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Egyptian Clover (*Trifolium alexandrinum* L.) Breeding in Egypt: A Review

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ABSTRACT

Berseem or Egyptian clover is a winter annual legume of major importance to Egyptian agriculture as a principle source of forage and for conserving soil fertility under intensive cropping. In this review, the published work by authors on breeding Egyptian clover (*Trifolium alexandrinum* L.) for productivity in Egypt has been summarized since 1980. The variability of the genotypes, genotypic and phenotypic correlation, Path coefficient, the breeding strategies, pollination and seed setting for Egyptian clover are presented. The genetics and productivity of Egyptian clover are recorded. The objectives of this review are enrichment the background for the investigators working in Egyptian clover breeding. Finally the achievements and the needed cooperation on the national level are also outlined.

Key words: Egyptian clover, *Trifolium alexandrinum*, variability, forage breeding, seed setting, genotype×temperature interaction

INTRODUCTION

Berseem clover or Egyptian clover (*Trifolium alexandrinum* L.) is the main annual winter forage leguminous crop in Egypt. Berseem clover is high nutritional quality for animal feed. Berseem also contributes to soil fertility and improves soil physical characteristics (Graves *et al*., 1996). Berseem forage is superior to grasses in protein and mineral contents (Laghari *et al*., 2000). Also, it cultivates to take forage and seed. Berseem clover is a major seed export crop in Egypt. Annual exports of berseem seed have to more than 12000 tons in 2001 and the main honey producing crop (El-Nahrawy, 2005).

Although, plant breeders have made significant improvement in yields of many crops, limited work has been done on yield potential or seed production in Egyptian clover improvement. This may be due to small floral parts which make artificial hybridization difficult. Besides, high degree of self sterility and incompatibility limits the extent to which these plants may be inbred. Therefore, Egyptian clover breeders have used mainly the selection or synthetics varieties procedures for improved forage and seed yield in their breeding programs.

GENETIC VARIABILITY, GENOTYPIC AND PHENOTYPE CORRELATION AND PATH-COEFFICIENT IN EGYPTIAN CLOVER

Radwan *et al*. (1983) studied the variability and combining ability in 331 farmers seed lots of berseem clover collected from different regions in Egypt, the lots were divided into six lots classes on the basis of seed weight. They concluded that, great discrepancy in means and coefficient of variation (c.v.) values was observed among seed lots. Meanwhile, specific leaf weight was the character that showed the least variation. The combined analysis of variance for fresh forage yield showed no significant difference among three cuts of the six seed lots. The highest actual gain from selection was 13.1 in one location and 19.2% in the second one.

Rammah *et al.* (1984) improved of multicut berseem clover through selection among 380 entries. They reported that, great variation was observed among the tested materials in each location. Also, 40% of the selected entries significantly increased the control variety (Giza 1) in yield in one location while, 18% of the selected entries significantly increased the control variety in another two locations.

Bakheit (1985a) study the genotypic stability of the 56 local accessions and found highly significant differences due to environments, (location, year) genotypes and their interactions some high yielding genotypes, exhibited great instability in forage yields, whereas average stability area contained other high yielding genotypes. However, one genotype exhibited nearly perfect stability for forage yields.

Fifty-six local accessions of multi-cut Egyptian clover collected from farmers in different governorates (Kena, Sohag, Assiut) were evaluated by Bakheit (1986) at four experiments under different environmental conditions i.e., location and season and found that considerable variation for plant height, seasonal fresh, dry and protein yields. The genetic variance exceeded the environment variance for all the studied traits. Heritability was high for all characters studied. Both phenotypic and genotypic correlations among traits showed that mean plant height was positively correlated with each of seasonal fresh, forage dry and protein yields but negatively correlated with mean protein percentage. Path-coefficient analysis revealed that seasonal fresh forage yield had the highest positive direct effect on seasonal protein yield (0.84) followed by mean dry matter percentage (0.46). The mean protein percentage showed the minimum direct (0.172) and indirect influence on seasonal protein yield.

