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Efficacy of Progeny Tests in Alfalfa *(Medicago sativa* L.*)* Breeding for Yield and Quality

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Summary: Alfalfa is one of the most important forage crops in the world and it is spread across the globe on over 30 million hectares. The objectives of this study were to assess the importance and effectiveness of progeny tests used in alfalfa breeding for dry matter yield and crude protein content and to determine which of these tests provides the best information for choosing parental components to improve the success of alfalfa breeding. The experiment tested 35 alfalfa genotypes of different geographic and genetic origin. The rank correlations obtained in our study indicate that open pollination and self pollination progeny tests are effective tools for evaluating alfalfa parents for dry matter yield and crude protein content, respectively. Alfalfa breeding for improved quality can be effective if crosses are made between genetically divergent populations in order to produce hybrid combinations that will be used as donors of genes for quality in the development of new synthetic cultivars, all the while making sure that yield is maintained as an essential factor in alfalfa breeding.
Key words: alfalfa, crude protein, dry matter yield, progeny testing

Introduction

Alfalfa (*Medicago sativa* L.) is an old crop that is grown for the purpose of producing forage. It is spread across the globe and grown on over 30 million hectares worldwide (Michaud et al. 1988), which creates the need for breeding and developing varieties possessing different characteristics. In alfalfa breeding, use is made of simple methods of selection, such as mass selection, individual selection, phenotypic selection, recurrent selection, and so on. The progress of alfalfa breeding is slow, most notably due to the use of the above selection methods (Katić et al. 2011). Alfalfa traits whose inheritance is complex in nature (such as yield and quality) require breeding procedures that include progeny tests (Woodfield & Brummer 2001, Milić et al. 2010 b).

The goal of progeny testing is to assess parental components based on the values found in the progeny. 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 (Milić et al. 2010b). Progeny tests provide information about the genetic value of the parents; hence their great importance in the breeding of perennial forage crops (De Araújo & Coulman 2002, Milić et al. 2010b). Half-sib and full-sib progeny tests are being used extensively but with many modifications to suit the crop and trait (Woodfield & Brummer 2001). De Araújo (2001) compared different progeny tests (polycross, open-pollinated, selfed) in bromegrass (Bromus riparius) and determined that the most progress in the breeding of this crop can be made by making use of open-pollinated progeny tests.

Although a number of different progeny tests are used in alfalfa breeding (Rumbaugh et al. 1988), alfalfa breeders commonly use tests of open-pollination progenies (Riday & Brummer 2002, Riday & Brummer 2006). Progenies of selected plants or half-sib families obtained through open pollination are tested. The remainder of the seed

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of parent plants selected on the basis of the progeny test is used to obtain the next generation, bearing in mind that half of the genes come from an unknown parent (Rumbaugh et al. 1988, Rotili et al. 1999). Progeny tests require the time for planting and separate generation analysis (three years), which prolongs the selection process; as a result of which they are less commonly used as the method for evaluating the selected parents (Milić et al. 2010b).

With the aim of assessing the efficacy of progeny tests in alfalfa breeding for increased yield and better quality, we analyzed parental populations and their progenies obtained through different types of pollination. Another goal of the study was to assess which progeny test provides the most useful information for choosing parental components to improve the success of alfalfa breeding.

Materials and Methods

The study was conducted at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad (45° 20' N and 19° 51' E and 84 m asl) during 2006-2008.

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). More detail on the experimental material and the trial set-up is provided in Milić et al. (2010a).

The yield of dry matter (g plant^{-1}) and crude protein content (g kg^{-1}) were recorded. Dry matter yield was analyzed over 10 cuttings - five in the second year of plant life (2007) and five in the third (2008). The crude protein content (g kg^{-1}) was determined in the first cut of 2008 using the standard method proposed by Kjeldahl.

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%.

Spearman's coefficients of correlation were calculated between parents and progeny tests (open pollination and selfing) for the studied traits.

Results and Discussion

The results we obtained showed that dry matter yield varied significantly among the parents and progeny tests (Tab. 1). The lowest yield (88.9 g plant^{-1}) was found in the progeny obtained by selfing, while the highest dry matter yield (115.3 g plant^{-1}) was recorded in the F_1 progenies developed by dialell crossing. The OP1 progeny had a lower dry matter yield than the F_1, but the difference was not significant. The percentage increase of dry matter yield relative to parent was 10.2% in the F_1 progeny and 7.2% in the OP progeny. The use of selfing on parental populations resulted in inbreeding depression for dry matter yield that reached a level of 15.0%.

