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ESTABLISHMENT OF PHENOTYPIC VARIABILITY AND CORRELATIONS OF SEED YIELD AND YIELD RELATED TRAITS IN ALFALFA (*Medicago sativa* L.) CLONAL PROGENIES

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ABSTRACT

The aim of present study was to evaluate phenotypic variability of seed yield and yield-related traits and to establish the relationships among them at eleven alfalfa clonal progenies. The study was conducted in experimental field at Institute of Agriculture and Seed Science Obraztsov chiflik - Ruse under conditions of open pollination (polycross), from 2014 to 2016. The traits plant seed yield, plant height, generative stem number, inflorescence number, pod number, seed number and 1000-seed weight were evaluated. The main effect and second interaction (year, genotype and year x genotype) had a significant influence on all morphological and generative traits. There was wide range of variability for all analized traits between progenies and over study period. PM30 progeny showed superior scores regarding all traits studied and four progenies distinguished with high phenotypic expression of seed yield, pod number, seed number and 1000-seed weight. The seed yield obtained was in range from 2.45 to 3.44 g plant^{-1}. These progenies are valuable germplasm source to be used in further breeding to develop a new synthetic alfalfa variety with stable seed yield. It was found seed yield strongly and positively correlated with plant height (r_p=0.752), pod number per inflorescence (r_p=0.700**) and seed number per pod (r_p=0.611**), which suggest that selection for improving alfalfa seed yield may be performed directly through selection on these three traits.**

Keywords: Alfalfa, clonal progenies, seed yield, variability, correlations.

INTRODUCTION

Common cultivated alfalfa (*Medicago sativa* L.) is an allogamous autotetraploid cross-pollinated perennial species of the family Fabaceae, which means “Best Fodder” in Arabic. Alfalfa has always been and continues to be the most valuable forage legume in world agriculture and a key component of sustainable agricultural systems due to its high yield, excellent forage quality, and ability to improve soil through nitrogen fixation (Bouton, 2012; Naydenova et al., 2022). It is considered as one of the world’s most versatile crops, because its wide adaptation to diverse environmental conditions ranging from burning hot deserts to cool high mountain valleys (Prosperi et al. 2006).

The alfalfa varieties are synthetic populations of wide genetic base (multiple hybridization of different number of selected parental genotypes) and a number of selection schemes have been proposed for their creation (without and with inbreeding, tests of progenies from polycross, topcross, etc.) (Annicchiarico et al., 2015). Developing synthetic varieties through the use of full-sib and half-sib families or clones as parents is a commonly used breeding method in alfalfa (Flajoulot et al. 2005).

Seed yield is an important property for the market success of forage legume varieties (Boelt et al., 2015). Therefore, ability of high seed producing of the new alfalfa varieties is critical in its effective commercial distribution and delivery to the farmers at a competitive price (Torricelli, 2007). According Bolanos-Aguilar et al. (2002), the low seed production is a problem in some alfalfa varieties and the progress in achieving higher seed yield is very limited over years.

The number of studies has been carried out to assess the influence of genetic factors, environmental conditions and the crop management on the seed yield (Andjelkovic et al., 2010; Abd El-Naby et al., 2016; Chen et al., 2016; Avci et al., 2017; Pajcin et al., 2020; Marinova, 2021).

The theoretical seed yield of alfalfa, is 12 t ha^{-1} (Lorenzetti, 1993), but the actual seed yield achieved under the most favourable conditions reaches only 4% of the potential yield (Bolanos-Aguillar et al. 2000). The main causes of low seed productivity of alfalfa are the poor pod set (only 40 to 60 % of the flowers set pods), and the low number of seeds per pod, (usually 3 to 4) (Bodzon, 2016).

Alfalfa seed yield is controlled genetically complex

qualitative characteristic and degree of its phenotypic expression is result of complex interaction between internal (genetic structure of variety) and external (environments, pollinators existence and crop management) factors. Rincker et al. (1988), reported that as quantitative characteristic with complex genetic basis, seed yield depends on the number of seeds per unit area and individual seed weight and ranged from 0 to 2110 kg ha^{-1}. The authors also stated that the seeds produced in the pods and the yield components include pod number per inflorescence, stem number per plant and the plant number per unit area.