Awad (1988) evaluated forty-one of multi-cut Egyptian clover ecotypes collected from farmers field in Upper Egypt and New Valley Governorates for forage yields and its components and found significant variations among berseem ecotypes in growth parameters (RGR, NAR, LAR), forage yields (fresh, dry, protein yields) and values of photosynthetic pigments (chlorophyll a, b, a/b and total carotenoid). Also, he found heritability estimates in broad sense for the first season were 67, 69 and 68 and 50, 45 and 50% in second season for fresh, dry and protein yields, respectively.

Bakheit and Mahdy (1988) evaluate another thirty four accessions of multi-cut Egyptian clover collected from seven governorates (Behera, Gharbia, Kafr El-Sheikh, Minia, Assiut, Sohag and Kena). They reported that highly significant differences among accessions in both separate and combined analysis in forage yield. Also, the year effect was highly significant. Six accessions were stable, but insignificantly out yielded the check variety Giza-1 over the two seasons.

Martiniello *et al.* (1992) evaluated thirty two genotypes for forage and seed yield (26 of southern Italy origin and 6 Egyptian) and found that the Italian genotypes are better adopted when its cut in the early growth stages, also its high in seed production. While the Egyptian populations produced the high forage and seed yields in the later cuts. The range of variation among the bio-agronomical traits was higher when the cut was applied at early flowering. The traits with wider range of variation were dry matter, plant height at cut, regrowth at the fourteenth day after cut, tiller density and seed yield. Considering a multiplicative selection index, six populations were well adapted and could be used as parents in breeding programs and in extended farm cultivation.

Bakheit and El-Hinnawy (1993) evaluated thirty two accessions of multi-cut Egyptian clover under normal irrigation and drought condition for forage yield. The results obtained revealed a considerable variation among accessions for both fresh and dry forage yield. In general, water stress decreased both yields. The combined analysis of variance revealed significant differences due to environments, accessions and their interactions. Some accessions surpassed significantly the check cultivar Giza-1 in fresh and dry forage yield. The estimates of genotypic stability parameters (α and λ) showed that most of the high forage yielding accessions exhibited instability in their performance.

Ahmed (2006a) studied the variability, correlations and path-coefficient analysis in two populations of multi-cut berseem clover and found the highly variability for plant height and lower for dry matter. Also, reported heritability was moderate magnitude for all studied traits. The expected gain from selecting the highest 10% of families were 29.3 and 37.5% for dry forage yield, 43.5 and 43.0% for plant height, 30.9 and 40.7% for stem diameter and 22.1 and 24.4% for dry matter percent of Khadarawi and Meskawi populations, respectively. The genotypic and phenotypic correlation between seasonal dry forage yield and each of plant height and number of tillers plant^{-1} were highly positive correlation. In contrast, the correlation was strong and positive at the genotypic level only with stem diameter, mean leaf area/plant and leaf/stem ratio. Path-coefficient analysis, at phenotypic and genotypic levels, showed that Khadarawi seasonal dry forage yield was directly affected by dry matter percent, seasonal fresh forage yield, plant height and number of tillers/plant in that order. Meanwhile, Meskawi seasonal dry forage yield was orderly directly affected by seasonal fresh forage yield and dry matter percent.

Abdel-Galil *et al*. (2007) evaluated the yield as well as the phenotypic and genotypic stability for sixteen Egyptian clover genotypes at four locations (Sakha, Gemmeza, Serw and Sids) during two seasons. They reported that, genotypes Hatour, Sakha 4, Gemmeza-1, Narmer and Giza-6 outyilded other genotypes with no significant differences among them regarding fresh herbage yield. However, no significant differences were detected among most of the entries in dry yields. The highest fresh and dry yields over two seasons were recorded at Sids location surpassing the other locations. The genotypes Sakha 96, Giza 15, Gemmeza-1, Sids, Syn. Assiut population, Cairo-3 and Hatour met the parameter of phenotypic and or genotypic stability.

IMPROVEMENT EGYPTIAN CLOVER BY USING SOME BREEDING METHODS

Breeding methods used by some authors to improved forage and seed yield in Egyptian clover as follows.

Katta *et al*. (1980) used twenty eight seed lots of berseem clover were selected according to forage yield and were used as parent seeds in hybridization. The highest seven parents in green yield were crossed in all possible combinations including reciprocals. Moreover, seven polycrosses were obtained for the same seven parents, also five synthetic or composite seeds were obtained. They reported that, average heterosis was highly significant for green and dry yield under dense planting while in space planting the significance disappeared. General and specific combining abilities were significant for green and dry yield at different cuts and annual yield, under dense planting. Under space planting, general and specific combining abilities were significant for certain cuts at different season. The general combining ability effects were much more important than specific combining ability. No significant differences were detected among either the seven poly-crosses or the five synthetics.