The results of our study indicate that there were no significant differences in crude protein content

Table 1. Dry matter yield (g plant^{-1}) and crude protein content (g kg^{-1}) in the parents and their progeny during 2007-2008
Tabela 1. Prinos suve materije (g biljci^{-1}) i sadržaj sirovih proteina (g kg^{-1}) roditelja i testova potomstava tokom 2007-2008.

Progeny test Test potomstva	Dry matter yield Prinos suve materije	%	Crude protein Proteini	%
Parent / roditelji	104.6	100.0	201	100.0
Open pollination / slobodna oplodnja	112,1**	107.2	200 ^{ns}	99.5
Crosses / ukrštanja	115.3**	110.2	190 $^{--}$	94.5
Selfing / samooplodnja	88.9 $^{--}$	85.0	202 ^{ns}	100.5
Average / prosek	105.2		198	
CV %	16.7		4.3	
LSD 0.05	4.0		6.0	
NZR 0.01	5.3		8.0	

** Significantly higher at 0.01; $^{--}$ significantly lower at 0.01; ^{ns} not significantly different

between the parents and open-pollinated and selfed progeny tests. The F_1 progenies had significantly lower crude protein content (190 g kg^{-1}) than the parents (201 g kg^{-1}) (Tab. 1).

Crude protein content did not increase significantly when selfing was used. The use of selfing resulted in a crude protein content of 202 g kg^{-1}, which is a 0.5% increase relative to the parental populations (Tab. 1.)

Study results indicate that crossing among full-sib or half-sib parents produces significant yield increases in alfalfa. Our analysis of genotypes through progeny tests (Tab. 2) has revealed significant differences in dry matter yield among the different tests. Generally, the response of all the studied genotypes in the S_1 generation was a significant lowering of dry matter levels (Tab. 2). All of the genotypes except Banat ZMS II had higher dry matter levels in direct crosses and open-pollination test as compared to the parents (Tab. 2). The open pollination progeny of the French cultivar Pecy had significantly higher dry matter yields than the parents and the S_1 progeny. Percentage-wise, the increase relative to the parental population was 23.6%, or 24.5%. In direct crosses and in open pollination, significantly higher yields of dry matter compared with the parents were found in the varieties Pecy (116.4 and 109.3 g $plant^{-1}$) and Zuzana (112.3 and 113.5 g $plant^{-1}$). The cultivar RSI 20 had significantly higher dry matter yields in the crosses (121.1 g $plant^{-1}$) than in the parental population (112.1 g $plant^{-1}$) (Tab. 2). Looking at how the genotypes behaved in the selfing test, it can be seen that the genotype worst affected by inbreeding in the S_1 generation was the cultivar Ghareh Yon Geh, as its dry matter yields were 20% lower than those of the parents following selfing. The varieties Zuzana and Pecy, on the other hand, responded best to the use of selfing – their S_1 progenies had yields that were about 10% lower than what was found in the parental populations. Our findings confirm those of De Araújo (2001), who compared several different progeny tests (polycross, open pollination,

Table 2. Dry matter yield (g $plant^{-1}$) and crude protein content (g kg^{-1}) in alfalfa genotypes from different progeny tests conducted during 2007-2008
Tabela 2. Prinos suve materije (g/biljci) i sadržaj sirovih proteina (g kg^{-1}) genotipova lucerke u različitim testovima potomstava tokom 2007-2008.