The genotypic (GCV) and phenotypic coefficients of variation (PCV) are essential biometric tools and the basis for breeding, because the greater traits variability in a population, the greater the opportunity for selection and improvement of the genotypes for given traits (Rasheed et al., 2023). Typically, the PCV value for a given trait is somewhat higher than the GCV value, demonstrating the influence of the environment in the degree of phenotypic expression of the trait (Monirifar et al., 2011; Ozturk and Yildirim, 2014; Hussain et al., 2021).

In breeding for improved seed yield it is particularly important to determine the effect of generative and morphological characters on seed yield as well as their interrelations (Bodzon, 2016). Knowledge of the relationships between important characteristics makes it possible to improve a greater number of traits simultaneously, especially for those with low genetic variability, in which the success of the selection is achieved by indirect methods (Parihar et al., 2015; Yildirim et al., 2023). The establishment of the relationships between the traits of populations consisting of a different genotypes kept under the same conditions is a basis for establish objective criteria for effective selection (Kosev and Vasileva, 2021).

According Bodzon (2004), breeding for improved seed yield potential of alfalfa should be based on simultaneous selection for several important traits as inflorescence number per stem, pod number per inflorescence, seed number per pod and 1000-seed weight closely related with seed yield.

The objective of present study was to evaluate phenotypic variability of seed yield and yield-related traits between alfalfa clonal progenies and acrross years and to establish the relationships among traits in conditions of open pollination (polycross nursery), for developement alfalfa varieties with high ability of seed producing.

MATERIAL AND METHODS

Plant material and experimental design

The experiment was carried out in experimental field at Institute of Agriculture and Seed Science Obraztsov chiflik – Rousse, Agricultural academy, Bulgaria at 2014-2016. The experimental field is located at 43°48^{I} N latitude 26°02^{I} E longitude and altitude 152 m.

The object of investigation were eleven alfalfa clonal progenies of native origin, developed by vegetative propagation of partly inbred (S_1) superior individual plants (genotypes) on February 2014 in the green house of the Institute. At first the cuttings were rooted in test-tubes of water and then planted in chests of soil. The rooted cuttings were transplanted in the experimental field of the Institute at the end of April.

The polycross nursery was designed as completely randomised block in four replications. The nursery contained total 880 plants, and each clonal progeny was represented by 20 plants in each replication (total of 220 plants). The plants originated from rooted cuttings from each clonal progeny were planted in two 5 - meter long rows with ten plants in each row at a distance of 50 cm between plants and inter-row spacing of 50 cm. For good rooting on the field the plants were immediately watered after planting. The plants were watered total five times in 6-7 days intervals. During growing seasons, the necessary crop management was performed. Phosphorous and potassium in the form of triple superphosphate (400 kg ha^{-1}) and potassium nitrate (600 kg ha^{-1}) were supplied with the ploughing in late autumn before the year of progenies planting. Nitrogen in the form of urea (200 kg ha^{-1}) was applied with the last tillage of experimental in the spring before planting the clonal progenies. Weeds were controlled with hand hoeing as needed throughout the all growing seasons. Pests control was carried out by treatment with appropriate insecticides.

Soil data and climatic conditions during study period

The soil type of experimental site was leached black soil, located on sandy clay. Active soil fertility was characterized by good potassium (33.17 mg 100 g^{-1} soil), insufficient nitrogen (16.84 mg 100 g^{-1} soil) and poor phosphorus (6.15 mg 100g^{-1} soil) nutrient regime. The humus content was low and ranged from 2.03% to 2.17% (for the layer from 0 to 40 cm). The soil reaction was slightly acid (pH - from 5.84 to 5.94).

Weather conditions during study period are presented in Table 1. In the year of alfalfa plants establishment, the total monthly precipitation in all months was close to the long-term average (LTA) (1896-2005) with slight deviations, except for May, when the rainfall amounts was significantly more compare with the LTA (66.1 mm).

