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PHENOTYPIC EVALUATION OF SEED PRODUCING ABILITY OF ALFALFA (*Medicago sativa* L.) CLONAL PROGENIES

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Abstract

The objective of present study was to assess seed yield and yield related traits of alfalfa clonal progenies. The phenotypic variation of traits within progenies was also determined. Eleven alfalfa clonal progenies of native origin were object of investigation. The experiment was carried out at the Experimental field of the IASS Obraztsov Chiflik, Rousse for three-year period. The traits plant seed yield (PSY), plant height (PH), generative stem number (GSN), inflorescence number (INP), pod number (PNI), seed number (SNP) and 1000-seed weight (TSW) were evaluated. There were statistically significant differences among clonal progenies for all studied traits. Five progenies (PM30, JM13, GM27, SL83 and PM18) were identified as very valuable genetic source with potential for developing high seed yielding varieties. There was established PSY, PNI and SNP exhibited moderate to high phenotypic variability within progenies, while PH and TSW expressed low variability. Data confirm possibility of used the traits number of pods per inflorescence and number of seeds per pod as criteria of germplasm selection for seed yield improvement.

Key words*: alfalfa, Medicago sativa, progenies, seed yield, variability.*

INTRODUCTION

Cultivated alfalfa (*Medicago sativa* L., 2n = 4x = 32) is an allogamous, open pollinated auto-tetraploid species with polysomic inheritance (Barnes et al., 1988; Zhu et al., 2005).

Alfalfa is widely known as the "Queen of the forages" because its ability to consistently produce high forage yield, excellent nutritional quality, and high adaptability to different climatic conditions (Tesfaye et al., 2006). It is one of the most important forage legumes in the world as major source of protein for livestock. Alfalfa ability to fix atmospheric nitrogen, as well as to improving soil structure with its long root system established it as the crop with the greatest contribution and excellent basis for sustainable agricultural systems (Tesfaye et al., 2006; Bouton, 2012; Naydenova et al., 2022).

Therefore, alfalfa has always been considered the most valuable forage legume in Bulgarian agriculture and continues to be even now. The leading role of alfalfa imposes the need for breeding new varieties that can establish their genetic potential for high forage and seed productivity in different environments, producing stable yields for several years.

The major targets of alfalfa breeding programs have been improving forage quality and maintaining high yield potential and good adaptability to biotic and abiotic stresses, while seed yield is considered to be of secondary importance (Annicchiarico et al., 2015).

The seed is the carrier of the genetic structure of the crops and the commercial success of an agronomically superior variety in forage legumes depends not only on their forage attributes, but also on their ability to produce seed (Torricelli et al., 2007; Boelt et al., 2015).

Low seed-producing ability is a special problem in some alfalfa varieties. Seed yield is a genetically complex trait and in the perennial, insect-pollinated forage legumes it is further highly influenced by complex interaction between genetic ability of varieties, flowering and pollination biology, environmental conditions, pollinators existence and crop management factors (Boelt et al., 2015).

Alfalfa seed productivity, compared with biomass productivity, is very low and over the years progress in achieving higher seed yield is very limited (Bolanos-Aguilar et al., 2002). Hacquet and Karagic (2014) reported that over the last 30 years, seed yield has significantly increased from an average from 200 to 500 kg

ha^{-1} in France. According to Lorenzetti (1993), the theoretical seed yield potential in alfalfa calculated from the number of flowers and the number of ovules is 12 000 kg ha^{-1}, but the actual seed yield achieved under the most favorable conditions only reaches 4% of this seed yield potential. The results obtained in a study of seed productivity in perennial legumes showed the coefficient of seed productivity varied from 8 to 30% in alfalfa species (Kolyasnikova, 2015).

There are a number of studies carried out to assess the influence of genetic factors, environmental conditions and the crop management on the components of productivity, respectively on the seed yield and seed quality (Čupić et al., 2005; Andjelkovic et al., 2010; Stanisavljevic et al., 2012; Abd El-Naby et al., 2016; Chen et al., 2016; Terzic et al., 2016; Bozhanska, 2017; Pajcin et al., 2020; Marinova, 2021). Tlahig et al. (2017) revealed that plant characteristics that increase pollination efficiency and seed yield have to be well understood and determined with a higher priority during breeding programs.