Genotype Genotip	Progeny test Test potomstva	Dry matter yield Prinos suve materije	%	Crude protein content Proteini	%
NS Banat ZMS II	Parent / roditelj	108.6	100.0	202	100.0
NS Banat ZMS II	Open pollination-SO	106.1	97.7	198	98.0
NS Banat ZMS II	Crosses-F_1	109.9	101.2	185	91.6
NS Banat ZMS II	Selfing-S_1	86.8	79.9	195	96.5
Ghareh Yon Geh	Parent / roditelj	112.2	100.0	183	100.0
Ghareh Yon Geh	Open pollination-SO	115.5	102.9	205	112.0
Ghareh Yon Geh	Crosses-F_1	116.8	104.1	191	104.4
Ghareh Yon Geh	Selfing-S_1	88.1	78.5	189	103.3
Zuzana	Parent / roditelj	96.6	100.0	206	100.0
Zuzana	Open pollination-SO	113.5	117.5	196	95.1
Zuzana	Crosses-F_1	112.3	116.3	195	94.7
Zuzana	Selfing-S_1	88.3	91.4	216	104.9
Pecy	Parent / roditelj	93.5	100.0	209	100.0
Pecy	Open pollination-SO	109.3	116.9	206	98.6
Pecy	Crosses-F_1	116.4	124.5	193	92.3
Pecy	Selfing-S_1	86.2	92.2	216	103.3
RSI 20	Parent / roditelj	112.1	100.0	206	100.0
RSI 20	Open pollination-SO	116.0	103.5	197	95.6
RSI 20	Crosses-F_1	121.1	108.0	186	90.3
RSI 20	Selfing-S_1	95.2	84.9	196	95.1
Average / prosek			105.2		198
CV %			16.7		4.3
LSD 0.05		8.9		14	
NZR 0.01		11.8		19	

and selfing) in bromegrass (*Bromus riparius*). The results of our study indicate that advances can be made in the breeding of forage crops through the use of the open pollination test. They also underscore the importance of crossing in alfalfa that leads to the accumulation of desirable genes in progenies resulting from open pollination and crosses, which is agreement with previous studies (Riday & Brummer 2002, Li & Brummer 2009, Milić et al. 2010b).

Crude protein content is an important indicator of the nutritional value of alfalfa (Julier et al. 2001, Katić et al. 2005a). An analysis of crude protein content in alfalfa shows that there are significant differences between the parents and progeny tests with respect to this trait. No significant differences in crude protein content have been observed between parents and open pollination progenies. The use of direct crossing does not lead to an increase of crude protein content; on the contrary, there have been significant negative differences observed in the hybrid progenies relative to the parents regarding this component of alfalfa quality. The reason for this is the presence of significant negative correlations between yield and crude protein content in alfalfa (Julier et al. 2001, Katić et al. 2005a). Also, some direct crosses have exhibited significant positive heterosis for dry matter yield (Milić et al. 2010 a), so it was expected that F_1 progenies would have a lower crude protein content than progenies obtained by using the other methods of pollination.

The Iranian variety Ghareh Yon Geh had significantly lower crude protein content (183 g kg^{-1}) than the other four parents (Tab. 2). The use of planned crosses led to a significant decrease in the crude protein content of progenies of the varieties NS Banat ZMS II, Pecy, and RSI 20 relative to the parents. Overall, planned hybridization reduced crude protein levels in all the genotypes except Ghareh Yon Geh.

The use of open pollination, on the other hand, did not result in significant reductions of crude protein levels in any of the progenies except for those of the Iranian variety Ghareh Yon Geh, which had higher protein content than the parental plants by a margin that came close to being significant. Selfing increased crude protein levels in progenies of the varieties Zuzana and Pecy (216 g kg^{-1}) as well as in S_1 progenies of the cultivar Ghareh Yon Geh, but the increase was not statistically significant.

The highest protein contents were recorded in the S_1 progenies (202 g kg^{-1}), followed by the parental populations (201 g kg^{-1}), open pollination progenies (200 g kg^{-1}), and F_1 hybrids (190 g kg^{-1}). When looking at the results of the present study, we must take into account the fact that the crude protein data originated from a single cut (first cutting of 2008), that the samples were taken from the replicates over a period of five days, and that the protein content of alfalfa decreases by about 3 g kg^{-1} per day (Katić et al. 2005b). This may be of consequence in the analysis of the crude protein data and the resultant small variations among the parents and different types of pollination. An analysis of the parental populations and genotypes obtained through direct crosses, open pollination, and selfing shows that genotypes can be identified that have higher crude protein levels and higher biomass yields. The results we obtained support the notion proposed by Veronesi et al. (2010), that there is variability among high-yielding alfalfa populations with respect to crude protein content and indicate that it is possible to make progress in breeding for both increased yield and increased quality in alfalfa.

Spearman's rank correlation coefficients show that there was a medium correlation between the dry matter yields of the parents and those of open pollination progenies ($r = 0.60$) as well as between the parental dry matter yields and those of the S_1 progenies ($r = 0.50$) (Tab. 3).

