Egypt. J. Plant Breed. 24(3):617–630(2020) SELECTION OF SOME ALFALFA POPULATIOS FOR FORAGE YIELD AND QUALITY USING MODIFIED MASS SELECTION

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ABSTRACT

Modified mass selection method was used to study the gain from selection and compare between the base population and the half-sib families of alfalfa in 2016/2017 season. Selection was practiced among half-sib families for the best eight families (10% intensity of selection). Seeds of selected half-sib families and both modified mass selection cycles C_1 and C_2 were evaluated for forage yield, some yield components and protein percentage. C1 significantly dominated over the base population as well as C₂ significantly dominated the C₁for plant height, leaf/stem ratio and forage yield. The realized gains after the two modified mass selection cycles were 6.89, 25.56, 15.87, 19.47 and 0.87% for plant height, leaf/stem ratio, fresh forage yield, dry forage yield, and protein percentage, respectively, over the base population. We may suggest from the results that modified mass selection resulted in a great improvement of forage yield, plant height and leaf/stem ratio, but in small gain for protein percentage. The values of genotypic coefficient of variation (GCV%) and phenotypic coefficient of variation(PCV%) were 5.28% and 7.03% for plant height, 1.30 and 1.79% for leaf/stem ratio, 7.76and 10.26% for fresh forage yield, 8.42 and 11.23% for dry forage yield and 5.53 and 6.55 % for protein percentage, respectively. Broad-sense heritability values for leaf/stem ratio and protein percentage were 72.94% and 71.25% which were higher than those for fresh and dry forage yields and plant height, at 56.47%, 57.07% and 56.15%, respectively.

Key words: Medicago sativa L., Base population, Modified mass selection, Selection cycles, Realized selection gains, Heritability, Genotypic and phenotypic coefficient of variability.

INTRODUCTION

Alfalfa is the most important forage crop worldwide. It grows as a green forage for hay, silage or pasture production. Alfalfa plants are characterized with high nutritive value and have a wide adaptability range (Li and Brummer 2012).The presence of large genetic variability in alfalfa leads to create various range of varieties adapted to specific environments and management systems. In order to make selection in alfalfa more efficient, breeders should carefully design and establish breeding programs which will lead to maximize the expected yield. For inclusion in varieties, individual parent plants of alfalfa or other open pollinated perennial forage species are evaluated as clonal materials or according to the performance of their half-sib(HS) progenies, S₁ progenies or, more rarely, full-sib progenies (Poehlman and Sleeper 1995, Casler and Brummer 2008 and Posselt 2010).

Alfalfa breeding programs have long placed minor emphasis on forage quality, as confirmed by negligible rates of genetic gain for its component traits (Annicchiarico*etal*2015). There is limited and somewhat inconsistent information on the possibility to jointly improve leaf/stem ratio and forage yield of alfalfa, despite its selection directed towards increasing frequencies of favorable alleles has improved vigor in alfalfa populations at several levels of inbreeding(Gallais1984 and El-Nahrawy and Bingham 1989) realized gains over the base population were 17.7 and 25.2% for fresh forage yield, 18.7 and 24.8% for dry forage yield and 25.4% for protein yield in the first and second cycles of recurrent selection, respectively (Bakheit and El Nahrawy 1997). The genetic advance of selection for the studied traits ranged from 3.3 to 20.3% (Abdel-Galil 2007). The realized gains after the two mass selection cycles were 14.94, 14.00 and 11.34% for fresh and dry forage yield and crude protein(%), respectively over the base population. Gains from family selection as percentage of base population were 17.24, 16.00and 16.49% for these traits in the same order (Bakheitet al 2011). Omara and Hussein (1982) reported that family selection was more rewarding than mass selection for forage yield where the response was 8.66% over the base family mean after one cycle of selection. A significant response to mass selection was obtained after the first cycle of selection which amounted to 20.58 and 5.11% over the base population mean in two successive yield trails. Bakheit (1985) reported that the realized gains of the first and second cycles of mass selection, for the fresh forage yield were 8.43 and 10.7% of the base population, respectively. Bakheit (1986) showed that the expected genetic advance for the best six accessions was 16.10% for plant height, 17.50% for seasonal fresh yield, 20.60% for seasonal dry yield and 21.0% for seasonal protein yield. Bakheit and El-Nahrawy (1997) found that realized gains over the base population were 17.7 and 25.2 for fresh forage yield, 18.7 and 24.8% for dry forage yield and 18.3 and 25.4% for protein yield in the