A prerequisite for effective selection and building an efficient breeding program is determining the extent of traits variability both within and among breeding materials (Ibrahim et al., 2014; Song et al., 2015). Annicchiarico et al. (2015) indicated that alfalfa breeding for seed yield depends on seed yield per plant (highly related to seed yield per inflorescence) which displays narrow-sense heritability of around 0.5. Moreover, seed yield is correlated with number of seeds per inflorescence, inflorescence length and pod number (Boelt et al., 2015). Liatukiene et al. (2009) suggested evaluation of flowers number per inflorescence as positively seed yield influencing trait for selecting of highly yielding populations and as the criteria for rejecting low yielding genotypes among the initial breeding material. According El-Hifny et al. (2019) seed yield per plant of alfalfa could be generally a function of number of pods per plant x number of seeds per pod x 1000-seed weight. On the other hand, studies of Bolanos-Aguilar et al. (2002) and Annicchiarico et al. (2013) indicated that genetic improvement for seed yield and forage yield at seed harvest stage are not antagonistic and displayed high (variety × environment) interaction.

The objective of present study was to assess seed yield and some agronomic, morphological and generative yield-related traits of alfalfa clonal progenies and to establish traits phenotypic variability within progenies under conditions of controlled, free pollination (polycross nursery), with a view breeding varieties with improved seed productivity.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out in the Experimental field at the Institute of Agriculture and Seed Science "Obraztsov Chiflik" – Rousse during the period of 2014-2016.

On February 2014 eleven alfalfa clonal progenies were developed by vegetative propagation of partly inbred (S_1) superior individual plants (genotypes) 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 a polycross nursery in the Experimental field at the end of April.

The Experimental field is located at 43°48^{l} N latitude 26°02^{l} E longitude and altitude 152 m. The soil type of experimental site was leached chernozem, located on sandy clay. Active soil fertility was characterized by good potassium (33.17 mg 100 g^{-1} soil), insufficient nitrogen (16.84 mg 1 000 g^{-1} soil) and poor phosphorus (6.15 mg 100 g^{-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).

The field experiment (polycross nursery) was arranged in a randomized complete block design with four replications. In each replication the plants, originated from rooted cuttings, for every clonal progeny were planted in two 5-meter long rows, at spaced 50 cm apart in row and inter-row spacing 50 cm. Therefore, each replicate included a total of 220 plants (20 plants for each progeny). After planting, the plants were immediately watered. For good rooting on the field the plants were watered total five times in 6-7 days intervals.

During growing seasons, the necessary crop management was performed.

Data collection and statistical analysis

In the three consecutive alfalfa growing seasons the following agronomic, morphological and generative characteristics were evaluated on individual plant basis: seed yield per plant (PSY, g), plant height (PH, cm), generative stem number per plant (GSNP), inflorescence number per stem (INS), pod number per inflorescence (PNI), seed number per pod (SNP) and 1000-seed weight (TSW, g). The traits were evaluated from the first alfalfa regrowth in the first year and the second regrowth in the second and third years.

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

At seed maturity, ten generative stems for each clonal progeny were randomly 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 inflorescence 10 pods were randomly selected. These pods were 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 means of seed yield per plant and 1000-seed weight were determined.

Experimental data were processed by the One-way analysis of variance (ANOVA). The significance of differences among clonal progenies was detected by LSD test at 0.01% confidence level. The phenotypic variation of traits within progenies was also determined. The degree of variation of traits was determined through phenotypic coefficient of variation (PCV). According to the scale of Mamaev (1973): up to 7% - very low, from 7.1 to 12% - low, from 12.1 to 20% - moderate, from 20.1 to 40% - high; over 40% - very high. Principal component analysis (PCA) was applied to identify the traits that were the main source of the variability. The STATGRAPHICS PLUS software was used.

Meteorological conditions during study

Meteorological conditions during the growing seasons in 2014, 2015 and 2016 are presented in Figure 1. For the study period significant differences in both the temperature sums and amount of rainfall and its distribution by months and years were observed. During the year of alfalfa plants establishment (March-August of 2014), the total monthly rainfall in all months was close to the long-term average (LTA) (1896-2005) with slight deviations, except for May, when was extremely rainy (166.7 mm), and amount of rainfall was significantly more compare to the LTA (66.1 mm). In April, May, June and July of the second alfalfa growing season there was significantly less rainfall than the LTA, while in August significantly more rainfall compared to the LTA was recorded. During 2016, in all months, a higher or similar amount of rainfall was recorded compare to the LTA, with the largest rainfall deficit in July (2.2 mm).

