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*Original scientific paper*

**IMPORTANCE OF PROGENY TESTING IN ALFALFA BREEDING**  
**(*Medicago sativa* L.)**

Dragan MILIĆ<sup>1</sup>, Slobodan KATIĆ<sup>1</sup>, Jan BOĆANSKI<sup>2</sup>, Đura KARAGIĆ<sup>1</sup>,  
Aleksandar MIKIĆ<sup>1</sup> and Sanja VASILJEVIĆ<sup>1</sup>

<sup>1</sup>Institute of Field and Vegetable Crops, Novi Sad, Serbia

<sup>2</sup>Faculty of Agriculture, University of Novi Sad, Serbia

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The objective of this paper was to assess the importance and effectiveness of progeny tests that can be used in alfalfa breeding for increased green forage yields and shoot number per plant. A total of 35 alfalfa genotypes of different geographic and genetic origins were analyzed. The results of the study have shown that crossing full-sib or half-sib parents leads

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*Corresponding author:* Dragan Milić, Institute of Field and Vegetable Crops, Novi Sad, Serbia email: dragan.milic@ifvcns.ns.ac.rs

to a significant increase in yield and shoot number per plant. Significant inbreeding depression was observed in the  $S_1$  progenies of all the genotypes involved. The choice of alfalfa parents must be based on progeny analysis. Which particular method will be used will depend on the objective of the breeding program.

*Key words:* alfalfa, progeny testing, shoot number, yield

## INTRODUCTION

The economically most important characteristics of alfalfa such as green forage and dry matter yields and other agronomically important characters such as plant height, number of shoots, and regrowth are all quantitative traits. Alfalfa traits whose inheritance is complex in nature (such as yield, for example) require breeding procedures that include progeny tests (WOODFIELD and BRUMMER 2001).

According to RIDAY and BRUMMER (2005), progeny tests play an important role in the choice of heterotic parental populations of alfalfa. The same authors also note the importance of these tests for the genetic gain obtained in each cycle of selection. Based on the information obtained from progenies of diallel crosses made in the U.S. among nine alfalfa germplasms of different origin, SEGOVIA-LERMA *et al.* (2004) discuss the degrees of expression of additive and nonadditive genetic variances and the extent of heterosis expression in diallel alfalfa hybrids.

The results of using the current breeding methods that involve progeny testing in alfalfa indicate that the effectiveness of breeding depends strongly on the population being used and the conditions under which selection is being carried out and that the success of selection (genetic gain) cannot be guaranteed (ROWE and HILL, 1999).

The goal of progeny testing is to assess parental components based on the values found in the progeny (MILIĆ *et al.* 2010a). Examining the relationships among progeny tests (correlations, heritability, components of additive and genetic variances) is of great importance in determining which of the tests is the most suitable (effective) for the purposes of breeding and developing synthetic varieties. Progeny tests provide information about the genetic value of the parents; hence their great importance in the breeding of perennial forage crops (DE ARAUJO and COULMAN 2002). PECETTI and PIANO (2005) have used open-pollinated and selfed progenies in order to make comparisons between such progenies and their parental populations and calculate heritability for the purposes of developing alfalfa cultivars tolerant of grazing.

The success in developing alfalfa cultivars depends the most on the breeding method used (KATIĆ *et al.* 2008). An effective method used for such purposes should include the following: 1) phenotypic analysis of the parental populations; 2) selection of parental components based on phenotype; and 3) synthesis of new populations. A further improvement of this procedure can be achieved by the inclusion of a fourth step consisting in determining the genetic values of parental components using progeny tests (polycross, top-cross, etc.) prior

to developing synthetics (ROTILI *et al.* 1999). The same authors also discuss the use of progeny testing in the reconstruction of heterozygosity in partially inbred alfalfa lines.