In April, May, June and July of the second alfalfa growing season there was significantly less precipitation than the LTA, while in August significantly more precipitation compared with the LTA was recorded. During 2016, in all months, a higher or similar rainfall amounts was recorded compare with the LTA, with the largest precipitation deficit in July (2.2 mm). The total amount of precipitation in the first and second growing season were 478.4 and 406.9 mm, respectively, which is by 94.3 and 22.8 mm more than LTA (384.1 mm). In 2016 the total amount of precipitation (375.4 mm) recorded was below than in the previous two years of study and close to the LTA.

Table 1. Climate data during three growing seasons (2014-2016)

Months	Rainfall (mm)				Temperatures (°C)			
	2014	2015	2016	LTA*	2014	2015	2016	LTA*
March	65.0	61.7	70.0	70.0	9.5	6.5	8.1	5.0
April	64.8	37.2	76.6	50.7	11.9	11.4	14.6	11.4
May	166.7	19.4	98.3	66.1	16.4	18.4	15.9	16.5
June	79.4	65.1	74.2	80.5	19.8	20.1	22.0	20.2
July	67.3	18.8	2.2	67.4	22.6	24.6	24.8	22.5
August	35.2	204.7	54.1	49.4	23.6	23.2	22.9	23.8
September	478.4	406.9	375.4	384.1	17.3	17.37	18.05	16.6
March-September	65.0	61.7	70.0	70.0	9.5	6.5	8.1	5.0

Legend: * LTA (Long term average) - the period from 1896 to 2005 was used

During the year of alfalfa establishment air temperature in all months were close to the LTA. In the second year the mean air temperatures at beginning of the growing season were similar to this in 2014, with mean monthly air temperature deviation in relation to the LTA in May and July +1.9 and +2.10 °C, respectively. The highest mean air temperature was recorded in 2016 (18.05 °C), as in April (14.6 °C), June (22 °C) and July (24.8 °C) is significantly higher than the LTA. The mean monthly air temperatures for the three alfalfa growing seasons (March–August) was 17.6 °C and it was higher by 1°C than the LTA (1896–2005) (16.6°C).

Data collection and statistical analysis

For the study period seven quantitative traits were evaluated of which four generative: seed yield per plant (SYP), pod number per inflorescence (PNI), seed number per pod (SNP) and 1000-seed weight (TSW), and three morphological traits: plant height (PH), generative stem number per plant (GSNP) and inflorescence number per stem (INS). The traits were evaluated during the establishment year, the second and third years.

Plant height (cm) and generative stem number were determined at full pod development stage (green pods) by measuring the length of the stems from soil surface to the tip. Generative stem number was calculated by counting the stems of 10 randomly selected plants for each genotype.

At seed maturity, five plants per progeny on each replicate were randomly sampled. Ten generative stems were selected and their inflorescences were counted to calculate the inflorescence number per stem.

In order to determine the traits pod number per inflorescence and seed number per pod, 20 inflorescences were collected of randomly selected stems. Ten inflorescences were selected and their pods were counted to calculate the number of pods per inflorescence. From the rest 10 inflorescence 10 pods were selected, threshed and their seeds were counted to calculate the seed number per pod. Ten plants per progeny were selected and their pods were threshed. The seeds were cleaned and weighted and the mean seed yield per plant (g) and 1000 seed weight (g) were determined.

Experimental data were processed by the One-way analysis of variance (ANOVA). The significance of differences among clonal progenies were detected by LSD test at 0.01% confidence level. In order to determine the degree of influence of the sources of variation genotype (G), year (Y) and genotype x year (G x Y) interaction on the studied traits a two-way analysis of variance was applied. Variation and correlation analysis was performed to establish the traits variability and the relationships among them. Phenotypic coefficients of variation (PCV) were estimated as a percentage of their corresponding phenotypic standard deviations to the trait grand mean. The magnitude of variation of the traits was determined according to the scale of Mamaev (1973) as follows: very low (up to 7%), low (7.1-12%), moderate (12.1-20%), high (20.1-40 %) and very high (over 40%). The relationships among studied characteristics, expressed by phenotypic correlation coefficients (r_p, %) were determined among all possible combinations of traits. The STATGRAPHICS PLUS software was used.

