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Differential Response of Two Base Populations of Egyptian Clover "*Trifolium alexandrinum*, L." to Select Half- Sib and S₁- Families.

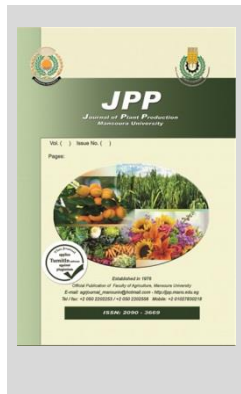
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ABSTRACT

Egyptian clover "*Trifolium alexandrinum*, L." is the most important forage crop in Egypt. The main objectives of the current study were to assess the differential response of two base populations of Egyptian clover to half-sib and S₁-families selection methods. At the fourth cutting, Sakha 4 population, expressed the higher gains from half-sib family selection vs. Helaly population. The obtained values were 30.88 and 39.83% vs. 18.40 and 37.93% for the former and the later populations relative to base population (C₀) and the average of the checks, respectively. Meanwhile, S₁- family selection, expressed 44.12 and 53.97% in Sakha 4 population, relative to base populations (C₀) and the average of the checks, respectively. However, 37.60 and 60.30% gains were presented by Helaly population, relative to base populations (C₀) and the average of the checks were 37.60 and 60.30%, respectively. Commonly, Sakha4 population was more responsive to any of the tested selection methods. This might indicate a higher level of variability within that population relative to Helaly population, which received much intensive improvement through selection, which limited the valuable variability.

Keywords: Base population, Egyptian clover, gain from selection, half-sib family, S₁-family.

INTRODUCTION

Egyptian clover "*Trifolium alexandrinum*, L." is the main forage crop in Egypt. It occupies about one third of the cultivated area during winter season (El-Nahrawy 2014). Barseem lasts for about seven months, providing valuable palatable forage as soiling, silage, hay, and grazing land.

Barseem landraces had been adapted to prevailing conditions in Delta and valley of the Nile River across Egypt. Breeding studied had focused on increasing green and dry yields, along with improving characters related to environmental adaptation. Different selection methods were practiced. Mass selection had been practiced by Abou El-Shawareb (1971), Omara and Hussein (1982), Bakheit (1985), Bakheit (1989 b), Ahmed (2000), Awad (2001), Bakheit *et al.*, (2007), Bakheit *et al.*, (2011). Ecotypic selection for efficient land races had been applied by EL-Nahrawy (1980), Mikhiel (1987). Abd EL- Galil (2007), Rajab (2010) and Ahmed *et al.*, (2021). Recurrent selection had been applied to germplasm of barseem by Bakheit and Mahdy (1988), Bakheit (1989 a), Bakheit (1989 b), Ahmed (1992), Bakheit and EL- Nahrawy (1997), Ahmed (2000), Ahmed (2006 a and b), Tag EL-Din (2006), Bakheit *et al.*, (2007), Bakheit *et al.*, (2011), Badawy (2013) and Ahmed *et al.*, (2021). All selection trials showed potentiality for improvement, indicating, sufficient valuable variability with Egyptian pool of barseem clover. Gain from selection in barseem populations reached 11.6, 19.8, 19.7 and 17.7% over the original population for three successive cuts and total yield Abou EL- Shawareb(1971). While, reached 8.43 and 10.7% of the original population after the first and the second cycles of mass selection Bakheit (1985). Ahmed (1992) recorded a realized gain of 27% over base from half-

sib family selection. Bakheit and EL- Nahrawy (1997), obtained 17.7 and 25.2% realized gain over the base population, in the first and the second cycles of recurrent selection. Ahmed (2006 b) reached a realized gain of 7.14 and 5.66% relative to the base population and the average of the checks per cycle of half-sib family selection. Bakheit *et al.*, (2011), recorded a realized gain of 14.94% and 17.24% over the base population from mass and family selection, respectively. Badawy (2013) revealed that the realized gain in seasonal total green forage yield due to S₁- family selection amounted to 19.47 and 27.76 % relative to the base population and the average of the checks, respectively. Ahmed *et. al* (2021), reached a cumulative realized gain over cuttings over the base population reached 9.09 and 67.32% from the first and the second cycles of selection for frequent- cutting tolerance.

The main objective of the recent study was to assess the differential response of two base populations of barseem clover to half-sib family and S₁-family selection methods.