Koraiem *et al.* (1980) reported 20.5% gain from one cycle of recurrent selection, based on general combining ability in Meskawi population (*Trifolium alexandrinum* L.) of over two locations.

Effectiveness of mass and family selection for fresh forage yield was determined for two generations in Egyptian clover by Bakheit (1985b). He reported that, the gain of the first and second cycles of mass selection for the fresh forage yield were 8.4 and 10.7% of the original population, respectively. The realized heritability and expected selection advance for first and second cycles of mass selection were 0.38 and 0.04 and 31.8 and 3.94%, respectively.

Family selection was more rewarding than mass selection and produced a response of 15.5% of the unselected base family mean after one cycle of selection. The difference between the two methods of estimating heritability, parent-progeny regression (0.48) and variance component (0.57) was not great.

Mahdy and Bakheit (1985) studied the inheritance of forage yield in Egyptian clover (*Trifolium alexandrinum* L.). The proposed model of Gardner and Eberhart (1966) was utilized on the two parents, namely Fahl and Miskawi and their S_1, S_2, F_1, Bc_1, Bc_2 and F_2 pollinations. The inbreeding depression of Fahl variety was more pronounced than that of Miskawi variety. Moreover, the superiority of the F_1-hybrid over the mid-parent and high parent was 30.8 and 23.7%, respectively. The genetic analysis revealed that the additive, dominance and epistatic gene action were significant while the heterosis parameter was not significant. Accordingly, the superiority of the F_1 hybrid over the mid parent and high parent could be due to the combined effects of dominance and epistatic gene action. The results of some morphological traits were also obtained.

Younis *et al.* (1986) subjected five populations of berseem clover to three cycles of visual selection. Results obtained on green and dry yield, leaf/stem ratio, plant height, crude protein and fiber%, ash% and fat% concluded that visual selection was more affective in increasing green and dry yield in berseem clover (single-cut) than berseem clover (multi-cut). The improved population of berseem clover (single-cult) increased dry yield 31.7% over their initial populations while the improved population of berseem clover (multi-cut) increased also dry yield from 17.7-23.9% over their initial populations. Values of crude protein% increased and values of crude fiber% reduced some what in improved populations from their initial populations while values of ash and fat% were almost the same in both improved and their initial populations.

Mikhiel (1987) recorded a positive response of 22% in forage yield after one cycle of half-sib selection. Also, estimates of heritability ranged from low to medium values for forage yields.

Bakheit and Mahdy (1988) studied the improving berseem clover within six superior farmer's seed lots. They found that, fifteen selected families significantly out yielded their respective base population with a range from 131.9-160.6%. Moreover, seventeen selected families significantly out yielded the check variety Giza-1. The observed gain from selection of the overall families mean as a percent increase from the base populations mean and the check variety Giza-1 amounted to 14.1 and 13.8%, respectively.

Mahdy (1988) applied selection for fresh forage yield of Egyptian clover under two plant densities. He found that the realized gain from selection in cycle 2 as a percentage from the base population mean amounted to 9.4 and 8.4% for the high and low densities, respectively. The difference in realized gain from selection between the high and low densities was not significant.

Sufficient genotypic and phenotypic coefficients of variabihty for further cycles of selection were remained between the cycle 2 families. The realized heritability of forage yield ranged from

6.8-18.7%. However, the broad-sense heritability as estimated from the variance components ranged from 65.6-92.7%. The large differences between the broad-sense and realized heritability estimates reflected the large dominance and epistatic gene effects on fresh forage yield.

Bakheit (1989a) using two method of breeding, recurrent selection and seed synthetics in berseem clover (*Trifolium alexandrinum* L.). Concerning the recurrent selection method he found that there are an increased in fresh forage yield, dry forage yield and protein yield by the rate of 13.9, 14.8 and 14.0% respectively in the first cycle as compared with the base population, being 21.7, 23.8 and 22.9% in the second cycle in the same order.