The total amount of rainfall in the first and second growing seasons 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 rainfall (375.4 mm) recorded was below than in the previous two years of study and close to 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). During the year of alfalfa establishment air temperature in all months were close to the LTA. In the second experimental 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 for growing season was recorded in 2016 (18.05°C), with mean air temperatures in April (14.6°C), June (22°C) and July (24.8°C) significantly higher than the LTA (11.4°C for April, 20.2°C for June and 22.5°C for July).

Figure 1. Meteorological data during the study period (2014-2016) and long-term average (LTA) 1896-2005

RESULTS AND DISCUSSIONS

Data of analysis of variance at evaluation of clonal progenies showed a different degree of phenotypic expression of seed yield per plant and the seed yield related traits both among clonal progenies and during study period.

The lowest means for all studied traits, with some exceptions, were ascertained in the first year. This is likely because the fact, that data in the first year were collected to plants originated from rooted cuttings after their field transplantation.

The values presented in Table 1 shown that seed yield per plant ranged from 3.72 (JM13) to 2.1 g plant^{-1} (PM65) in the year of alfalfa plants establishment and indicated significant differences at P≤0.01 among clonal progenies.

Table 1. Seed yield in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Seed yield, g plant^{-1}					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	2.88 c	102.89	3.91 cde	110.88	2.7 a	112.50
SL89	2.59 de	92.53	2.9 fg	82.24	2.5 ab	104.17
SL92	2.76 cd	98.60	2.84 fg	80.54	1.9 c	79.17
SL99	2.41 ef	86.10	2.52 g	71.46	2.6 ab	108.33
PM30	3.60 a	128.61	4.16 a	117.97	2.8 a	116.67
PM18	2.80 cd	100.03	4.01 abcd	113.71	1.8 c	75.00
PM49	2.62 de	93.60	3.23 efg	91.60	2.6 ab	108.33
PM65	2.10 g	75.02	3.66 cde	89.61	2.1 bc	87.50
GM14	2.18 fg	77.88	3.46 def	98.12	2.6 ab	108.33
GM27	3.13 b	111.82	4.50 ab	127.61	2.3 abc	95.83
JM13	3.72 a	132.90	4.10 abc	116.27	2.5 ab	104.17
Mean	2.80		3.53		2.40	
LSD 99%	0.23		0.84		0.56	

*The different letters in same column indicate significant differences at P≤0.01

The differentiation of the progenies was also considerable during the second growing season. Progeny GM27 exhibited the highest ability in seed producing (4.5 g), followed by PM30 (4.16 g) and JM13 (4.1 g). Data indicated that the magnitude of differences among clonal progenies decreased in third year of study. It can be noted that PM30 (2.8 g) distinguished with the highest degree of phenotypic expression of the trait, while PM18 was with the lowest one (1.8 g). The results of present study corresponding to the values reported in

the literature. Balanos-Aguillar et al. (2000) reported seed yield per plant ranged from 0.30 to 30.75 g in the study of 214 genotypes, and Torricelli et al. (2007) from 0.36 to 32.53 g PSY. The reported means shown that in the first growing season JM13 had the highest plants (63.1 cm), followed by GM27 and PM30 with stems length of 61.0 and 60.3 cm, respectively (Table 2). The excesses over other progenies were considerable (P≤0.01). PM49 was characterized by the lowest plants (49 cm).