RESULTS AND DISCUSSION

Data of analysis of variance at evaluation of the alfalfa clonal progenies indicated a different degree of phenotypic expression of seed yield per plant and yield related traits both among clonal progenies and for period of study. The values presented in Table 2 shown that the highest three-year average seed yields of 3.52 and 3.44 g plant^{-1} were obtained in PM30 and JM13, respectively. High seed yield was also recorded at GM27 and SL83, exceeding mean for clonal progenies (2.91 g) by 13.8 and 8.59 %. The seed yield was reported in the literature varied in narrow range from 0.30 to 40 g. The means established in present study corresponded to values obtained by Tlahig et al. (2017), whereas El-Hifny et al. (2019) found mean seed yield per plant of 1.2 g at evaluation of seven alfalfa genotypes under three different sowing dates over the two seasons.

The variability of morphological and generative properties both among studied alfalfa progenies and across years, express by the phenotipic coefficients of variation is presented in Table 2-4.

Table 2. Means, standard divisions and phenotypic coefficients of variability of seed yield among progenies and for study period

Clonal progenies	Seed yield (g plant^{-1})			
	Mean	% to mean for progenies	SD	PCV for study period (%)
SL83	3.16 bc	108.76	0.65	20.64
SL89	2.66 de	91.57	0.21	7.88
SL92	2.50 e	85.96	0.52	20.85
SL99	2.51 de	86.30	0.10	3.80
PM30	3.52 a	121.03	0.68	19.42
PM18	2.87 cd	98.68	1.11	38.56
PM49	2.82 cde	96.84	0.36	12.71
PM65	2.45 de	84.35	0.61	24.95
GM14	2.75 de	94.44	0.65	23.76
GM27	3.31 b	113.80	1.11	33.56
JM13	3.44 ab	118.27	0.84	24.30
Mean	2.91			
$LSD_{0.01}$	0.37			
SD	0.39			
PCV (%)	13.37			

Means followed by same letter in the columns are not significantly different at $p \leq 0.01$

Reported values indicated that seed yield per plant varied moderately among clonal progenies (PCV=13.37%). Data correspond with the results of Basafa and Taherian (2009), who found CV of 13% for seed weight per plant. The authors postolate that phenotypic variability of seed yield among alfalfa genotypes is due to crop management practices and high level of genetic impurity. The values of PCV indicated that clonal progenies exhibited from very low to high variability regarding trait during study period. A large variation in SY among varieties and environments has been reported by Bolanos-Aguilar et al. (2002). The highest stability for SYP in the present study was observed in SL99 (PCV=3.8%). It was found the seed weight per plant varied significantly across years in GM27 (PCV=33.56%).

The power of influence of sources of variation was expressed by η (%). The reported values of 33.16, 20.10 and 16.08% for factors year (Y), genotype (G) and genotype x year (G x Y) interaction, respectively, revealed their significant impact on the degree of phenotypic expression of seed yield (Table 5). Ilic and Dukic (2006) found that the degree of penotypic expression of seed yield is highly influenced by year, as well as by genotype x year interaction when investigated 10 genetically divergent genotypes. A significant influence of the year and the genotype on seed weight per plant was also reported by Lakić et al. (2022) but the authors argued that the genotype x year interaction had no significant effect on the trait.

There were established considerable differences ($p \leq 0.01$) between clonal progenies regarding plant height as they were classified into 7 homogenous groups (Table 3). The means ranged from 67.23 to 53.87 cm. The clonal progenies GM27 and SL83 distinguished with the highest plants and the excesses in values for the trait to the other progenies were statistically significant. It was outlined trend for high genetic potential of JM13 progeny, which ranked third with trait value exceeding by 9% the mean for progenies.

Regarding trait variation among progenies, the PCV value showed that plant height exhibited lower phenotypic variability (PCV<10%) compared to the other studied quantitative traits, with exception of TSW. Data indicated different magnitude of variability for PH during study period, as only SL99 (PCV=3.8%) exhibited low variability. The degree of variation for all other progenies was determined as mederate to high.

