

**BREEDING EVALUATION OF ALFALFA (*Medicago sativa* L.)
POLY-CROSS PROGENIES BY MULTIVARIATE ANALYSIS
BASED ON AGRO-MORPHOLOGICAL TRAITS**

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Abstract

The alfalfa varieties are mostly synthetic populations of wide genetic base and a number of breeding schemes have been proposed for their creation. The progeny testing plays an important role in new alfalfa variety development because it gives information about the genetic value of the parental components based on the values established in their progeny.

From 2018 to 2021 nine polycross progenies (23-M, S11-17, S11-34, S12-1/8, 9K11, 8K7, AI17, 9P17 and 3P17) originated in a polycross nursery were tested at the experimental field of the Institute of Agriculture and Seed Science "Obraztsov chiflik" – Rousse. The field experiment was performed in a randomized block design in four replicates and harvesting plot size of 10 m². The objective of the study was to evaluate the alfalfa polycross progenies based on main agro- morphological traits performance and to predict their genetic potential as a source of parental material for new alfalfa variety breeding.

9K11 and 8K7 progenies showed superior scores regarding green mass and dry matter yield, plant height, stem producing ability and regrowth rate, while S11-34 and 3P17 progenies distinguished with high phenotypic expression of crude protein content and leaf/stem ratio. These progenies are valuable germplasm source to be used in a further breeding programme for new alfalfa variety development.

Key words: alfalfa, polycross progeny, traits, multivariate analysis, correlation

Introduction. Alfalfa is allogamous species of genus *Medicago*, family Fabaceae and it is the most widely grown perennial legume forage crop in the world. It is widely known as the “Queen of the forages” due to high biomass production, excellent forage quality and wide adaptation to different environments [1].

The common (*Medicago sativa* L.) is a perennial, cross pollinated autotetraploid ($2n = 4x = 32$) with expressed self-incompatibility and severe inbreeding depression [2]. As an autotetraploid cross pollinated crop, alfalfa has its specific requirements in breeding methods and selection of progenies that are different to diploid plant species [3]. Due to the autotetraploid alfalfa genome, breeding for enhanced agronomic traits is challenging and progress historically using conventional breeding has been slow [4]. Nevertheless, these methods are basic in creating new varieties and will continue to play an important role in the alfalfa breeding [3].

The economically most important characteristics subject to improvement in alfalfa breeding are quantitative traits, caused by the action of a large number of genes whose degree of phenotypic expression is modified under the influence of environmental conditions [5]. A number of authors report that the variability of the quantitative traits is due to a genetically determined response to the changing environmental conditions (soil, climate, agricultural techniques, etc.) [6, 7].

Several breeding schemes for new synthetic varieties creation were proposed for some open- pollinated fodder legumes, including alfalfa. They may imply the phenotypic (mass) selection of individual plants, selection on replicated clonal material, and various types of genotypic selection based on half-sib (HS) or full-sib (FS) families or progenies issued by one or more selfing generations (S1, S2, etc.) [8, 9].

The identification and classification of plant germplasm by phenotypic traits and the selection of breeding materials with excellent economic and morphological characteristics have become a common and effective research method [10, 11].

Phenotypic evaluation of germplasm on the basis of mean values and variability of the most representative traits is a crucial step in the choice of appropriate genetic material to the achievement of the purposes of the alfalfa breeding programme [12]. PARIHAR et al. [13] pointed out that a prerequisite for building an efficient breeding programme is knowledge of the degree and nature of relationships between important characteristics, especially for those with low genetic variability. Principal Component Analysis (PCA) has been widely used in the studies on magnitude of traits variability in germplasm collections of many species [14, 15]. According to MARTÍNEZ-CALVO et al. [16], multivariate analysis is a very useful method because it reveals the relationships and correlation among traits studied, allows a better understanding of the structure of the collection and identification of the most relevant variables.

The objective of the study was to evaluate alfalfa polycross progenies originated in polycross nursery, based on main agro-morphological traits performance

and to predict their genetic potential as a source of parental material for new alfalfa variety breeding.

Material and methods. Nine polycross progenies (23-M, S11-17, S11-34, S12-1/8, 9K11, 8K7, AI17, 9P17 and 3P17) were tested from 2018 to 2021 at the experimental field of the Institute of Agriculture and Seed Science “Obraztsov chiflik” – Rousse. Prista 3 variety as control variety was included.