first and second cycles of recurrent selection, respectively. Hamd Alla *et al* (2012) revealed that one cycle of mass selection for root characteristics was effective in increasing fresh forage yield/plot by 9.9 and 22.9% and dry forage by 11.9 and 22.9% overall cuts in selections from local and exotic populations, respectively (Bakheit and Ali 2013).Realized gains from C₁ and C₂of mass selection were 4.9 and 14.4% for fresh forage and 5.3 and 13.2% for dry forage yield over the base population. Bakheit *et al* (2016) found that the realized gains of the recurrent selections were 18.6, 11.7, 14.6 and 24.1% for leaf/stem ratio, fresh and dry forage and protein yields, respectively, over the base population.

The success of selection programs for forage yield and its components depend mainly on large genetic variability that has been found for morphological traits along with forage yield. The heritability of the selected traits, the nature of correlations between different characters and the intensity of selection applied are also important for the success of selection (Abdel Galil 2007, Veronesi *et al* 2010, Bakheit *et al* 2011, Hamd Alla *et al* 2012, Annicchiarico 2015 and Badawy 2017).

Abdel-Galil (2007) indicated that there was relative variation among the tested genotypes. The environmental variation ranged from 2.4 to 30.5%. The values of genotypic coefficient of variation for fresh and dry forage yields revealed relative variations among the cultivars under study which were less influenced by environment. Rajab (2010) observed that the highest phenotypic coefficient of variation value (P.C.V.) was recorded for fresh forage yield as 5.075%, 9.036% and 6.792% in the first, second and third seasons. Badawy (2017) estimated genetic parameters for fresh yield, dry yield, plant height and dry leaf/stem ratio. P.C.V. values were 4.75, 4.65, 1.224 and 3.07% and G.C.V. values were 3.21, 3.72, 0.774 and 2.24%, respectively.

Badawy (2013) reported that fresh forage yield expressed the highest estimates of heritability while, the obtained values for dry forage yield were of lower magnitude. Abd El- Naby et al (2014) found that broad- sense

heritability was 0.90 and 0.84 for plant height and total fresh weight plant⁻¹, respectively. Ahmed *et al* (2015) estimated broad- sense heritability on berseem clover, the highest values obtained for fresh forage yield, dry forage yield and leaf/stem ratio were 98.23, 98.7 and 100% respectively. Badawy (2017) found that heritability for fresh forage yield reached 69.56%.

The objectives of this study were, i): studying the effect of two cycles of selection for forage yield in alfalfa ecotypes. ii): estimating genetic variability among selected genotypes, heritability and genetic improvement for forage yield, its components and quality, and iii): identifying the promising genotypes and their use in further breeding programs.

MATERIALS AND METHODS

Field trials were conducted in Nubaria Agricultural Research Station (North West of Nile delta, Egypt) The first trial was conducted to evaluate five hundred and twenty five populations during two years from 2009 to2011. The seed lots were collected from Dakhla, Kharga, Elbharei and Siwa oasis and one French population (non-winter dormant). In 2011, twenty five populations (5% intensity of selection) were selected depending on stability of fresh and dry forage yields and quality using modified mass selection method. Equal seeds from each selected genotype were bulked to form C₁seed. The same time, seeds of the twenty- five genotypes were sown as half-sib families. Each family consisted of 200 plants. Cultural practices were applied at optimum levels to realize high alfalfa productivity. In 2011-2014 selected entries (25 populations) were evaluated and selection was practiced among half-sib families for the best 30% families (Eight families). The best twenty fresh forage producing plants from the best eight families were selected and transplanted in an isolated plot to avoid cross pollination with unselected plants. Seeds were harvested from each selected plant separately and mixed to from the first cycle of family selection (C_2) .