Based on analysis of variance, it is evident that the factor G had statistically significant impact on PH (η=36.48%). The analized trait was also considerably influenced by Y (η = 32.88%) and G x Y interaction (η=27.28%) (Table 5).

There were found significant differences in degree of phenotypic expression of the generative stem number per plant (Table 3). The means given in Table 3 indicated that GM14 had the highest ability in stem producing (47.47 stem plant^{-1}) and SL89 the lowest (29.80 stem plant^{-1}). PM65 and PM30 ranked second at reported value of 41.57 and 41.37, respectively.

The coefficient of variation, as a measure of dispersion, determined the variability of GSNP among clonal progenies as moderate (PCV=14.32%). It can be seen that the trait varied considerably (PCV>65%) at all progenies over study period (Table 5). The year, as a source of variation had exclusively strong influence on stems number (η=91.19%). The influence of factors genotype and genotype x year interaction was also statistically significant but much low than year.

Table 3. Means, standard divisions and phenotypic coefficients of variability of morphological traits among progenies and for study period

Clonal progenies	Plant height (cm)				Generative stem number per plant				Inflorescence number per stem			
	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)
SL83	66.73 a	111.22	7.02	10.51	35.27 cd	95.57	25.17	71.37	10.27 b	105.97	4.95	48.22
SL89	58.87 de	98.11	4.41	7.49	29.80 f	80.76	22.45	75.35	10.00 bc	103.22	2.95	29.51
SL92	53.87 h	89.78	6.19	11.48	37.40 c	101.36	28.53	76.29	9.17 de	94.62	4.34	47.30
SL99	55.77 fg	92.94	8.31	14.89	33.60 de	91.06	24.10	71.73	8.30 f	85.67	1.56	18.82
PM30	63.83 c	106.39	11.90	18.64	41.37 b	112.10	29.66	71.70	11.97 a	123.52	3.11	25.98
PM18	57.80 e	96.33	9.51	16.46	35.77 cd	96.93	24.15	67.52	9.50 cd	98.06	4.15	43.69
PM49	55.03 g	91.72	5.42	9.85	36.30 cd	98.37	26.51	73.04	9.30 de	96.00	2.43	26.16
PM65	56.33 f	93.89	6.81	12.09	41.57 b	112.65	29.09	69.98	9.23 de	95.31	1.75	18.96
GM14	59.03 d	98.39	6.79	11.50	47.47 a	128.64	34.86	73.45	8.80 ef	90.84	4.49	51.06
GM27	67.23 a	112.06	5.93	8.82	35.63 cd	96.57	26.00	72.97	9.43 cde	97.37	4.04	42.80
JM13	65.50 b	109.17	6.07	9.26	31.73 ef	86.00	23.80	75.00	10.60 b	109.42	2.52	23.81
Mean	60.00				36.90				10.27			
$LSD_{0.01}$	1.08				2.80				0.65			
SD	4.93				5.25				1.50			
PCV (%)	8.22				14.32				14.56			

Means followed by same letter in the columns are not significantly different at p≤0.01

Table 4. Means, standard divisions and phenotypic coefficients of variability of gnerative traits among progenies and for study period

Clonal progenies	Pod number per inflorescence				Seed number per pod				1000 seed weight (g $plant^{-1}$)			
	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)
SL83	7.20 b	106.21	2.12	29.40	3.70 a	120.07	0.44	11.78	1.92 ab	106.19	0.19	9.62
SL89	6.10 cd	89.99	2.43	39.79	2.50 de	81.13	0.35	13.86	1.69 f	93.49	0.14	8.38
SL92	5.83 d	86.05	1.91	32.72	2.70 cd	87.62	0.53	19.60	1.75 de	96.62	0.13	7.47
SL99	5.80 d	85.56	1.39	23.89	2.97 bc	96.27	0.12	3.89	1.84 c	101.41	0.20	10.62
PM30	8.07 a	119.00	2.30	28.55	3.83 a	124.40	0.72	18.87	1.89 b	104.53	0.19	9.83
PM18	7.20 b	106.21	2.77	38.52	3.10 b	100.60	0.72	23.26	1.79 cd	98.83	0.16	8.71
PM49	6.83 b	100.80	1.83	26.84	2.84 bcd	92.19	0.56	19.58	1.77 d	97.72	0.14	7.77
PM65	6.90 b	101.79	2.91	42.10	2.36 e	76.44	0.04	1.63	1.68 f	92.94	0.27	15.74
GM14	6.70 bc	98.84	2.50	37.28	3.10 b	100.60	0.60	19.35	1.70 ef	93.86	0.18	10.73
GM27	7.10 b	104.74	2.42	34.15	3.07 b	99.52	0.90	29.23	1.96 a	108.21	0.31	15.84
JM13	6.83 b	100.80	1.79	26.19	3.73 a	121.15	1.07	28.64	1.92 ab	106.19	0.03	1.31
Mean	6.78				3.08				1.81			
$LSD_{0.01}$	0.63				0.34				0.05			
SD	0.82				0.55				0.10			
PCV (%)	12.12				17.96				5.61			