Table 2. Plant height in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Plant height, cm					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	60.0 b	109.47	74.0 b	95.27	66.2 a	111.30
SL89	54.0 cd	98.52	62.6 d	91.92	60.0 c	100.87
SL92	48.1 fg	87.76	60.4 e	85.38	53.1 d	89.27
SL99	47.3 g	86.30	56.1 g	117.33	63.9 b	107.43
PM30	60.3 b	110.02	77.1 a	104.09	54.1 d	90.95
PM18	55.0 c	100.35	68.4 c	86.14	50.0 e	84.06
PM49	49.0 f	89.40	56.6 fg	97.70	59.5 c	100.03
PM65	52.4 e	95.60	64.2 d	88.57	52.4 d	88.10
GM14	52.7 de	96.15	58.2 f	110.79	66.2 a	111.30
GM27	61.0 b	111.29	72.8 b	110.18	67.9 a	114.16
JM13	63.1 a	115.12	72.4 b	95.27	61.0 c	102.56
Mean	54.81		65.71		59.48	
LSD 99%	1.55		1.87		1.85	

*The different letters in same column indicate significant differences at P≤0.01

In the second year PM30 ranked first, with plant height of 77.1 cm. The difference versus other progenies was statistically significant. The high phenotypic expression of trait of SL83, GM27 and JM13 was kept during the year. There were observed some deviations from outlined trends in 2016. This finding was the most clearly expressed in PM30 progeny, which exhibited lower potential than both some progenies and this one expressed in the two previous growing seasons.

Concerning generative stem number per plant, the values obtained were in wide range, from 8.8 in the year of alfalfa plants establishment to 79.6 in second growing season (Table 3).

Table 3. Generative stem number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Generative stem number per plant					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	8.8 bc	94.62	58.9 de	94.17	38.1 cd	98.06
SL89	7.6 c	81.72	52.5 e	83.94	29.3 g	75.41
SL92	9.7 ab	104.30	66.7 bc	106.64	35.8 de	92.14
SL99	9.7 ab	104.30	57.9 de	92.57	33.2 ef	85.45
PM30	10.1 ab	108.60	69.1 b	110.48	44.9 b	115.56
PM18	9.6 ab	103.23	57.2 de	91.45	40.5 c	104.23
PM49	8.8 bc	94.62	61.7 cd	98.65	38.4 cd	98.83
PM65	9.9 ab	106.45	67.1 bc	107.28	47.7 b	122.77
GM14	10.4 a	111.83	79.6 a	127.27	52.4 a	134.86
GM27	9.7 ab	104.30	61.7 cd	98.65	35.5 de	91.37
JM13	8.0 c	86.02	55.6 de	88.90	31.6 fg	81.33
Mean	9.30		62.55		38.85	38.85
LSD 99%	1.49		6.51		3.75	

*The different letters in same column indicate significant differences at P≤0.01

In 2014 GM27 and PM30 had the largest number of generative stem 10.4 and 10.1, respectively, while GM14 stood out with the smallest one (7.6). Data of analysis of variance indicated considerable differences between clonal progenies in the second year and they were classified into 5 homogenous groups. In the year GM14 exhibited the highest trait phenotypic expression, significantly exceeding the other progenies. The results in 2016 were in

line to that in 2015, whit some exceptions. It can be note GM14, PM65 and PM30 distinguished with the highest ability in stem producing across years.

Based on the analysis of variance, it was establish considerable differences in inflorescence number per stem among clonal progenies in all years (Table 4). In first growing season the highest value was found for PM30 (8.4) and the lowest one for GM14 (3.8). Progeny JM13 ranked second at reported value of 8.3 INS. The trait values for the both years 2015 and 2016 clearly confirmed the priority of PM30.

Table 4. Inflorescence number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Inflorescence number per stem					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	5.3 d	84.49	15.2 a	121.42	10.3 cd	100.27
SL89	7.1 bc	113.19	13.0 bcd	103.85	9.9 def	96.37
SL92	4.2 e	66.96	12.2 cde	97.46	11.1 c	108.05
SL99	6.5 c	103.62	9.3 g	74.29	9.1 fg	88.58
PM30	8.4 a	133.91	14.1 ab	112.64	13.4 a	130.44
PM18	5.4 d	86.09	13.7 b	109.44	9.4 def	91.50
PM49	7.7 ab	122.75	12.1 def	96.66	8.1 g	78.85
PM65	7.5 abc	119.57	11.0 f	87.87	9.2 ef	89.56
GM14	3.8 e	60.58	12.5 cd	99.85	10.1 cdef	98.32
GM27	4.8 de	76.52	11.3 ef	90.27	12.2 b	118.76
JM13	8.3 a	132.32	13.3 bc	106.25	10.2 cde	99.29
Mean	6.27		12.52		10.27	
LSD 99%	1.05		1.12		1.1	

*The different letters in same column indicate significant differences at P≤0.01

The means across years outlined trend for high ability for stems producing with a greater number of inflorescences, likewise at JM13, SL83 and SL89. The results of present study are with accordance to data reported by Đjurović et al. (2007), who obtained 9.37 mean inflorescences number per stem. Bekovic et al. (2016) reported that INS ranged from 9.84 to 14.39 at inter-row spacing of 20 and 60 cm, respectively.