The second trial was sown on Oct. 10th, 2015. A randomized complete block design with four replicates was used. The best plants in each plot based on fresh forage weight (10% selection intensity) were selected. Selections were transplanted in an isolated plot to avoid pollination with unselected plants. Seeds were harvested by hand at seed maturity stage. In 2014/2015 season, equal parts of seeds from each of the selected plants were bulked to form the first cycle of modified mass selection (MMC₁). Similar cultural practices, selection procedure and intensity of selection were adopted as described previously to obtain MMC₂. In 2016/2017 season, to evaluate the response to selection, the base population and two modified mass selection cycles (MMC₁ and MM C_2) were tested in a randomized complete block design with four replications. Plot size was 3.0 x4.0 m with rows 20 cm apart. The Seeding rate was 48 kgha⁻¹. Seeds were inoculated with Rhizobium melolitii prior to seeding. Starter dose of nitrogen (48 kgha⁻¹) was applied directly after the full establishment. A base dose of Super phosphate $(15.5\%P_2O_5)$ at the rate of 360 kgha⁻¹ was applied before sowing. 120 kgha⁻¹ of Potassium sulphate $(46\% K_2 O)$ was applied at three equal doses, yearly. Data were recorded at cutting. Nine cuts were taken from each population. The first cut taken at 20 February after 85 days from sowing (15 November 2016). The other cuts were taken at 30-35 day intervals, and the last cut was taken at 26 November 2017. Ten guarded plants were randomly dogged out from each plot to study plant height (cm), leaf/stem ratio (LSR). The plants in each plot were harvested and weighed to determine the forage yield (kg/plot)and a representative sample from each plot was dried at 65°Ctillconstant weight to determine dry forage yield .Crude protein percentage (%) was determined using standard method (A.O.A.C 1990).

Statistical analysis for all the studied traits were performed according to El-Nakhlawy (2010) using ANOVA procedure of SAS software (2014). The realized gains from selection measured as the

deviation percentage of the overall cycle mean from the base population (Falconer 1989) as follows: Realized gain (%) relative to base population = $C1-C0/C0 \times 100$.

The genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) were calculated according to Burton (1952)

as follows = G.C.V.= σ_G /grand mean x100

P.C.V. = σ_p /grand mean x 100

Where; σ_G : represents genetic standard deviation.

 σ_p : represents the phenotypic standard deviation.

Heritability in broad- sense according to Johnson *et al* (1955) as follows;

 $\mathbf{H} = \sigma^2_{\rm G} / \sigma^2_{\rm p} \mathbf{x} \ \mathbf{100}$

Where: σ^2_{G} is genotypic variance and σ^2_{p} is phenotypic variance.

RESULTS AND DISCISSIONS

Evaluation of modified mass selection

Analysis of variance (Table 1) shows significant differences among the selected cycles ($p \le 0.01$) for plant height, leaf/stem ratio, fresh forage yield, dry forage yield and protein percentage.

Table 1. Analysis of variance of plant height, leaf/stem ratio, fresh and dry forage yields and protein percentage of the base population, first (C1) and second (C2) of modified mass selection.

		MS						
SOV	df	Plant height	Leaf/stem	Fresh forage	Dry forage	Protein		
		_	ratio	yield	yield	(%)		
Replicates	3	0.30	4.37	3.41	0.22	0.007		
Among cycles	2	21.26**	127.92**	20.988**	3.93**	0.037**		
Base vs Selected	1	26.04**	18.20*	7.848**	2.02**	0.073**		
$C_1 \text{ vs } C_2$	1	16.47**	255.83**	34.11**	5.83**	0,001 ns		
Error	6	0.28	2.81	1.11	0.10	0.003		

Ns: not significant at $p \le 0.05$. *, **: significant at $p \le 0.05$ and $p \le 0.01$, respectively.

Also, significant differences were found between the base population and the two cycles of modified mass selection ($p \le 0.01$) for all the studied traits. Besides, significant differences were shown between C₂ and C₁ populations ($p \le 0.01$) for all the studied traits, except protein content. The previous results are in a harmony with those obtained by Wang *et al* (1991), Bakheit and El-Nahrawy (1997), Kimbeng and Bingham (1998), Awad (2001), Bakheit *et al* (2011) and .Bakhiet and Ali (2013).