The first generation of the synthetic (Syn.F_1) showed an increase over parents of 3.7% in forage yield 4.4% in dry yield and 6.3% in protein yield relative to the check variety Giza-1. Corresponding means in Syn.1 F_2 were not different. In the other hand, the synthetic productivity exceeded that of the check cultivar in dry forage yield by up to 21.3%. Moreover, this synthetic would be adapted to a wider range of environment factors due to the presence of genetic variability and would give a more constant yield from year to year. In addition, this synthetic could be used as reservoir of desirable gene combination. In conclusion when comparisons between the two methods of breeding with the check cultivar were undertaken, the results showed that the synthetic F_2 variety approximately equal the second cycle of recurrent selection.

Bakheit (1989b) applied modified mass and family selection for seed yield production of mono-cut berseem clover (*Trifolium alexandrinum* L.) c.v Fahl for two generations. Two hundred plants (5% intensity of selection) were selected for seed yield in the first season. In the second season, selection between and within half-sib families was practiced. In addition, equal parts of seeds from each of the 200 selected plants were bulked to form the C_1 modified mass selection, after establishing, the same procedure was adopted to form the C_2 generations. The cycles 1 and 2 of half-sib families and modified mass selection along with the base population family were evaluated for forage and seed yields. The results showed that, the realized gains from modified mass selection were 6.1 and 9.5% for fresh forage yield, 5.6 and 10.9% for protein yield and 13.2 and 16.2% for seed yield in cycles 1 and 2, respectively, over the base population. The realized gain from family selection in cycle 2 as a percentage of the base population mean amounted to 11.3, 13.4, 17.5 and 3.2% for forage, protein and seed yield and seed index, respectively. The broad sense heritability as estimated from the variance components was 89.6, 63.0 and 76.7% for dry forage, seed yield and seed index, respectively. Although, all these traits (fresh, dry, protein and seed yields and seed index) had positive correlated with each other, weak correlation were found between seed yield and forage yields. Furthermore, close associations were found among forage yield traits.

Ahmed (1992) concluded that maternal-line selection with S_1 as recombiners, was superior to both half-sibs and controlled mass selection in Meskawi variety (*Trifolium alexandrinum* L.). The realized gain from that study was 22% in both green and dry forage yield.

Bakheit (1993) studied genetic analysis of diallel crosses in Egyptian clover (*Trifolium alexandrinum* L.). Five multi-cut genotypes including commercial cultivar Giza-1 of Egyptian clover, were crossed in a complete diallel. The main objectives of this study were to determine the relative importance of both general and specific combining ability and reciprocal effects and to determine the magnitude of heterosis for forage yield in crosses of selected collections and Giza-1 after two generations of selfing. He found that both additive and non-additive gene effects were involved in determining the performance of fresh and dry forage yield. The significant heterosis obtained over the better of mid-parental values for fresh and dry forage yields were correlated with positive significant specific combining ability effects of the respective hybrids. Five hybrids showed highly significant heterosis over the better parents for dry seasonal yield. Significant positive general combining ability effects were shown by the parent Giza-1 "good general combiners" and could be exploited for breeding programs. The reciprocal effects were only significant for first and fourth cuts in both fresh and dry forage yields.

Martiniello and Iannucci (1998) found that genetic variance in dry matter among maternal half-sib populations in short cycle (harvest made when the stems had seven or eight internodes) was 51% greater than in long cycle (harvest made when 5-10% of the tillers had followed). Narrow-sense heritability was 35% higher in short cycle than long cycle for dry matter and 26% higher for seed weight than seed yield. The magnitude of the genetic variance components and genetic correlations suggested that selection among plants of maternal half-sib populations would be more effective for improving dry matter in short than in long cycle harvests. The selection applied in the study was not effective for increasing seed yield per se, however, the trait may be increased by selection indirectly for seed weight.