The means presented in Table 5 shown that in the establishment year the highest pod number per inflorescence was determined for PM30 (6.1), followed by JM13 (5.8) and SL83 (5.6).

Table 5. Pod number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Pod number per inflorescence					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	5.6 ab	109.61	9.6 abcd	103.83	6.4 bc	106.99
SL89	4.1 e	80.25	8.8 de	95.18	5.4 de	90.27
SL92	4.4 de	86.12	8.0 ef	86.53	5.1 e	85.26
SL99	5.0 bcd	97.86	7.4 f	80.04	5.0 e	83.59
PM30	6.1 a	119.40	10.6 a	114.65	7.5 a	125.38
PM18	5.5 abc	107.65	10.4 ab	112.49	5.7 cde	95.29
PM49	5.4 abc	105.69	8.9 cde	96.26	6.2 bcd	103.65
PM65	3.9 e	76.33	9.7 bcd	104.92	7.1 ab	118.69
GM14	4.7 cde	91.99	9.5 abcd	102.75	5.9 cde	98.63
GM27	5.7 ab	111.57	9.9 abc	107.08	5.8 cde	96.96
JM13	5.8 ab	113.52	8.9 cde	96.26	5.7 cde	95.29
Mean	5.11		9.25		5.78	
LSD 99%	0.84		1.07		0.98	

*The different letters in same column indicate significant differences at P≤0.01

Significant differences between clonal progenies were found in the second year, when they were classified into 7 homogenous groups. The maximal and minimal trait values reported were 10.6 and 8.0 PNI for PM30 and SL92, respectively. During 2016 progenies PM30 and PM65 ranked first and second, respectively. In the same year for two clonal progenies (PM18 and GM27) were observed deviations in the expressed potential for the trait versus

exhibited potential in previous two years. It is noticeable SL83 and PM49 exceeded mean for clonal progenies by 6.99 and 3.65%. Trait values for the study period confirm the superiority of PM30. The results across years of study are in line with those found in previous studies. Đjurović et al. (2007) found mean 7.31 number of pods per inflorescence and Bekovic et al. (2016) reported that PNI ranged from 5.96 to 8.14 at inter-row spacing of 20 and 60 cm, respectively.

According to Bodzon (2016), seed yield per plant significantly depends on the pod number per inflorescence and the seed number per pod. Means of the trait seed number per pod over the three years of study are presented in Table 6.

Table 6. Seed number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Seed number per pod					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	4.0 ab	122.11	3.9 abc	115.22	3.2 a	123.85
SL89	2.7 efg	82.42	2.7 efg	79.77	2.1 e	81.27
SL92	2.9 def	88.53	3.1 def	91.58	2.1 e	81.27
SL99	3.1 de	94.63	2.9 efg	85.68	2.9 abcd	112.24
PM30	4.2 ab	128.21	4.3 ab	127.04	3.0 abc	116.11
PM18	3.3 cd	100.74	3.7 bcd	109.31	2.3 e	89.01
PM49	3.0 def	91.58	3.3 cde	97.49	2.2 e	86.00
PM65	2.3 g	71.23	2.3 g	68.93	2.4 de	92.89
GM14	3.7 bc	112.95	2.5 fg	73.86	3.1 ab	119.98
GM27	2.5 fg	76.32	4.1 ab	121.13	2.6 bcd	100.63
JM13	4.3 a	131.27	4.4 a	129.99	2.5 cde	96.76
Mean	3.28		3.37		2.58	
LSD 99%	0.53		0.61		0.56	