Means of plant height, leaf/stem ratio, fresh forage yield, dry forage yield and protein percentage of the base population and the selected populations are presented in Table (2).

and second (C2) of modified mass selection.									
	Means								
Generation	Plant height (cm)	Leaf/stem ratio (%)	Fresh forage yield (Kg/plot)	Dry forage yield (Kg/plot)	Protein (%)				
Base population (C ₀)	66.14	44.24	37.172	9.916	18.40				
First cycle (C ₁)	67.83	52.51	39.445	10.765	18.58				
Second cycle (C ₂)	70.70	55.55	43.072	11.847	18.56				
RLSD: (0.05)	0.91	2.90	1.82	0.55	0.09				
(0.01)	1.38	4.39	2.76	0.82	0.14				

Table 2. Means of plant height, leaf/stem ratio, fresh and dry forage yield and protein percentage of the base population, first (C_1) and second (C_2) of modified mass selection.

Means of both C_1 and C_2 were significantly greater than the base population (C_0) for all five studied alfalfa traits. Means of the C_2 were significantly higher than those of the C₁for plant height, leaf/stem ratio, fresh and dry forage yields at $p \le 0.05$. An insignificant difference was detected between C_1 and C_2 for protein percentage ($p \le 0.05$). These results indicated the positive effects of modified mass election to improve alfalfa forage yield and the other two studied yield components. These results are in agreement with those reported by Wang *et al*(1991), Bakheit and El-Nahrawy (1997), Awad (2001), Bakheit*et al* (2011) and Hamd Alla *et al* (2012).

The realized gains from selection estimated as the percentage deviation of the mean of the base population are shown in Table (3). The results indicated that, direct selection was effective in improving the studied traits after the second cycle of modified mass selection by 6.89% for plant height, 25.56% for leaf/stem ratio, and 15.87% for fresh forage yield, 19.47% for dry forage yield and 0.87% for protein percentage. However, the realized gain from selection after C₁ cycle were 2.55, 19.58, 6.11, 18.56 and 0.98% for the previous traits, respectively.

Table 3. Realized gain (%)from modified mass selection for plant
height, leaf/stem ratio, fresh and dry forage yield and protein
percentage in the two cycles of selection measured in
percentage from the base population.

C1 2.55** 19.58** 6.11** 18.56** 0.9	Base population	Cycle of selection	Plant height (cm)	Leaf/stem ratio (%)	Fresh forage yield, (kg/plot)	Dry forage yield, (kg/plot)	Protein (%))
	C.	C1	2.55**	19.58**	6.11**	18.56**	0.98**
C ₀ C ₂ 6.89** 25.56** 15.87** 19.47** 0.8	C ₀	C ₂	6.89**	25.56**	15.87**	19.47**	0.87**

**: significant at p≤ 0.01

Evaluation of family selection

As for family selection, results of the analysis of variance (Table 4) showed significant differences among families ($p \le 0.01$) for all the studied traits, except for protein percentage. Also, the differences between the eight selected families and the base population were significant (p<0.01), except for protein percentage.

Data of means of plant height, leaf/stem ratio, fresh forage yield, dry forage yield and protein content of the base population and the eight families are presented in Table (5). As for plant height, mean values ranged from 71.40 to 75.60cm for the half-sib (H.S.) families compared with 58.83 cm for the base population. All H.S. families were significantly ($p \le 0.01$) taller than the base population. Considering leaf/stem ratio, it ranged from 52.31 to 57.88 for the H.S. families compared with 52.40 for the base population. Furthermore, six H.S. families were significantly higher than the

base population after one cycle of family selection. Only the families G.6, G.7 and G.8 were not significantly different from the base population for leaf/stem ratio.

Table 4.	Anal	ysis of v	ariance	e of pl	lant heigl	nt, leaf/stem	ratio,	fresł	n and
	dry	forage	yield	and	protein	percentage	for	the	base
	popu	ulation a	nd eig	ht sel	ected fam	ilies of alfal	fa gro	wn i	n the
	2018	3/2019 se	ason.						