Ahmed (2000) did a comparison of single trait of selection (total green forage yield) via combining ability test, with multiple trait selection (total green forage yield, dry weight of root nodules and seed yield) by using independent culling levels or index selection for the improvement of berseem clover (*Trifolium alexandrinum* L.) were obtained from 100 poly crosses isolated from a base population represented the second generation of random mating for a seed synthetic composed from 23 farmer's seed lots of Meskawi (multi-cut) type. He found, selection for multiple trait was significantly much rewarding than single trait selection. A realized gains of 12.2, 17.4, 17.2, 28.5, 14.1, 17.9 and 8.% from index selection were obtained vs: 6.6, 6.0, 3.8, 10.3, 7.9, 55.1 and 4.5% from single trait recurrent selection for total green forage, dry forage, protein and seed yields, seed index, dry weight of nodules and leaves/stem ratio, relative to the base population. Although, index selection gave higher magnitudes of realized gain relative to either the base population or the check varieties, these figures were not significantly different from those obtained with independent culling levels. The amount of effort required for index selection method is somewhat greater beside that, breeder should wait until all observations are recorded to construct an index. These are not essential with independent culling.

Ahmed (2006b) studied the response to three methods of recurrent selection in a Khadarawi berseem (*Trifolium alexandrinum* L.) population. He reported that, one cycle of selection was conducted for each of the following methods: half-sib with S_1 as recombiners (H.S.), S_1-familie (S_1) and S_1-families selection. Selection for all programs was based on protein yield (ton-fad.). A 20% selection intensity was common in the three methods. Response to selection was measured for protein yield and correlated responses on fresh forage, dry forage, seed yield and leaves/stem ratio. All selection methods were successful in improving significantly the population performance for protein yield, S_1 family selection had the largest magnitude of response of 0.346 ton fed^{-1} cycle^{-1} (37.3%) which was insignificantly different from the realized response for S_1 family selection of 0.322 ton fad^{-1}, cycle^{-1} (34.7%). H.S. family selection gave the lowest gain of 0.157 ton fed^{-1}, cycle^{-1} (16.94%). Taking into account both cost unit^{-1} gain and length of time required S_1 families selection had the highest rate of gain.

Radwan *et al.* (2007) improved plant vigour associated with selection for self-fertility in two berseem (*Trifolium alexandrinum* L.) populations. They reported that the open pollinated and selfed (S_1) progenies of these plants were not significantly different in mean fresh yield, but showed wide variation among plants and a significant correlation between open pollinated and selfed (S_1) for self-fertility and fresh yield. Populations developed by selfing and selected for self-fertility and resulting from their multiplication by open pollinated from one or two generations and synthetic populations combining to contain new populations. S_1 and S_2 lines selected for self-fertility produced significantly greater dry matter yield than unselected open pollinated progenies with higher yield often associated with significant increases in plant height, tillering and root weight. Traits over two-year showed that in both varieties dry yield increased with generations of selfing both for inbred (S_1 to S_5) populations selected for self fertility and for their sib-populations, comprising open pollinated seed of the plants selfed in each generation, exhibited a linear increase in dry yield with generation of selfing, but the rate of increase per generation was significant only for Ahaly population. Dry matter yield averaged over the selfed and open pollinated populations from synthetic 79 was 19.6 and 35.7% and from Ahaly was 13 and 29.7% significantly higher than the source variety. Results strongly suggest that selection for self fertility in berseem might bring about positive correlated responses of plant vigour traits. The implications for improving berseem forage and seed yields were discussed.

Bakheit *et al.* (2007) applied two cycles of modified mass selection and one cycle of family selection on a population of the mono-cut variety (Fahl) of Egyptian clover. They found that, the realized gains for C_1 and C_2 mass selection, respectively, were 13.6 and 8.5% for seed yield, 4.9 and 14.4% for fresh forage yield, 5.3 and 13.2% for dry forage yield, 4.1 and 11.6 for protein yield and 9.1 and 13.2% for 1000-seed weight over the base population. Gains from family selection as percentage of base population were 18.5, 8.9, 9.9, 11.6 and 3.8% for these traits. Broad sense heritability as based on variance components among half-sib families were 76.8, 76.1, 98.2, 98.7 and 84.3 for seed yield, fresh forage yield, dry forage yield, protein yield and 1000-seed weight, respectively. Also, he reported that all studied traits correlated positively with us except the correlation between seed yield and forage yield. Moreover, one half- sib family was higher than the base population by 30.1, 34.2, 3.8, 36.4 and 16.4% for seed yield, fresh forage yield, dry forage yield, protein yield and seed weight, respectively. The results suggest that family selection may be more effective for improving seed yield than modified mass selection.