MATERIALS AND METHODS

The main objective of the recent study was to assess the response to selection in two basic commercial populations of *Meskawi* barseem clover "*Trifolium alexandrinum*, L." The experimental site was Sakha Agricultural Experimental Station delta of Nile, Egypt. The two base populations were released as commercial varieties. Sakha4 was released by early ninetieth of the twentieth century (1990 's) whereas, Helaly was released by the beginning of the twenty first century (2000 's). Sakha 4 was developed depending upon selection for leafiness. While Helaly realized depending on forage production vigor. Two basic selection methods were

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applied to each of the two populations separately. These were S₁ line selection (S₁L.S) and half-sib line selection (H.S.L.S). Details of each selection protocol will be illustrated in the following.

a- Half- sib family selection:

During winter season of 2016/ 2017, 500 separate single plants were selected in the nursery field of each population, after three successive cuttings. These plants were characterized by growth vigor and high tillering. Other unselected plants were uprooted before flowering. For each selected plant 10 inflorescence were paged in muslin to conserve self- pollinated seeds, however other unpagged inflorescences were harvested for each plant separately to indicate half- sib family. Depending on seed weight of each single plant, the heaviest seed producing 100plants were saved (20% selection intensity).

In winter season of 2017/ 2018, the selected 100 half-sib families were randomly divided to five groups (each of 20 families). Each set of families was evaluated in a Randomized Complete Block Design (RCBD) with four replicates. Each family was assigned to a single row plot of two meters' length and 0.2 meter apart. Seeding rate was 15 Kg. faddan⁻¹ (2.86g.row⁻¹). Each row was guarded by two rows sown with the base population's seeds. Four cuttings were taken in which plant height, number of tillers 0.25 meter and green forage yield were measured for families. Depending on overall performance of families, the best 20% were identified (4 families in each set a total of 20 families).

In that season, S₁ seeds of the evaluated half- sib families were sown in an isolated field. Depending on the selection results, unselected families were uprooted before flowering a cage of fine cloth was placed over the pollination block, whereas a heave if honeybee was placed inside to permit random cross-pollination. Harvested seeds were designated as the first cycle of half-sib family selection (C₁.H.S.).

b- S₁-family selection:

During the base season of 2016/2017, 500 separate plants in each base were selected depending on tillering, and growth vigor the whole plant was caged by a fine cloth page fixed to the ground by wood sticks. Tripping was applied by hand shaking of inflorescences. Selfed seeds were harvested for each plant separately to represent S₁- family. The highest seed producing 100 families were identified.

In 2017/2018, S₁- families were evaluated as five groups (20 families each) in a randomized complete block experiment using part of families seed. Plot size was one row of one-meter length and 0.2 meter apart. Seeding rate was 13Kg. faddan (0.69 g. plot⁻¹). Each plot was guarded by two rows sown by the original population's seed. Four cutting were taken, in which plant height, number of stems. 0.5 m⁻¹, and green forage yield were evaluated. Families unselected were uprooted before flowering Depending on the characters, the best four families in each set were identified (20% intensity).

In 2018/2019 winter season, the remnant seeds of the twenty selected S₁- families were mixed with equal seed number in an isolated block. Sowing was on rows at 0.2m apart a wood cage was placed over the mating block before

flowering for isolation. A heave of honeybee was incubated inside the cage to ensure random mating. Harvested seeds was designated as the first cycle of first- selfed family selection (C₁-S₁).

In 2019/2020 winter season selected cycles in each base population (four entries) along with the base population (two entries) and three commercial checks (Gemiza 1, Serw 1, and Giza 6) were sown in randomized complete blocks experiment in three replications. Each entry plot was one row of four meters long and 0.2 meter apart. Data were recorded for green forage yield (four successive cuttings).

Analysis of variance was performed as stated by Cochran and Cox, (1957). Combined analysis over sets was performed when the assumption of errors homogeneity cannot be rejected. Data of selection cycle's evaluation were performed over cuttings and base populations.

RESULTS AND DISCUSSION

Results were analyzed for each separate population to assess the homogeneity of errors prior to combined analysis. (Table 1) showed the analysis of variance for six entries of each population (base, (C₁H.S.), (C₁.S₁) Gemiza 1, Serw 1, and Giza-6) over four successive cutting for green forage yield, plant height and dry forage yield. Errors of different base populations were not homogenous Bartlett (1937). This would drive discussion for each separate population. The recorded variables in any of the studied cuttings were significantly different (P≤ 0.01) in both studied populations. Also, significantly different forage characters (fresh forage yield, plant height and dry forage yields) were realized among the tested entries (P≤ 0.01). Entries maintained similar ranked and/or magnitude of forage characters among the studied cuttings (insignificant cutting × entry interaction).