*The different letters in same column indicate significant differences at P≤0.01

The result obtained shown a wide range of values (from 4.3 for JM13 to 2.3 for PM65) among clonal progenies in establishment year. In the second growing season, the reported values for the trait were in the same range. The clonal progenies were classified into 7 homogeneous groups, with significant differences between them. The highest seed number per pod of 4.4 and 4.3 were reported for JM13 and PM30, respectively, while PM65 had the lowest values for the trait (2.3 in both years). The range of values found is consistent with those reported by Bekovic et al. (2016). According the authors, depending on the inter-row spacing SNP ranged from 3.64 (20 cm) to 4.18 (60 cm). It can be note that the high trait phenotypic expression at SL83 and PM30 was kept in 2016. There were established deviations from outlined trends for some clonal progenies. Reported means across years outlined trend for high potential of PM30, JM13 and SL83.

Concerning 1000-seed weigh, the values presented indicated the clonal progenies exhibited different potential across the years (Table 7). Data of analysis of variance revealed highly significant differences (P≤0.01) among progenies in first growing season, when they were distributed in 8 homogeneous groups. In the year SL99 ranked first with TSW of 2.06 g, whereas PM65 had the smallest one (1.47 g). The values in second growing season were in the range from 2.26 g for GM27 to 1.75 g for SL99. During 2016 JM13 distinguished with the highest TSW (1.92 g). The lowest trait value in GM14 was ascertained. The trait values in present study are in line with these reported by Stanisavljević et al. (2012). Iannucci et al. (2002) reported significant differences in TSW among five alfalfa varieties, at the highest mean (2.45 g) for Iside variety. The clear expressed differences in the degree of traits phenotypic expression between the clonal progenies and growing seasons clearly indicate that genotype and environmental factors had a strong influence on observed traits.

Table 7. 1000-seed weight in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	1000-seed weight, g					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	1.92 bc	107.59	2.11 b	107.90	1.74 b	102.74
SL89	1.54 gh	86.30	1.82 de	93.07	1.72 b	101.56
SL92	1.81 de	101.43	1.84 de	94.10	1.60 de	94.47
SL99	2.06 a	115.44	1.75 e	89.49	1.70 bc	100.38
PM30	1.72 f	96.38	2.09 b	106.88	1.87 a	110.41
PM18	1.88 cd	105.35	1.88 cd	96.14	1.61 de	95.06
PM49	1.74 ef	97.50	1.92 cd	98.19	1.65 cd	97.42
PM65	1.47 h	82.37	1.98 c	101.26	1.60 de	94.47
GM14	1.61 g	90.22	1.91 cd	97.68	1.58 e	93.29
GM27	1.98 ab	110.95	2.26 a	115.57	1.64 cde	96.83
JM13	1.9 bc	106.47	1.95 c	99.72	1.92 a	113.37
Mean	1.78		1.96		1.69	
LSD 99%	0. 08		0.11		0.08	

*The different letters in same column indicate significant differences at P≤0.01

It is evident that the degree of phenotypic expression of all traits was the highest in the second year and the lowest in first one, with some exception. The lower values for SYP, SNP, TSY during 2016 compared with those reported in year of clonal progenies establishment can be explain by the fact that the flowering-seed set-seeds ripening period (July-August) was characterized as less favorable for seed production than in 2014 and 2015. A number of authors also consider that variation in alfalfa seed yield is primarily because weather conditions during the alfalfa growing season for seed, especially in the flowering and seed maturing stages (May-August) and the total amount and distribution of rainfall were the most important (Bolanos-Aguilar et al., 2002; Karagić et al., 2010).

In conclusion, it can be noted that five progenies (PM30, JM13, GM27, SL83 and PM18) were identified as superior in most of analyzed traits and they represent valuable breeding materials with the potential to develop high seed yielding varieties.

The phenotypic coefficients of variation for analyzed traits, used as the measure of within-progeny variability are presented in Tables 8-9. Data showed different magnitude of variability within clonal progenies for all agronomic, morphological and generative traits across years.