The objectives of this study were to assess the importance and effectiveness of progeny tests used in alfalfa breeding for green forage yield and shoot number and to determine which of these tests provides the best information for choosing parental components to improve the success of alfalfa breeding.

#### MATERIALS AND METHODS

The study was conducted during the 2006-2008 period at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad.

The experiment tested 35 alfalfa genotypes, which included five varieties of different geographic origin (parents): NS Banat ZMS II (Serbia), Ghareh Yon Geh (Iran), Zuzana (Czech Republic), Pecy (France), and RSI 20 (Spain), and their progenies from diallel crosses (20 hybrids), one generation of selfing ( $S_1$ ), and open pollination (half-sibs).

In 2003 and 2004, hand pollination was carried out using the full diallel method and the parental components were selfed to produce the  $F_1$  and  $S_1$  seeds, respectively. At the same, seed of parental genotypes was produced by open pollination ( $OP_1$ ).

The  $F_1$ ,  $S_1$  and  $OP_1$  seeds and the parents were planted in a comparative trial using a randomized block design with three replications. Twenty plants were analyzed per replicate (60 per genotype). The yield of green forage (g/plant) and shoot number per plant were recorded. The traits were analyzed over 10 cuttings - five in the second year of plant life (2007) and five in the third (2008). The differences among the progeny tests and parents were analyzed using the LSD test for two-factor ANOVA with progeny test as Factor A and genotype as Factor B. The differences among the genotypes across tests were analyzed by the LSD test and represented as test x genotype interaction. The efficacy of the progeny tests and genotypes in progeny testing was also expressed as a relative percentage value with the parental value being 100%.

#### RESULTS AND DISCUSSION

The results we obtained showed that green forage yield and shoot number varied significantly among the parents and progeny tests (Tables 1 and 2). The lowest yield (350.7 g/plant) and shoot number (51) were found in the progeny obtained by selfing, while the highest yield (451.1 g/plant) and the most shoots (58) were recorded in the progeny developed by diallel crossing.

The  $OP_1$  progeny had a lower green forage yield than the  $F_1$ s, but the difference was not significant (Table 1.). The  $F_1$  progeny had more shoots than the  $OP_1$  one, which indicates that crosses within the same genotype occurred during open pollination (sibing).

The percentage increase of green forage yield relative to parent was 9.1% in the F<sub>1</sub> progeny and 8.1% in the OP<sub>1</sub> progeny. Percentage-wise, the number of shoots in the OP<sub>1</sub>s increased by 5.4%, whereas in the F<sub>1</sub> progeny the increase was considerably higher – 12.4%, as indicated by the LSD test.

Table 1. Green forage yield (g/plant) and shoot number/plant in the parents and their progeny during 2007-2008.

Progeny test	Green forage yield g/plant	Average %	Shoot number	Average %
Parent	413.5	100	51.6	100
Open pollination	446.8**	108.1	54.4**	105.4
Crosses	451.1**	109.1	58.0**	112.4
Selfing	350.7 <sup>-</sup>	84.4	51.0 <sup>ns</sup>	98.8
Average	415.5		53.7	
CV %	16.0		16.2	
0.05	15.1		2.0	
LSD 0.01	19.9		2.6	

\*\* Green forage yield and shoot number significantly higher than in parent at 0.01

<sup>-</sup> Green forage yield significantly lower than in parent at 0.01

<sup>ns</sup> Shoot number non significantly different than in parent

An analysis of the genotypes using progeny tests showed that there were significant differences among the tests (Table 2.). Generally, it can be noted that all of the genotypes tested responded to selfing by reducing their yields of green forage (Table 2.). These findings are in agreement with those of BINGHAM *et al.* (1994), which indicated that strong inbreeding depression occurred in autotetraploid alfalfa due to a loss of desirable dominant allelic interactions and epistatic allelic combinations. All the genotypes in our study with the exception of NS Banat-a ZMS II produced higher yields in the F<sub>1</sub> and OP<sub>1</sub> generations than their parents. The varieties Pecy (439.9 g/plant) and Zuzana (459.4 g/plant) produced significantly higher yields in the F<sub>1</sub> generation than the parental populations (Table 2.).