Table 1. Analysis of variance for six entries of each base population (Saka4, Helaly) over cuttings for fresh forage yield

S.O.V.	d.f.	Fresh forage yield (kg plot ⁻¹)	
		Sakha 4	Helaly
Cutting (C)	3	28.67**	22.70**
Rep/ cutting	9	0.915	0.38
Entries (E)	5	6.98**	3.62**
C × E	15	0.047 ^{n.s}	4.6 ^{n.s}
Error	60	0.28	2.8

n.s and **: not significant and highly significant at 0.01 level probability.

Orthogonal comparison between base population vs. selection schemes, half- sib family vs. S₁ – family selection, and selection vs. checks were presented in (Table 2). Selections were significantly different from the base population in fresh forage yield during successive cuttings from the second to the fourth across the two different studied populations. The type of selected family (half- sib or S₁-family), Significantly affected the obtained fresh forage yield in all cuttings of the two studied population, except for, the second and the fourth cuttings of Sakha 4 population and the third cutting of Helaly population. Selections of the two studied populations were significantly surpassing the tested checks in fresh forage yield of all cuttings, except for, the first cutting of Helaly population.

Table 2. Mean squares of orthogonal comparison between; base population (C₀) vs. Selection Schemes, half- sib vs. S₁family selection, and selections vs. Checks for each studied population in each cutting.

Comparison	d.f.	Fresh forage yield (kg plot ⁻¹)							
		1 st cutting		2 nd cutting		3 rd cutting		4 th cutting	
		Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly
C ₀ vs. Selection	1	0.107 ^{ns}	0.010 ^{ns}	0.540 ^{**}	0.107 [*]	0.667 ^{**}	0.350 [*]	0.135 [*]	0.327 ^{**}
H.S vs. S ₁	1	0.0001 ^{**}	0.001 [*]	0.020 ^{ns}	0.0001 ^{**}	0.080 [*]	0.151 ^{ns}	0.045 ^{ns}	0.180 ^{**}
Selection vs. Checks	1	0.192 [*]	0.037 ^{ns}	0.588 ^{**}	0.108 [*]	1.728 ^{**}	1.027 ^{**}	0.867 ^{**}	0.705 ^{**}
Error	15	0.028	0.032	0.043	0.020	0.018	0.043	0.023	0.017

ns, * and **: not significant, significant and highly significant at 0.05 and 0.01levels, respectively

Means of fresh forage yield of the first cutting for the evaluated entries of each base population and realized gain (%) from selection relative to the base (C₀) and the average of the checks were presented in (Table 3). Half- sib family selection gave a realized gain from selection of comparable magnitude between the two base populations, whether relative to C₀ and the average of the checks 128.66 and 25.00% gain were recorded relative to C₀ and the average of checks in Sakha 4 population. While 229.82 and 21.29 % gains relative to C₀ and the average of the checks, respectively were recorded in Helaly population. S₁- family selection, expressed higher magnitude of gain relative to any of base population (C₀) or the average of the checks. The realized gain from S₁-family selection in Sakha4 population reached 142.07 and 32.33% relative to any of bases and average of the checks. While Helaly population expressed a realized gain of 232.46 and 22.26 % relative to the base population (C₀) and the average of the checks, respectively.

Table 3. Means of fresh forage yield of the first cutting for the evaluated entries of each base population, and realized gain from selection relative to base population (C₀) and the average of checks.

Entries	1 st cutting					
	Means (kg plot ⁻¹)		*Realized gain% relative to			
	Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly
Base population (C ₀)	0.820	0.570				
C ₁ -H.S	1.375	1.880	128.66	229.82	25.00	21.29
C ₁ -S ₁	1.985	1.895	142.07	232.46	32.33	22.26
Checks						
Gemmiza 1	1.600	1.600				
Serw 1	1.500	1.575				
Giza 6	1.400					
Average	1.500	1.550				

*Plot = (3 × 0.2 = 0.6m²)1.575

*: Realized gain% relative to base = C₁ - C₀ / C₀ × 100.

relative to checks = C₁ - check average / check average × 100. C₁-H.S: First cycle of half-sib family selection. C₁-S₁: First cycle of S₁- Family selection.

Regarding the second cutting (Table 4), the calculated realized gain from half- sib family selection in Sakha 4 population reached 14.07 and 17.29% relative to C₀ and the average of checks. Meanwhile, the recorded values in Helaly population were 20.00 and 15.00% relative to C₀ and the average of the checks, respectively. S₁- family selection expressed higher realized gains reached 25.19 and 28.71% relative to C₀ and the average of the checks in Sakha4 population vs. 29.57 and 24.17% relative to C₀ and the average of checks in Helaly population, respectively.