Table 8. Phenotypic variability of traits seed yield, plant height, generative stem number and inflorescence number within eleven alfalfa clonal progenies

Clonal progenies	Phenotypic coefficients of variation within progeny (PCV, %)											
	Seed yield per plant			*Plant height*			*Generative stem number per plant*			*Inflorescence number per stem*		
	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
SL83	9.79	14.56	25.00	2.48	2.01	2.45	12.90	9.35	6.83	17.90	6.79	9.21
SL89	7.79	22.59	28.26	2.62	2.16	2.22	11.10	8.53	7.55	14.01	9.59	7.45
SL92	6.44	28.17	16.64	2.07	2.37	3.00	13.79	8.60	6.94	10.04	8.47	7.89
SL99	6.32	28.28	17.67	1.74	2.72	2.50	12.90	8.94	9.82	8.11	7.26	9.62
PM30	5.07	16.43	12.49	2.35	2.48	3.07	12.74	9.06	9.00	12.80	7.05	10.07
PM18	7.72	16.67	19.42	2.97	3.02	2.98	11.20	8.91	8.41	15.62	6.92	7.44
PM49	7.59	24.65	17.67	2.15	2.79	2.77	10.44	7.33	7.49	10.69	8.22	9.11
PM65	6.46	18.24	21.88	2.87	2.41	2.58	11.12	9.95	6.41	16.92	7.42	6.87
GM14	9.62	20.20	17.67	2.69	2.54	3.09	15.17	8.67	8.78	20.76	4.22	8.67
GM27	5.65	18.89	18.33	2.56	1.69	2.64	19.47	9.82	9.03	16.43	9.37	9.31
JM13	5.78	18.00	18.86	1.39	2.37	1.89	15.59	6.52	8.06	12.76	6.19	11.13

It was found seed yield per plant exhibited very low or low phenotypic variability (PCV from 9.79% for SL83 to 5.07% for PM30) in the establishment year (Table 8).

In second growing season the PCV values determined the trait variability as very high in five progenies (PCV from 28.28% for SL99 to 20.2% for GM14). It was established during

third year high degree of variation in SL89 (28.26%), SL83 (25%) and PM (21.88%). In both years in other progenies seed yield varied moderately. The very high PCV values for seed weight per plant (80.7%) within 99 alfalfa accessions were reported by Pelican et al. (2016).

The values of phenotypic coefficients of variation, presented in Table 8 indicated very low variability of plant height (PCV<7%) within all progenies in all years. Regarding generative stem number and inflorescence number, data showed the both traits exhibited moderate variability within progenies, with some exceptions in first year. It was observed deviations in the degree of trait variation in the next growing seasons. Data indicated that both traits varied mostly low. The calculated PCV revealed significant differences in variation degree of INS within the clonal progeny GM14 across years, from very high (PCV = 20.76%) in first growing season to very low (PCV = 4.22%) in second one. According to Bodzon (2016), the variability of pod number per inflorescence and seed number per pod determines about 60% of total variability of seed yield per plant.

The phenotypic coefficients of variation for generative traits pod number per inflorescence, seed number per pod and 1000-seed weight are presented in Table 9. Comparing data over the years, it can be seen the magnitude of phenotypic variability of pod number per inflorescence within clonal progenies is determines as moderate in 2014 and 2016, except SL92, PM18 and SL83. In the second growing season the trait exhibited low variability within all progenies.

Data showed high phenotypic variation (21-21.52%) of seed number per pod within PM65 progeny across all years. High trait variability was also evident for four progenies (PM18, GM14, GM27 and JM13) but only in one growing season. Seed number exhibited moderate or low variability within other progenies in all years. The values of Phenotypic coefficients of variation indicated very low variability of the trait 1000-seeds weight (PCV<7%) across years. Data obtained correspond with the results of Stanisavljević et al. (2012), who found CV of 3.2 and 5.9% for the trait at experiment conducted in two locations with different environmental conditions. PCV values less than 12% for TSW were reported by Iannucci et al. (2002). According to the authors, the low variability is due to the seed size in legumes depends mainly on genetic factors. Based on the obtained results, it can be noted that pod number per inflorescence and seed number per pod are reliable and successful selection criteria for increasing the seed yield of alfalfa because the relatively good variability.