Means followed by same letter in the columns are not significantly different at p≤0.01

There were considerable differences among clonal progenies concerning inflorescence number per stem (Table 4). Means for study period shown that PM30 and JM13 distinguished with significantly higher phenotypic expression of the trait than other progenies, with 11.97 and 10.6 inflorescence stem^{-1}, while SL99 was with the lowest one (8.3), at average value for the progenies of 10.27. Abd El-Naby et al. (2016) in a study of 10 genotypes found that INS ranged from 8 to 16 inflorescence stem^{-1}, at mean 11.93.

Table 5. Results of analysis of variance for studied traits.

Trait	Sources of variation	SS	df	MS	F exp.	η (%)	Sign.
SYP	Genotype (G)	51.51	10	5.15	19.46	20.10	**
	Year (Y)	84.99	2	42.49	160.58	33.16	**
	Interaction (G * Y)	41.23	20	2.06	7.79	16.08	**
	Error	78.60	297	0.27			
PH	Genotype (G)	7299.40	10	729.94	322.22	36.48	**
	Year (Y)	6578.86	2	3289.42	1452.08	32.88	**
	Interaction (G x Y)	5458.95	20	272.95	120.49	27.28	**
	Error	672.80	297	2.27			
GSNP	Genotype (G)	7426.33	10	742.63	52.43	4.33	**
	Year (Y)	156559.66	2	78279.83	5526.69	91.19	**
	Interaction (G * Y)	3493.01	20	174.65	12.33	2.03	**
	Error	4206.70	297	14.16			
INS	Genotype (G)	297.02	10	29.70	34.74	9.24	**
	Year (Y)	2201.75	2	1100.87	1287.75	68.49	**
	Interaction (G x Y)	462.18	20	23.11	27.03	14.38	**
	Error	253.9	297	0.86			
PNI	Genotype (G)	133.69	10	13.37	19.65	9.18	**
	Year (Y)	1045.82	2	522.91	768.46	71.79	**
	Interaction (G x Y)	75.24	20	3.76	5.53	5.16	**
	Error	202.10	297	0.68			
SNP	Genotype (G)	74.75	10	7.48	31.89	32.51	**
	Year (Y)	41.41	2	20.70	88.35	18.01	**
	Interaction (G x Y)	44.19	20	2.21	9.43	19.22	**
	Error	69.60	297	0.23			
TSW	Genotype (G)	3.10	10	0.31	60.38	25.04	**
	Year (Y)	3.89	2	1.94	379.15	31.42	**
	Interaction (G x Y)	3.87	20	0.19	37.75	31.26	**
	Error	1.52	297	0.01			

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; η - degree of influence of the factor;
**significant at 0.01 level

There were established significant differences in degree of trait variation for clonal progenies over study period. The values of PCV ranged from 18.82% (moderate variability) for SL99 to 51.06% (very high) for GM14. Among progenies the trait varied moderately (PCV=14.56%). Pelikán et al. (2014) in a study of 99 alfalfa accessions found high variability (33.1%) of INS. According Bolanos-Aguilar et al. (2000), under a spaced plant design, the number of fertile stems and inflorescences may be more variable, depending on the size of the individual plants while, in dense canopies, the number of fertile stems per unit area is likely to be more stable.