The investigated polycross progenies were originated in a polycross nursery at free-limited pollination of alfalfa clones and have been developed by vegetative propagation of partly inbred (S1) superior individual plants.

The field experiment was performed in a randomized block design in four replications and harvesting plot size of 10 m². Sowing was carried out on March 17 at interrow spacing of 12.5 cm and sowing rate of 25 kg ha⁻¹. The alfalfa green mass in early flowering stage was harvested. A total 12 cuts were performed – two cuts during year of alfalfa establishment (2018), four cuts during the second growing season (2019) and three cuts during the third (2020) and fourth (2021) growing seasons.

In all growing seasons at each cut seven agro-morphological traits were measured as follows: plants height (PH), ability for stem producing, expressed by vegetative stem number (VSN) per m², green mass yield (GMY), dry matter yield (DMY), regrowth rate (RR) after cutting, leafiness (leaf/stem ratio – LSR), crude protein content (CPC).

The green mass and dry matter yields (t ha⁻¹) were reported by weighing the green biomass from each replicate at all regrowths. To determine dry matter yield, plant samples were taken before each mowing. The samples were weighed on electronic balance and dried to constant weight to assess dry matter content (%).

The plants height (cm) was recorded by measuring the length of the stems from the soil surface to the top in five places in each harvesting plot.

Regrowth rate (cm) was determined by measurement of plant height ten days after each cut. Five measurements per each harvesting plot were made. The results were averaged to determine a mean plants height.

Crude protein content (%) in the dry matter was determined by the Kjeldahl method (according to BDS/ ISO-5983).

For leaf to stem ratio (LSR) determination the forage samples of 500 g were randomly taken in each cut, from each plot of all polycross progenies. The leaves were separated from the stems, then leaves and stems samples were dried to constant weight. The dry separated leaves and stems were weighed. LSR was calculated by dividing the dry leaves weight by the dry stems weight.

The data recorded were analyzed by ANOVA, using STATGRAPHICS Plus software. The significance of differences between half-sib progenies was detected by LSD test at 0.01% confidence level. Principal component analysis (PCA) was performed to identify the traits that were the main source of the variability.

Multivariate analysis was applied to determine the relationships among traits analyzed.

Results and discussion. Results from the four-year study of nine polycross progenies are presented in Table 1. The means of the agro-morphological traits used to evaluate the progenies were averaged from 12 regrowths. Data of analysis of variance indicated a different degree of phenotypic expression of all traits among polycross progenies.

The values show that the highest mean annual green mass yield of 61.56 t ha⁻¹ were obtained in 9K11 progeny, followed by 8K7 and S12-1/8. High GMY was also recorded for AI17 (60.09 t ha⁻¹), exceeding mean for progenies (59.28 t ha⁻¹). The differences versus Prista 3 control variety were statistically significant.

According to the results presented in Table 1, annual dry matter yield ranged from 16.64 t ha⁻¹ to 14.58 t ha⁻¹. Data revealed that 9K11 and S12-1/8 ranked first, significantly exceeding at $P \leq 0.01\%$ control variety and mean value determined for progenies. The results for DMY were in same range obtained from TUCAK et al. [17] who studied twenty selected alfalfa populations and two control varieties.