		MS						
SOV	df	Plant	Leaf/stem	Fresh forage	Dry forage	Protein		
		height	ratio	yield	yield	(%)		
Replicates	3	5.59	124.90	83.14	3.02	1.12		
Families	8	106.35**	247.23**	192.89**	7.83**	4.089 ns		
Base vs selected families	1	330.53**	99.97**	201.05**	6.17**	3.629 ns		
Between selected families	7	74.32**	268.27**	191.73**	8.07**	3.50 ns		
Error (families)	24	5.59	6.21	4.23	1.65	2.22		
Error (selected families)	21	4.62	6.11	4.09	1.47	1.71		

Ns: not significant at $p \le 0.05$. *, **: significant at $p \le 0.05$ and $p \le 0.01$, respectively

Table 5. Means of plant height, leaf/stem ratio, fresh and dry forageyield and protein percentage for the base population and eightselected families of alfalfa.

Genotype	Plant height (cm)	Leaf/stem ratio (%)	Fresh forage yield (kg/plot)	Dry forage yield (kg/plot)	Protein percentage (%)
	• •		H.S.		
G.1	71.40	54.72	41.549	10.803	18.691
G.2	74.86	55.12	44.824	11.967	18.886
G.3	73.23	57.06	43.810	11.522	18.949
G.4	75.60	56.78	43.740	10.962	18.631
G.5	72.90	57.88	42.662	11.101	18.623
G.6	74.10	55.03	41.040	11.074	18.410
G.7	72.10	52.31	43.527	11.678	18.622
G.8	73.90	53.53	41.429	10.660	18.527
Bass Population	58.83	44.28	38.674	9.258	18.022
RLSD (0.05)	3.45	3.63	3.001	1.672	ns
(0.01)	4.68	4.88	3.980	2.391	ns

As for, fresh forage yield (kg/plot), it ranged from 41.040 kg/plot for G.6to 44.824kg/plot for G.2. Five families were significantly higher than the base population (38.674kg/plot) in fresh forage yield. Dry forage yield for the families ranged from 10.660kg/plot for G. 8 to 11.967 kg/plot for G.2, while the dry yield of base population was 9.258 kg/plot. All families except two families(G.1 and G.8) were significantly higher than the base population in dry forage yield. Insignificant differences were detected among families for protein means. Protein percentage means ranged from 18.022 % for the base population to 18.949% for G.3. Similar results were reported by other investigators (Awad 2001, Bakheit *et al* 2011, Annicchiarico 2015 and Bakheit *et al* 2016).

A summary for the main genetic parameters is presented in Table (6). The genotypic and phenotypic variances expressed as GCV% and PCV% were 5.28% and 7.03% for plant height, 1.30 and 1.79% for leaf/stem ratio, 7.76and 10.26% for fresh forage yield, 8.42 and 11.23% for dry forage yield, 5.53 and 6.55% for protein content, respectively.

 Table 6. Different genetic parameters for different traits of alfalfa genotypes.

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Genetic parameter	Plant height (cm)	Leaf/stem ratio (%)	Fresh forage yield (Kg/plot)	Dry forage yield (Kg/plot)	Protein percentage (%)
Range	58.83-75.60	44.28-57.88	38.674 - 44.824	9.258-11.967	18.022-18.949
Mean	71.87	54.98	42.362	11.12	18.618
σ^2 G	0.144	0.509	10.78	0.876	0.057
$\sigma^{2}E$	0.836	2.211	30.89	1.135	0.037
σ^2 GE	0.293	1.006	16.33	0.612	0.092
σ ² Ph	0.255	0.978	18.89	1.56	0.080
GCV (%)	5.28	1.30	7.76	8.42	5.53
PCV (%)	7.03	1.79	10.26	11.23	6.55
H (%)	56.47	72.94	57.07	56.15	71.25
2					

 $\sigma_{\rm E}^2$: environment variance, $\sigma_{\rm G}^2$: genotypic variance. $\sigma_{\rm GxE}^2$ genotypic x environment variance, $\sigma_{\rm P}^2$: phenotypic variance,

P.C.V.: phenotypic coefficient of variability and G.C.V.: genotypic coefficient of variability, .H: heritability.