The results indicated significant impact of all sources of variation on the inflorescence number per stem but the variability of the trait was the most influenced by year (η=68.49%). It can be explain by the weather conditions, mainly rainfall and its distribution during flowering - seed set - seeds ripening period (June-August) accros the growing seasons. These results are with accordance by findings of Bolanos-Aguilar et al. (2002) and Karagić et al. (2019).

The results of three-year for pod number per inflorescence shown significant differences between clonal progenies (Table 4). It was found that PM30 had the highest pods per inflorescence (8.07) and SL99 the lowest (5.80), at average value for the progenies of 6.78 pods inflorescence^{-1}. The results are in line whit these reported by Tlahig et al. (2017) who obtained that PNI varied from 5.56 to 8.95 pods inflorescence^{-1}. Karagić et al. (2019) reported a mean of 7.6 pods inflorescence^{-1} and Jevtić et al. (2014) stated values for the trait of 4.74, 7.68 and 6.97 pods inflorescence^{-1} on the lowest, middle and peak inflorescences, respectively. The reported values of PCV determined variability of trait between studied progenies as moderate (PCV=12.12%) and over the study period as very high for PM65 (PCV=42.10%) and high for other progenies. The high trait variability (CV=22.8%) among populations has been demonstrated by Pelikán et al. (2014).

The effects of G, Y and G x Y interaction on pod number per inflorescence were statistically significant (Table 5). It was found that the strongest influence on the pods developemment had year. On the contrary, Lakic et al. (2022) reported significant influence of genotype on NPI in a stuty on within-population variability of seed yield related traits in 10 alfalfa genotypes.

Concerning seed number per pod, data of analysis of variance revealed significant differences ($p \leq 0.01$) among progenies (Table 4). For study period PM30 characterized with the largest amount of seeds (3.83), followed by JM13 (3.73), whereas PM65 had the lowest (2.36). In previous researches has been reported that the number of seed per pod varied from 1.85 to 9.16 seed pod^{-1} (Dordas, 2006), from 5.5 to 5.7 seed pod^{-1} (Rashidi et al., 2009) and from 1.63 to 2.41 seed pod^{-1} (Abasov et al., 2019). Karagić et al. (2019) at studying 20 populations, which represent a part of the European alfalfa core collection, determined mean of 2.6 seed pod^{-1} in year of alfalfa stand establishment. Data shown that between clonal progenies SNP varied moderately (PCV=17.96), but more strongly than other analized traits. It is noticeable that over study period clonal progenies expressed different magnitude of trait variability (from very low to high). The highest stability of trait was established in PM65 (PCV=1.63%).

Data of two-way analysis of variance shown that the seed number per pod was determined more on genetic factors (η=32.51%) than on factors Y and G x Y interaction (Table 5). The results obtained are in agreement with those reported by Abd El-Naby et al. (2016). Avci et al. (2017) reported that the year effect was significant for seed number per pod, whereas row spacing and sowing rate did not significantly affect degree of phenotypic expression of trait during growing seasons.

The values presented in Table 4 indicated the clonal progenies exhibited different potential regarding 1000-seed weight, as the differences between them were statistically significant ($p \leq 0.01$). The highest phenotypic expression of the trait was recorded in GM27 (1.96 g) and the lowest was ascertained in PM65 (1.68 g) with a mean of 1.81 g for progenies. The results correspond with those of Sengul (2006) who reported that 1000-seed weight varied from 1.63 to 2.06 g with a mean of 1.85 g. Tlahig et al. (2017) obtained lower values for the trait from 1.45 to 1.74 g. In term of 1000-seed weight variability data shown that the trait expressed very low variation (PCV=5.61%) among clonal progenies. Data obtained correspond with the results of Iannucci et al. (2002) who reported PCV values less than 12% for TSW. On the contrary, Abbasi et al. (2003) revealed large phenotypic variation of the trait among alfalfa accessions.