T a b l e 1

Means of the investigated traits of nine alfalfa polycross progenies 2018–2021

Half-sib progenies	Agro-morphological traits						
	GMY, t ha ⁻¹	DMY, t ha ⁻¹	PH, cm	VSN	RR, cm	CPC, %	LSR
Prista 3	58.55 ^{de}	15.82 ^{bc}	75.63 ^b	429.25 ^{abc}	18.22 ^e	20.64 ^{bcd}	0.826 ^d
23-M	57.44 ^f	15.39 ^c	78.72 ^b	433.83 ^{ab}	17.41 ^f	21.86 ^a	0.969 ^{ab}
S11-17	57.66 ^{ef}	15.35 ^c	80.21 ^b	429.08 ^{abc}	18.02 ^e	20.81 ^{bcd}	0.878 ^{cd}
S11-34	59.70 ^{bc}	15.76 ^{bc}	79.50 ^{ab}	425.08 ^{bc}	18.94 ^d	21.92 ^a	1.019 ^a
S12-1/8	60.30 ^b	16.28 ^{ab}	80.10 ^a	433.01 ^{ab}	19.86 ^c	20.80 ^{bc}	0.897 ^c
9K11	61.56 ^a	16.64 ^a	78.46 ^a	443.26 ^a	21.08 ^a	20.58 ^{cd}	0.926 ^{bc}
8K7	60.47 ^b	15.83 ^{bc}	79.09 ^{ab}	440.74 ^a	20.16 ^{bc}	21.29 ^{ab}	0.988 ^{ab}
AI17	60.09 ^b	15.36 ^c	80.04 ^a	434.26 ^{ab}	19.92 ^{bc}	21.07 ^d	0.866 ^{cd}
9P17	59.02 ^{cd}	15.44 ^c	78.39 ^b	441.83 ^a	20.44 ^{ab}	21.85 ^a	0.975 ^{ab}
3P17	57.26 ^f	14.58 ^d	78.37 ^c	416.67 ^c	16.84 ^g	21.77 ^a	1.004 ^a
Mean for progenies	59.27	15.65	78.85	432.70	19.02	20.98	0.935
LSD 0.05	0.72	0.41	0.96	11.44	0.45	0.75	0.051
LSD 0.01	0.97	0.55	1.30	15.4	0.59	1.01	0.063
SE	0.25	0.14	0.33	3.96	0.15	0.26	0.02
PCV (%)	5.34	6.43	2.62	6.80	7.66	2.83	6.66

Values within columns followed by different letter are significantly different at $P \leq 0.01$

Legend: GMY – green mass yield, DMY – dry matter yield, PH – plants height, VSN – vegetative stem number, RR – regrowth rate, LSR – leaf/stem ratio, CPC – crude protein content

The polycross progenies differed significantly in terms of plant height as S11-17 were the tallest plants (80.21 cm) developed and the lowest (75.63 cm) were Prista 3 variety. Concerning vegetative stem number per m², the values obtained were in range, from 443.26 for 9K11 to 416.67 pcs. for 3P17 with a mean of 432.7 pcs. A trend for high stem producing ability was outlined for 9P17.

The results for regrowth rate revealed significant differences between progenies as they were classified into seven homogenous groups. It was found that 9K11 distinguished with the fastest regrowth after cut (21.08 cm), while 3P17 has the weakest plant regeneration (16.84 cm). The values were in agreement with other findings [17, 18].

The most useful forage quality trait is crude protein content in alfalfa biomass. Therefore, the main goal in alfalfa breeding for the improvement of forage quality is increasing protein concentration. Variations were found among polycross progenies in CPC. Maximum CPC was achieved in S11-34 (21.92%) while minimum (20.58%) in 9K11. The differences were statistically significant ($P \leq 0.01$). SAYED et al. [18] reported that the value of crude protein content of alfalfa genotypes ranged from 17.11 to 26.96% when studying twenty-one alfalfa genotypes.

The proportion of leaves is a major factor determining the forage quality as a larger quantity of leaves indicates better quality [18]. The values indicated the polycross progenies exhibited different potential regarding leaf/stem ratio, as the differences between them were statistically significant ($P \leq 0.01$). The best leafiness was ascertained in S11-34 (1.019), while Prista 3 control variety distinguished with the lowest phenotypic expression of the trait (0.826).

Data obtained were in line with the values reported by KEBEDE et al. [19] who found that the leaf to stem ratio ranged from 0.83 to 1.03 with a mean of 0.97.

It can be concluded that 9K11 and 8K7 progenies showed superior scores regarding green mass and dry matter yields, plant height, stem producing ability, and regrowth rate while S11-34 and 3P17 progenies distinguished with high phenotypic expression of crude protein content and leaf/stem ratio.

Traits variability evaluation is a necessary indicator of reliability of certain trait as a criterion for selection. It was found that between progenies the magnitude of phenotypic variability of all agro-morphological traits was low (from 7.66 to 2.62%). The PCV values revealed that PH and CPC exhibited the lowest phenotypic variability (PCV < 3%) compared to the other traits.