These results showed a sufficient genotypic variation among selected families after one cycle of family selection for different traits. Broad-sense heritability values were high for leaf/stem ratio and protein percentage with values of 72.94 and 71.25%, respectively. But, for fresh and dry forage yields, heritability values were medium with values of 57.07 and 56.15%, respectively. Regarding plant height, heritability was 56.47%. The medium heritability values of forage yield and plant height demonstrated more environmental effects on these traits. Similar results were reported by Awad (2001) and Bakheit *et al* (2011 and 2016), Annicchiarico (2015) and Badawy (2013 and 2017).

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انتخاب بعض عشائر البرسيم الحجازي للمحصول وجودته بأستخدام طريقة

الانتخاب الاجمالي المعدل

مفيدة عبد القادر صيام و محمد ابو زيد النحراوى

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تم استخدام طريقة الانتخاب الاجمالى المعدل لدراسة العائد من الانتخاب والمقارنة بين العشيرة الاصلية و العائلات المنتخبه انصاف الأشقاء فى ٢٠١٦ / ٢٠١٢ . تم الأنتخاب بين العائلات انصاف الأشقاء وانتخاب أفضل ثمان عائلات (كثافة الأنتخاب ١٠%) ثم ثقييم بذور العائلات انصاف الأشقاء المنتخبة ودورتا الأنتخاب الأجمالى المعدل للمحصول العالى وبعض مكونات المحصول ونسبة البروتين. تفوقت الدورة الأولى بشكل كبير على العشيرة الأصلية وتفوقت الدورة الثانبة على الدورة الأولى فى صفة أرتفاع النبات ونسبة الأوراق للسيقان ومحصول العشيرة الأصلية وتفوقت الدورة الثانبة على الدورة الأولى فى صفة أرتفاع النبات ونسبة الأوراق للسيقان ومحصول العشيرة الأصلية وتفوقت الدورة الثانبة على الدورة الأولى فى صفة أرتفاع النبات ونسبة الأوراق للسيقان ومحصول العلف. و كان العائد من الانتخاب بعد دورتين انتخاب تحسن بنسب مئوية ٨,٩ و ٢٥,٥٠ و ١٩,٥١ و ١٩,٤٧ و ٢٨,٠% لارتفاع النبات و نسبة الأوراق للسيقان و محصول العلف الأخضر و محصول العلف الجاف و نسبة البروتين على التوالى مقارنة بالعشيرة الأصلية و تشير النتائج الى ان الانتخاب الاجمالى المعدل أدى الى تحسين و ٢٨,٠% لارتفاع النبات و نسبة الأوراق للسيقان و محصول العلف الأخضر و محصول العلف الحاف و نسبة البروتين على التوالى مقارنة بالعشيرة الأصلية و تشير النتائج الى ان الانتخاب الاجمالى المعدل أدى الى تحسين و ٢٨,٠% لارتفاع النبات و نسبة الأوراق للسيقان و لكن العائد من الانتخاب كان منخفض لنسبة البروتين على التوالى مقارنة العثات و نسبة الأوراق للسيقان و لكن العائد من الانتخاب كان منخفض لنسبة و مربر في النتاجية العلف و ارتفاع النبات و نسبة الأوراق للسيقان و لكن العائد من الانتخاب كان منخفض لنسبة مالبروراق و ٢٠,٧ و ٢،١٠ لمحصول العلف الأخضر و ٢٠,٧ لارتفاع النبات و من الانتخاب و ٥،٠ و مارم و ٥، و مارم و ١٠,٠ العلف الباض و معيم العلف الأخضر و ٢٠,١ و من المائذ من العلف الجاف و ٢٠,٠ و ٥، ٦,٣ لنسبة البروتين على التوالى. و بلعت قيم كفائة التوريث بمعاه الواسع لارتفاع النبات و نسبة الأوراق مامر و ٥,٠ 9 و ٥، ٥,٠ % و ١٠,٠ 0، و ٢، ٥، 9 م ١٠,٠ % و نسبة البروتين مارم 0، و سبة الأوراق

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