Over study period 1000-seed weight exhibited very high stability (PCV=1.31%) in JM13 (Table 5). The trait varied moderately in two progenies and low in the others. It was established that all sources of variation had significant influence on TSW but the impact of factor Y (η=31.42%) and factor G x Y interaction (η=31.26%) was stronger than that of genetic factors. El-Hifny et al. (2019) also found that year (environment) had the stongest effect on TSW than other sources of variation (genotype, sowing date and their interaction).

According Bodzon (2016), the quantitative traits determining seed productivity are polygenically determined and for this reason determining of its effects on seed yield and relationships is decisive for the effectiveness of selection for increased seed yield.

Phenotypic correlation coefficients among analyzed traits are presented in Table 6.The estimate of degree and nature of relationship between analized traits revealed positive and significant phenotypic correlation between seed yield and all generative and morphological traits. Data obtained is in accordance with the results reported by Bodzon (2016) of positive correlation of SYP with 6 generative and 6 morphological traits. There was found weak correlation between seed yield and generative stem number per plant (r_p=0.150). Contrary, Zambrana (1972) reported on high and positive relationship between seed yield and number of fertile stems. Lakić et al. (2022) established positive correlation (r=0.47) between SYP and NPI. Phenotipic correlation coefficient confirmed correlation between SYP and INS (r_p=0.362*). Liatukiene et al. (2009) showed that the weak to medium correlations are more suitable for selecting of parental material.

Table 6. Phenotypic correlation coefficients among 7 characters for the tested genotypes

Traits	SYP	PH	GSNP	INS	PNI	SNP	TSW
SYP	1						
PH	0.752**	1					
GSNP	0.150	-0.165	1				
INS	0.362*	0.630**	-0.066	1			
PNI	0.770**	0.683**	0.241	0.570**	1		
SNP	0.611**	0.802**	-0.303	0.510**	0.290	1	
TSW	0.412*	0.749**	0.151	0.323*	0.661**	0.083	1

*, **significant correlation at 0.05 and 0.01 level of probability, respectively

The plant height was highly and positively correlated with all generative and morphological traits, except for the GSNP (r_p=-0.165). The correlation coefficients revealed a moderate positive correlation of inflorescence number per stem with pod number per inflorescence (r_p=0.570**) as well as with seed number per pod (r_p=0.510**). Furthermore inflorescence number per stem was positively but insignificantly related with 1000-seed

weight (r_p=0.323). There was also found a strong positive relationship between pod number per inflorescence and 1000-seed weight (r_p=0.661**). The correlation between pod number per inflorescence and seed number per pod was weak and statistically no confirmed. Insignificant assosiation between these two traits was established by Bodzon (2016). Khrbeet et al. (2016) found significant negative correlation between TSW and SY (-0.669), SNP (-0.589) and PNI (-0.603). Zhang et al. (2008) also reported a significant negative relationship between SY and TSW.

CONCLUSION

Evaluation of genotypic and morphologic properties of the alfalfa progenies were established that the sources of variation (genotype, year and genotype x year interaction) had a statistically significant influence on all morphological and generative traits analyzed. The degree of phenotypic expression of the traits seed yield per plant and seed number per pod were influenced more on genetic factors than factors Y and G x Y interaction. The factor G x Y interaction had the most significant impact on the 1000-seed weight and plant height.

The largest magnitude of phenotipic variability between clonal progenies was ascertained for seed number per pod (PCV=17.96%) and the lowest for 1000-seed weight (PCV=5.61%).

PM30 progeny showed superior scores regarding all traits studied and JM13, GM27, SL83 and PM18 distinguished with high phenotypic expression of the traits seed yield, pod number per inflorescence, seed number per pod and 1000-seed weight. These progenies were evaluated as a valuable germplasm source to be used in further breeding to develop a new synthetic alfalfa variety with stable seed yield.

It was found seed yield strongly and positively correlated with plant height (r_p=0.752**), pod number per inflorescence (r_p=0.700**) and seed number per pod (r_p=0.611**), wtich suggest that selection for improving seed productive ability in alfalfa may be performed directly through selection on these three traits.

This study would be continued whit testing the polycross progenies propagated by seeds in the polycross nursery, to determine the progenies with the best combining ability, regarding important agro-morphological traits and to be used as parental components in the synthesis of alfalfa synthetic populations.

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