The OP<sub>1</sub> progeny of the French variety Pecy (half-sib) had a significantly higher yield of green forage than their parents and the S<sub>1</sub> progeny, with the percentage increase relative to parental population being 23.6 %. Shoot number per progeny varied significantly among the genotypes (Table 2.). In all the genotypes, the highest number of shoots was recorded in the full-sib crosses.

No inbreeding depression for shoot number was observed, as there was no difference in the number of shoots between the parents and the S<sub>1</sub> progeny. A possible reason for this may lie in the fact that the plants were grown in a nursery in field conditions (individual plants). Alfalfa traits are also known to exhibit different levels of susceptibility to self-pollination (ROTILI *et al.* 1999). As the number of shoots is an important biological characteristic, it can be assumed that selfed

genotypes in which desirable dominant alleles are lost and lethal and sublethal genes become expressed deteriorate in field conditions and thus do not contribute to the formation of mean values, as a result of which no inbreeding depression for this trait is manifested.

Table 2. Green forage yield (g/plant) and shoot number/plant in alfalfa genotypes from different progeny tests conducted during 2007-2008.

Genotype	Progeny test	Green forage yield g/plant	Average %	Shoot number	Average %
NS Banat ZMS II	Parent	424.7	100	48.3	100
NS Banat ZMS II	Open pollination	427.8	100.7	51.9	107.5
NS Banat ZMS II	Crosses	422.7	99.5	55.9	115.7
NS Banat ZMS II	Selfing	359.1	84.6	48.7	100.8
Ghareh Yon Geh	Parent	446.1	100	55.0	100
Ghareh Yon Geh	Open pollination	465.3	104.3	57.8	105.1
Ghareh Yon Geh	Crosses	470.9	105.6	57.7	104.9
Ghareh Yon Geh	Selfing	345.0	77.3	54.8	99.6
Zuzana	Parent	388.0	100	50.7	100
Zuzana	Open pollination	464.1	119.6	54.4	107.3
Zuzana	Crosses	439.9	113.4	61.3	120.9
Zuzana	Selfing	346.1	89.2	50.8	100.2
Pecy	Parent	370.9	100	48.9	100
Pecy	Open pollination	431.4	116.3	52.4	107.2
Pecy	Crosses	459.4	123.6	56.1	114.7
Pecy	Selfing	346.0	93.3	48.0	98.2
RSI 20	Parent	437.8	100	55.3	100
RSI 20	Open pollination	445.3	101.7	55.5	100.4
RSI 20	Crosses	462.8	105.7	59.0	106.7
RSI 20	Selfing	357.5	81.7	52.5	94.9
	Average	415.5		53.7	
	CV %	16.0		16.2	
	0.05	33.7		4.4	
LSD	0.01	44.4		5.8	

Study results indicate that hybridization between varieties of different geographic origin can be used to increase alfalfa yields. RIDAY and BRUMMER (2002) point to yield increases and heterotic effects that result from crossing genetically divergent diverse genotypes of alfalfa. The same authors also argue that increased yield (heterosis) can be achieved by progenies produced by crossing morphologically similar parents in which heterosis is manifested through the combination of alleles that contribute to an increased number and size of plant organs. The same effect can

be produced by crossing morphologically and genetically heterogeneous parental populations, in which case the yield increase is a reflection of the effects of partial dominance or overdominance resulting in the manifestation of heterosis (RIDAY and BRUMMER 2002; MILIĆ *et al.*, 2010b).

Our results showed that the alfalfa yield increase depends on the crossing method (full- or half-sib) and is under the influence of both of the aforementioned mechanisms by which heterosis is manifested. The use of progeny tests (full-sib, half-sib, and inbred progeny testing) in breeding yields more information on the action of additive and nonadditive genes (WOODFIELD and BRUMMER 2001).