Table 9. Phenotypic variability of traits pod number, seed number, and 1000-seed weight within eleven alfalfa clonal progenies

Clonal progenies	Phenotypic coefficients of variability within progeny (PCV, %)								
	Pod number per inflorescence			*Seed number per pod*			*1000-seed weight*		
	2014	2015	2016	2014	2015	2016	2014	2015	2016
SL83	12.49	10.06	10.93	11.79	14.56	13.18	3.54	2.02	2.7
SL89	18.00	8.96	15.62	17.89	17.89	15.06	4.96	4.12	2.5
SL92	11.74	10.21	14.47	10.90	10.20	15.06	4.06	4.64	1.9
SL99	16.33	11.40	15.20	10.20	19.57	19.57	4.38	3.16	2.6
PM30	12.10	9.11	12.96	10.04	11.23	15.71	3.16	8.87	2.8
PM18	9.58	11.29	16.64	14.64	13.06	21.07	2.16	2.16	1.8
PM49	12.95	9.84	14.82	15.71	14.64	19.84	2.63	3.73	2.6
PM65	18.92	9.78	12.33	21.00	21.12	21.52	4.69	4.69	2.1
GM14	14.36	7.44	12.51	13.06	21.08	18.31	3.99	3.38	2.6
GM27	14.44	11.12	14.15	21.08	13.85	19.86	4.76	2.79	2.3
JM13	13.60	8.29	12.60	11.23	15.89	21.08	3.66	3.66	2.5

The results of Principal component analysis are presented in Table 10. Data show that three main components PC 1, PC 2 and PC 3 explain 82.3% of the total variation of all studied traits. PC1 accounted for 37.85% of total variation. According to the corresponding eigenvector values, it was mostly equated with seed yield (0.625) and seed number per pod (0.521). The

second component (PC2) accounted for 25.77% of total variation and was strongly, positively associated with two traits: generative stem number (0.636) and pod number per inflorescence (0.530). PC3 accounted for 14.34% of total variation and was mostly explained by TSW (0.687) and INS (-0.598).

Table 10. Principal component analysis (PCA) of seed yield and yield-related traits

Traits	Component		
	PC1	PC2	PC3
SYP	0.625	-0.213	0.074
INS	0.303	0.115	-0.598
PNI	0.322	0.530	-0.210
SNP	0.521	0.060	0.311
TSW	0.361	-0.231	0.687
PH	0.317	-0.449	0.465
GSNP	0.154	0.636	0.354
Eigenvector value	2.65	1.80	1.31
% of variance	37.85	25.77	18.65
Comulative percentage	37.85	63.62	82.26

The results of the PC analysis presented in Table 11 showed that the clonal progenies are related differently to the three main components. The first main component included five progenies four of which (PM30, GM27, JM13 and GM14) were positively associated with PC 1. The negative values of PC 1 were found for PM18 (-2.107) and SL92 (-2.079). It was established that clonal progeny PM65 was positively connected to PC 2 (2.136), and SL99 (-1.676) and SL89 (-1.514) negatively. The third main component was represented by three progenies as GM14 (2.181) was positively connected to PC 3, and PM30 (-1.864) and JM13 (-1.401) negatively.

Table 11. Explained significant components by varieties

Clonal progenies	Component		
	PC 1	PC 2	PC 3
SL83	0.319	-0.451	0.639
SL89	-0,574	-1.514	-0.666
SL92	-2.079	0.008	-0.606
SL99	0.199	-1.676	0.883
PM30	2.159	1.246	-1.864
PM18	-2.107	1.075	-0.223
PM49	-0.671	-0.157	0.489
PM65	-0.926	2.136	0.188
GM14	1.816	0.659	2.181
GM27	1.918	-0.764	0.280
JM13	1.884	-0.932	-1.401

CONCLUSIONS

There were statistically significant differences among clonal progenies for all studied traits. Based on traits values five progenies (PM30, JM13, GM27, SL83 and PM18) were identified as very valuable genetic source, with the potential to develop high seed yielding varieties. A tendency for a high ability of producing generative stems and seeds per pod for GM14 was outlined.

The clear expressed differences in the degree of traits phenotypic expression between the clonal progenies and growing seasons, determined in the present study, confirm the significant influence of a genotype and its variable response to the changes in the meteorological conditions across years.

It was established seed yield, pod number per inflorescence and seed number per pod exhibited moderate to high phenotypic variability within progenies, while plant height and 1000-seed weight expressed the lowest variability. Data confirm possibility of using the number of pods per inflorescence and number of seeds per pod as criteria of valuable germplasm selection at breeding of high seed yield varieties.

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