Abdel-Galil *et al.* (2008) developed a synthetic population through selection in seven Egyptian clover varieties. They reported that enormous improvement was achieved where synthetic 2 had higher values in all studied traits than best parent. The realized gains from selection ranged from 13.2-34.4% for fresh forage yield, 11.4-37.7% for dry forage yield and from 2.8-8.9% for protein percentage. Heritability in broad-sense were high for seasonal fresh, dry yields and protein percentage (88.7, 88.2 and 65.0%, respectively). The environmental variations were 11.3, 11.7 and 35.4% for fresh and dry yields and protein percentage, respectively. The expected genetic advance for fresh, dry yields and protein percentage were 8.1, 2.3 and 0.8%, respectively.

Abdalla *et al.* (2009) investigated effects of inbreeding and selection for self-fertility on forage yield of the two berseem varieties and their derivative populations. The results of inbreeding and selection for fertility and forage yield of different populations in different seasons indicated that the better performance of selections over their original parents in fresh and dry yields was due mainly to the great improvement in number of tillers per plant (in some cases increase of tillers was more than 100%) and to a less degree to an increase in plant height. This will result in an improvement of vegetative growth, flowering and seed yield of selected genotypes. Comparison between Open Pollinated (OP) and Manual Tripping (MT) plants of same generations indicated absence of inbreeding depression, irrespective of expected reduced heterozygosity in MT plants: This was explained as inbreeding tolerance, due to selection for good vigor accompanying inbreeding, due to recessive abnormal genes (segregants) being wiped up in early inbreeding generations (I1-I3) and due to possibility of high fertility and good forage yield are controlled by dominant genes that could be fixed in inbred and OP populations. The results also indicated that selection for high fertility of seed set could be accompanied by selection for good forage yield. An approach was suggested to develop new composite berseem varieties characterized by high seed fertility and good forage yield.

Abd El-Naby *et al.* (2009) used seven parents and three inbreeding generations of Egyptian clover to estimate the relative changes due to increasing homozygosity on agronomic traits. They reported that inbreeding depression of I1 generation was different from each population to another. High significant differences were observed among and within population for seed setting% in all modes of pollination. Also, breeder may select for self-fertile plants and then evaluating for high forage and dry yields to obtain better improved varieties.

POLLINATION AND SEED SETTING IN BERSEEM CLOVER

Information concerning the mode of pollination is essential in setting up an effective breeding programme. Egyptian clover, *Trifolium alexandrinum* L. has been recognized for a long time as a cross-pollinated crop by insect. Contrary to this information, Beri *et al.* (1985) in India has recently demonstrated that Egyptian clover is self fertile crop where a slight percentage of cross-pollination also occurs. An attempt, has, therefore, been made by Author (Bakheit, 1989c) to study the mode of pollination in Egyptian clover using a genetic marker and to determine seed set in the presence and absence of honey bees. With this respect (Bakheit, 1989c) found that the cross pollination in berseem clover was approximately 82%. Also, he found variation between studied genotypes for open pollination, selfing with each of manual tripping and without tripping. Open pollination surpassed any selfing pollination methods for seed setting over all studied genotypes. Here too, the seed setting were 51.9, 0.96 and 17.1% under uncaged (0pen), caged and caged plus hand tripping, respectively.

Dixit *et al.* (1989) reported that the Egyptian clover commonly known as berseem in India, is a predominantly self-pollinated autogamous fodder legume. It has a simple valvular type mechanism for pollination wherein tripping agencies like bees are needed for better seed setting. The seed setting efficiency and seed yield of berseem (g.m-2) under natural tripping or open tripping condition was extremely high (22.38) as compared to completely cages (0.52) which clearly indicates the significance of pollinators/triping agencies in seed yield of berseem crop. Further, the influence of pollinations was well marked with diploid strains of berseem as compared to tetraploid because of difference in fertility levels (tetraploids are generally less fertile).