According to our findings, which support those reported in LI and BRUMMER (2009), selection within parental populations and the crossing of these populations to obtain population hybrids (semihybrids) can be a more effective way of breeding alfalfa for yield than hybridization based on recurrent selection. Study results suggest that crossing full- or half-sib parents brings about significant increases in yield and shoot number per plant. Our findings show the importance of crossing in alfalfa and can be explained by the accumulation of alleles with desirable genes in progenies produced by open pollination and crossing. This is in agreement with the results of other authors (ROWE and HILL 1999; RIDAY and BRUMMER 2002; LI and BRUMMER 2009; MILIĆ *et al.* 2010b).

The selection of half-sib alfalfa genotypes (open pollination) significantly simplifies the breeding process and increases its efficacy. Use of the open pollination progeny test in evaluating and choosing parental populations for breeding is of outstanding importance (DE ARAUJO and COULMAN 2002). This has been confirmed by our results as well, especially in light of the possibility of being able to produce large amounts of seed. When using such seed in breeding, however, it must be borne in mind that half the genes in the next generation come from an unknown source (as the contribution of the male is not known). Progenies resulting from one generation of selfing ( $S_1$ ) can be used for evaluating and choosing parents, and use should be made of parents whose progenies exhibit little inbreeding depression.

The choice of alfalfa parents must be based on progeny analysis, and which particular method will be used will depend on the objective of the breeding program.

The results of the present study are in agreement with those of RIDAY and BRUMMER (2005), according to which the expected genetic gain per selection cycle is greater when progeny testing is employed than when simple recurrent phenotypic is used.

## CONCLUSIONS

Study results indicate that crossing among full-sib or half-sib parents produces significant yield increases and a higher number of shoots per plant. In our

study, the highest green forage yields and shoot number per plant were recorded in full-sib crosses.

Significant inbreeding depression was observed in the  $S_1$  progeny of all the genotypes studied. Progenies developed by one generation of self pollination ( $S_1$ ) can be used for assessing and selecting parents. In such cases, parents whose progenies exhibit little inbreeding depression should be used.

The use of half-sib crosses improves the efficacy of alfalfa breeding. The open pollination test is of great importance in assessing alfalfa populations, especially since it makes it possible to produce larger quantities of seed.

Analysis of progeny tests is a key step in alfalfa breeding that can significantly influence the choice of parental populations to be used for developing alfalfa synthetics capable of producing higher yields and having greater nutritional value.

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## ZNAČAJ PROGENIH TESTOVA U OPLEMENJIVANJU LUCERKE (*MEDICAGO SATIVA L.*)

Dragan MILIĆ<sup>1</sup> Slobodan KATIĆ<sup>1</sup>, Jan BOĆANSKI<sup>2</sup>, Đura KARAGIĆ<sup>1</sup>,  
Aleksandar MIKIĆ<sup>1</sup> i Sanja VASILJEVIĆ<sup>1</sup>

<sup>1</sup> Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija

<sup>2</sup> Poljoprivredni fakultet, Univerzitet Novi Sad, Srbija

### I z v o d

Cilj rada je bio da se utvrdi značaj i efikasnost progenih testova u oplemenjivanju na prinos zelene krme i broj izdanaka lucerke. U okviru istraživanja analizirano je 35 genotipova lucerke različitog geografskog i genetičkog porekla. Rezultati oglada ukazuju da ukrštanje roditelja u punom ili polusrodstvu dovodi do značajnog povećanja prinosa i broja izdanaka po biljci. Ispoljena je značajna inbred depresija u S<sub>1</sub> potomstvu kod svih ispitivanih genotipova. Izbor roditelja mora biti zasnovan na analizi potomstava lucerke a primena odgovarajućeg metoda zavisi od cilja oplemenjivanja lucerke.

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