Rank correlations between the inbred progenies and the parental populations were medium ($r = 0.50$). Those between open pollination and S_1 progenies were high ($r = 0.80$), indicating a similar distribution of genotypes between the two progeny tests with respect to dry matter yield.

Table 3. Rank correlations between parents (P) and progeny tests - open pollination (OP) and selfing (S_1)
Tabela 3. Korelacije ranga između roditelja (R), i testova slobodne oplodnje (SO) i samooplodnje (S_1)

Trait Osobina	P/OP R/SO	P /S_1 R/S_1	OP/S_1 SO/S_1
Dry matter yield (g $plant^{-1}$) Prinos suve materije	0.60	0.50	0.80
Crude protein content (g kg^{-1}) Sadržaj proteina	-0.10	0.83	-0.33

Rank correlation values for crude protein content varied depending on the progeny test and parent. Negative correlations were recorded between the parents and open pollination progenies (r = -0.10), suggesting there was a weak link among the genotypes in terms of rank for crude protein content during the study years. Also, negative rank correlations were found between open pollination and selfing (r = -0.33), which shows that there was no link in the ranking of progenies from these two tests with regard to crude protein content. However, highly positive rank correlations for protein content were observed between the parents and S_1 progenies (r = 0.83), indication that there was significant congruence in the ranks of parental populations and inbred progenies. The strong link between the parents and inbred progenies for crude protein content is in agreement with Rotili et al. (1999). It indicates that desirable genes for crude protein content accumulate in the parental populations and that they remain fixed through self pollination.

The most efficient way to evaluate parental populations for crude protein content is to make use of S_1 progenies, while the use of open pollination for this component of the nutritive value of alfalfa would not make sense.

Conclusions

Differences in dry matter yield between the parental populations and progenies obtained by different pollination methods were significant. The highest yields of dry matter were found in progenies produced by direct crosses.

No significant differences were recorded between alfalfa populations obtained by open pollination and those resulting from direct crosses. The results of our study indicate that open pollination tests are important for evaluating parental alfalfa populations in breeding for dry matter yield, especially because they make it possible to perform larger seed increases.

Dry matter yields decreased significantly after only one generation of self pollination, as indicated by the occurrence of inbreeding depression in the S_1 progenies of all the genotypes studied. The use of selfing in the first generation significantly increased the crude protein content of alfalfa.

The rank correlations obtained in our study indicate that open pollination and self pollination progeny tests are effective tools for evaluating alfalfa parents for dry matter yield and crude protein content, respectively.

Alfalfa breeding for improved quality can be effective if crosses are made between genetically divergent populations in order to produce hybrid combinations that will be used as donors of genes for quality in the development of new synthetic cultivars, all the while making sure that yield is maintained as an essential factor in alfalfa breeding.

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Efikasnost testova potomstava u oplemenjivanju lucerke *(Medicago sativa* L.*)* na veći prinos i kvalitet

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Izvod: Cilj rada je bio da se proceni efikasnost testova potomstava u oplemenjivanju lucerke na veći prinos i bolji kvalitet. Razlike su značajne u prinosu suve materije između roditeljskih populacija i potomstava nastalih različitim sistemima oplodnje. Najveći prinos suve materije dobijen je kod potomstava nastalih direktnim ukrštanjem. Između populacija lucerke nastalih slobodnom oplodnjom i direktnim ukrštanjem nije bilo značajnih razlika. Rezultati ukazuju na značaj testa slobodne oplodnje u oceni roditeljskih populacija lucerke u oplemenjivanju na prinos suve materije. Primena samooplodnje u prvoj generaciji značajno povećava sadržaj sirovih proteina kod lucerke. Korelacije ranga ukazuju na mogućnost efikasne ocene roditelja za prinos suve materije primenom testa potomstava, a za sadržaj sirovih proteina korišćenjem testa samooplodnje. Oplemenjivanje lucerke na veći kvalitet može biti efikasno ukoliko se vrše ukrštanja između genetički divergentnih populacija sa ciljem da se hibridne populacije koriste kao donori gena kvaliteta u stvaranju novih sintetičkih sorti, ali sa očuvanjem prinosa kao nezaobilaznog faktora u oplemenjivanju lucerke.

Ključne reči: lucerka, prinos suve materije, progeni testovi, sadržaj proteina