The highest magnitude of realized gain from selection was reached by the third cutting (Table5). Half- sib family selection/ expressed 31.33 and 204.5% relative to (C₀) and the average of checks in Sakha 4 population. While the respective recorded values reached 11.88 and 229.65% in Helaly

population. While S₁- family selection recorded a realized gain of 49.33 and 246.2% of (C₀) and the average of checks, respectively in Sakha 4 population, vs. 32.5 and 290.46% respective gains in Helaly population.

Table 4. Means of fresh forage yield of the second cutting for the evaluated entries of each base population and realized gain from selection relative to base population (C₀) and the average of checks.

Entries	2 nd cutting					
	Means (kg plot ⁻¹)		*Realized gain (%) relative to			
	Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly
Base population(C ₀)	1.350	1.150				
C ₁ -H.S	1.540	1.380	14.07	20.00	17.29	15.00
C ₁ -S ₁	1.690	1.490	25.19	29.57	28.71	24.17
Checks						
Gemmiza 1	1.360	1.260				
Serw 1	1.380	1.250				
Giza 6	1.200	1.090				
Average	1.313	1.200				

*Plot = (3 × 0.2 = 0.6m²)1.575

*: Realized gain% relative to base = C₁ - C₀ / C₀ × 100.

relative to checks = C₁ - check average / check average × 100. C₁-H.S: First cycle of half-sib family selection. C₁-S₁: First cycle of S₁- Family selection.

Table 5. Means of fresh forage yield of the third cutting for the evaluated entries of each base population and realized gain from selection relative to base population (C₀) and the average of checks.

Entries	3 rd cutting					
	Means (kg plot ⁻¹)		*Realized gain (%) relative to			
	Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly
Base population(C ₀)	1.500	1.600				
C ₁ -H.S	1.970	1.790	31.33	11.88	204.5	229.65
C ₁ -S ₁	2.240	2.120	49.33	32.50	246.2	290.42
Checks						
Gemmiza 1	0.750	0.620				
Serw 1	0.630	0.540				
Giza 6	0.560	0.470				
Average	0.647	0.543				

*Plot = (3 × 0.2 = 0.6m²)1.575

*: Realized gain% relative to base = C₁ - C₀ / C₀ × 100.

relative to checks = C₁ - check average / check average × 100. C₁-H.S: First cycle of half-sib family selection. C₁-S₁: First cycle of S₁- Family selection.

At the fourth cutting (Table 6), Sakha 4 population, expressed the higher gains from half-sib family selection vs. Helaly population. The obtained values were 30.88 and 39.83% vs. 18.40 and 37.93% for the former and the later populations relative to (C₀) and the average of the checks, respectively. Meanwhile, S₁- family selection, expressed 44.12 and 53.97% in Sakha 4 population, relative to (C₀) and the average of the checks, respectively. Also, 37.60 and 60.30% gains were presented by Helaly population, relative to (C₀) and the average of the checks, respectively.

Table 6. Means of fresh forage yield for the evaluated entries of each base population and realized gain from selection relative to base population (C₀) and the average of checks.

Entries	4 th cutting					
	Means (kg plot ⁻¹ *)		*Realized gain (%) relative to			
	Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly
Base population(C ₀)	1.360	1.250				
C ₁ -H.S	1.780	1.480	30.88	18.40	39.83	37.93
C ₁ -S ₁	1.960	1.720	44.12	37.60	53.97	60.30
Checks						
Gemmiza 1	1.220	1.150				
Serw 1	1.240	1.070				
Giza 6	1.360	1.000				
Average	1.273	1.073				

*Plot = (3 × 0.2 = 0.6m²)1.575

*: Realized gain% relative to base = C₁ - C₀ / C₀ × 100.

relative to checks = C₁ - check average / check average × 100. C₁-H. S: First cycle of half-sib family selection. C₁- S₁: First cycle of S₁- Family selection.

One of the major problems is to match the components of selected population to growing environmental conditions since, the performance of a specific population is not fixed over a wide range of environments. Consequently, it is necessary to identify a target population for each breeding scheme Comstok (1964). Two major factors influence this breeding problem. The first factor is that the combination of optimum levels of this trait expression in one population is impossible. The second factor, is that the adaptability of this trait does not extend sufficiently over environments to produce uniformly homeostatic responses breeding populations, therefore, generally have had and will continue to have non- uniform value response functions (Roberds and Namkoong, 1989).

