombinacionih sposobnosti (PKS) za većinu ispitivanih osobina u analizama cele biljke. Analiza  $F_1$  potomstava ukazuje na značaj genotipa za skoro sve praćene komponente kvaliteta (osim sadržaja ADF-a). Visoko značajni majčinski efekti registrovani su za sadržaj NDF-a i ADF-a na nivou cele biljke, ali u listovima i stabljikama ne. Način nasleđivanja je bio pretežno aditivni, zato što su efekti OKS veći od efekata PKS za većinu praćenih osobina. Najveće vrednosti heritabilnosti zabeležene su za sadržaj NDF-a ( $h_n=0,19$ ), i sirovih proteina ( $h_n=0,17$ ) u celoj biljci i lišću (0,11 and 0,08), dok u stabljikama osobine najveće naslednosti su sadržaj ADF-a ( $h_n=0,29$ ) i ADL-a ( $h_n=0,16$ ). Dobijeni rezultati ukazuju da sadržaj sirovih proteina, NDF and ADF bi bili efikasniji kriterijum u oplemenjivanju na nivou cele biljke, dok bi sadržaj ADF-a i ADL-a bilo važnije istraživati u stabljikama u oplemenjivanju na poboljšanu hranljivu vrednost lucerke.

*Ključne reči:* heritabilnost, lucerka, OKS, PKS, varijansa

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