The results of Principal component analysis are presented in Table 2. Data show that two main components (PC1 and PC2) gave Eigenvalues greater than 1.0 and explained 83.72% of the total variability among the progenies for all investigated traits. The first PC, which is the most important component, accounted for 63.08% of the total variability and according to the corresponding eigenvector values it was mostly resulted from the variation in RR (0.439), GMY (0.426), PH (0.423), and DMY (0.416).

T a b l e 2

Principal component analysis (PCA)
of agro-morphological traits

Traits	Component	
	PC1	PC2
Green mass yield	0.426	0.201
Dry matter yield	0.416	0.050
Plant height	0.422	0.011
Vegetative stem number	0.380	0.225
Regrowth rate	0.439	0.218
Crude protein content	-0.312	0.557
Leaf/stem ratio	-0.178	0.738
Eigenvector value	4.42	1.45
% of variance	63.08	20.64
Cumulative percentage	63.08	83.72

Similar result was reported by PROSPERI et al. [20] who detected that the first PC explained 56.4% of the total variability and was associated with biomass production. In the present study the second main component (PC2) accounted for 20.64% of total variability and mainly resulted from the variation in leaf/stem ratio (0.738) and crude protein content (0.556). TUCAK et al. [14] found that the second PC accounted for 16.24% of the variability. This portion of variation mainly resulted from the variation in number of stems, shape of leaf and width of central leaflet. Generally, the PCA separated the majority of progenies for studied traits in the two main principal components but the progenies were related differently to the PC1 and PC2 (Table 3).

T a b l e 3

Explained significant components by progenies

Half-sib progenies	Component	
	PC 1	PC 2
Prista 3	-0.171	-1.718
23-M	-0.156	0.490
S11-17	-0.833	-1.573
S11-34	-0.527	1.430
S12-1/8	1.601	-0.165
9K11	3.305	0.194
8K7	1.115	1.224
AI17	1.439	-1.307
9P17	0.051	1.421
3P17	-2.415	0.004

The first main component included five progenies four of which (9K11, S12-1/8, AI17, and 8K7) were positively associated with PC1. The negative values of PC1 were found for 3P17 (-2.415).

It was established that the second main component was represented by six progenies, as S11-34 (1.430), 9P17 (1.421) and 8K7 (1.224) was positively connected to PC2. Negative connection to PC2 was found for S11-17 and AI17 progenies.

The estimate of degree and nature of relationship between analyzed traits established positive and significant phenotypic correlation of GMY with DMY (rp = 0.805), PH (rp = 0.765), VSN (rp = 0.636) and RR (rp = -0.891) (Table 4). The phenotypic correlation coefficients revealed moderate negative correlation (rp = -0.477) between GMY and CPC. Furthermore GMY was negatively but insignificantly related (rp = -0.067) with LSR.

Table 4

Phenotypic correlation coefficients between agro-morphological traits in poly-cross progenies

	GMY	DMY	PH	VSN	RR	CPC	LSD
GMY	1						
DMY	0.805**	1					
PH	0.765**	0.773**	1				
VSN	0.636**	0.630**	0.229	1			
RR	0.891**	0.742**	0.777**	0.852**	1		
CPC	-0.477*	-0.490*	-0.513*	-0.336	-0.438*	1	
LSR	-0.067	-0.259	-0.321	-0.113	-0.132	0.756**	1

High and positive correlation was found between plant height and regrowth rate while relationship between plant height and vegetative stem number was weak and statistically not confirmed. A strong positive relationship was also ascertained between CPC and LSR (rp = 0.756). Data revealed that CPC and LSR were negatively related to all other traits analyzed.

Conclusions. 9K11 and 8K7 progenies showed superior scores regarding green mass and dry matter yield, plant height, stem producing ability, and regrowth rate, while S11-34 and 3P17 progenies distinguished with high phenotypic expression of crude protein content and leaf/stem ratio. These progenies are valuable germplasm source to be used in a further breeding programme for new alfalfa variety development.

Two main principal components (PC1 and PC2) explain 83.72% of the total variability among the progenies for all investigated traits. The first PC mainly resulted from the variation in regrowth rate, green mass yield, plant height, and dry matter yield. The PCA separated the majority of progenies for studied traits in the two principal components, and therefore selection in these progenies is useful to improve these traits.

A positive and significant phenotypic correlation was established of GMY and DMY with PH, WSN and RR, and negative relationship with CPC and LSR.

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