Commonly, Sakha 4 population was more responsive to any of the two tested selection methods and that might indicate higher level of variability within that population relative to Helaly population, which received much intensive improvement through selection, that limited the valuable variability. Success of breeding scheme depends on the existence of genetic variability for selecting superior genotypes (Otoboni et al., 2020). (Jighly et al., 2019), found that, the more speed breeding rounds were performed in each breeding cycle, the faster the shrinkage in the genetic diversity of the breeding population, which may limit the long- term genetic gain (Goddard, 2009). This was comparable for Sakha 4 and Helaly populations.

Also, S₁- family selection was more effective in improving green forage yield rather than half – sib family selection, irrespective of the base population.

As a total fresh forages yield, values of realized gains, whether relative to (C₀) or the average of the checks for Sakha 4 population were of larger magnitude relative to the corresponding values of Helaly population. Realized gains relative to (C₀) were 42.45, 56.56% vs.21.00, 36.21% for half-sib family and S₁- family selection in Sakha4 and Helaly populations respectively. Meanwhile realized gains relative to the average of the checks, reached 51.38 and 66.39% vs. 26.03 and 41.86% for half-sib family and S₁- family selection in Sakha 4 and Helaly populations, respectively.

The major factor that may affect the proposed selection gains includes: 1) selection intensity, 2) genetic properties of base population, and 3) environmental

conditions (Silva et al., 2020). Consequently, gains from selection are directly related to the difference between selected group's mean and the base population mean. S₁ – family selection, concentrate on highly heritable traits than contribute to yield, i.e.; plant height and growth vigor.

Table 7. Means of fresh forage yield for the evaluated entries of each base population and realized gain from selection relative to base population (C₀) and the average of checks.

Entries	Total fresh yield					
	Means (kg plot ⁻¹ *)		*Realized gain (%) relative to			
	Sakha 4	Helaly	Sakha 4	Helaly	Sakha 4	Helaly
Base population(C ₀)	5.03	4.570				
C ₁ -H.S	7.165	5.53	42.45	21.00	51.38	26.03
C ₁ -S ₁	7.875	6.225	56.56	36.21	66.39	41.86
Checks						
Gemmiza 1	4.930	4.630				
Serw 1	4.750	4.435				
Giza 6	4.520	4.110				
Average	4.733	4.388				

*Plot = (3 × 0.2 = 0.6m²)1.575

*: Realized gain% relative to base = C₁ - C₀ / C₀ × 100.

relative to checks = C₁ - check average / check average × 100. C₁-H.S: First cycle of half-sib family selection. C₁- S₁: First cycle of S₁- Family selection.

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اختلاف استجابة عشيرتنا اساس من البرسيم المصري لانتخاب العائلات نصف المنسبة وعائلات الجيل الاول من التلقيح الذاتي

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الملخص

البرسيم المصري "*Trifolium alexandrinum*, L." هو محصول العلف الرئيسي في مصر , وتهدف هذه الدراسة الي انتخاب افضل التراكيب الوراثية ذات الصفات المميزة بهدف الحصول على تركيبي متفوق على العشيرة الأصلية وتقدير الإستجابات المختلفة لعشيرتي أساس من البرسيم المصري إلى انتخاب العائلات النصف منسبة وانتخاب عائلات الجيل الأول الناتجة من التلقيح الذاتي. عند الحشة الرابعة وقد أظهرت عشيرة "سحا4" أعلى إستجابة لانتخاب العائلات النصف منسبة بالمقارنة بعشيرة "هلاي" حيث بلغت القيمة المتحصل عليها 30.88 و 39.83% في مقابل 18.40 و 37.93 للعشيرة الأولى والثانية على الترتيب منسوبة إلى متوسط عشيرة الأساس ومتوسط الأصناف الإختيارية. بينما سجل انتخاب عائلات الجيل الأول للتلقيح الذاتي إستجابة بلغت 44.12 و 53.97% في عشيرة "سحا4" منسوبة إلى عشيرة الأساس ومتوسط الأصناف الإختيارية على الترتيب. في حين سجلت عشيرة "هلاي" تحسن بلغ 37.60% و 60.30% منسوبة إلى عشيرة الأساس ومتوسط الأصناف الإختيارية وبصفة عامة، فإن عشيرة "سحا4" أظهرت إستجابة أعلى لأي من نظم الانتخاب المختبرة. وقد يدل ذلك على وجود مستوى أعلى من التصنيفات داخلها مقارنة بالعشيرة "هلاي" والتي تمت فيها دورات عديدة من التحسين مما قلل من التصنيفات المفيدة فيها.