The effect of inbreeding on head fertility and seed weight was studied in berseem clover (*Trifolium alexandrinum* L.) by Iannucci (2001) to identify highly self-compatible genotypes to create new parental populations for the production, through intercrossing of hybrid varieties. Plants of three populations of Egyptian origin (Local population Giza 10 and two breeding populations; Moltifoliate and synthetic variety) and three populations originating from southern Italy (population 14 and 22 and Sacromonte) were both naturally cross pollinated and inbred by selfing, each under isolation, for four generations (S_0, S_1, S_2 and S_3). Head fertility and seed weight showed a gradual decline with selfing, decreasing by 44 and 14% from S_0 to S_3, respectively. Giza 10 and Sacromonte varieties appeared to be the most and least affected, respectively, by selfing. Results indicated that the two modes of determining self-fertility were not equal effective and the percentage of seed-set obtained under hand-tripping flowers by tooth picks conditions was very high (92%) and absence of the tripping mechanism led to a drastic reduction in self-fertility.

Inbreeding resulted in an increase in the number of completely self-incompatible plants, although differences based on geographical origin were obtained. Egyptian population was the least self-fertility. Berseem populations studied were allogamous, but selection for highly self-fertile plants was possible at the advanced selfing generations.

Abd El-Naby (2003) reported that significant differences among populations were detected for seed setting under open-pollinated, selfing by auto-tripping, F_1 under auto-tripping and F_1 of total self-fertility. Also, she reported the contribution of auto-tripping to total self-fertility dropped from about 44% in the S_1 to about 16% in the S_2 populations of S-79 selected for self-fertility and 24% for the Ahaly populations. As well as, suggest that self-fertility from manual tripping and from auto-tripping are conditioned by two different genetic systems. Auto-tripping appeared to be controlled by a relatively few number of genes with complementary gene action which is easily disturbed by inbreeding. In contrast, numerous dominant genes appear to control the response to manual tripping which accounts for the relatively lower rate of response of fertility from manual tripping to inbreeding.

Roy *et al.* (2005) used four treatments of pollination i.e. open pollination, caging, hand tripping and controlled bee visits. Compared with the open pollinated condition, reduction in seed set in different populations ranged from 12.3-99.2% under caged condition. Eight of 16 populations registered more than 90%, reduction in seed set. Hand tripping and/or controlled bee visits improved seed set considerably in a few populations, whereas, in one population, no significant effect of these four treatments was observed. The results indicated considerable variation for self-compatibility, together with a requirement of tripping for pollination and seed set, even in self-compatible lines.

Abdalla *et al.* (2008) investigated effects of inbreeding and selection for self-fertility in the two berseem varieties and their derivative populations. They reported that selection for manual tripped was effective in self-fertility in all populations in all generations over original parents and 4 check varieties. Advanced inbreeding generations were more fertile than early ones. Selection for manual tripped was accompanied by improving fertility of open pollination. Seed set was highest under open pollination followed by manual tripped but seed set of seed set was very poor. Some individual plants set seeds up to 88% in manual tripped materials. Self-sterile plants were found only in open pollination and the four check varieties. The materials used may be considered self-compatible due cross-fertilized and need tripping to result in self-seed set. *T. alexandrinum* (or progenitor) was assumed to be self-incompatible species that has been forced to inbreeding. The enigma of fertility and sterility in berseem was discussed and several explanations and hypotheses were presented and employed in a try to understand reasons for controversial results in the literature. Different genes for self-compatibility, self-incompatibility, unilateral incompatibility, female rejecting and floral structure may be operating and affecting seed set.

Abdalla and Abd El-Naby (2012) reported that selection for Manual Tripping (MT) was effective in improving Self-Fertility (SF) in all populations and generations over original parents. Selection for MT was accompanied by improving fertility of Open Pollinated (OP) populations. Self-seed set (ST) was very poor. Some individual selections set seeds up to 88% in MT materials. The enigma of fertility, sterility and absence of inbreeding depressions in berseem was discussed. This was explained as inbreeding tolerance, due to selection for good vigor accompanying inbreeding. The results indicated that selection for high MT seed set could be accompanied by selection for good forage yield. An approach was suggested to develop new composite varieties characterized by high seed fertility and high forage yield. Improved selections for seed production was mainly due to profuse flowering and more flowers/inflorescence, whereas improved forage yield was mainly due to more tillers/plant and to less degree of plant height. An approach was outlined to produce new composites with high seed setting and good forage. *T. alexandrinum* is self-compatible, but needs tripping to stimulate self seeds. This is mainly due to the relative position of male and female organs and the presence of bubbles on stigma. Molecular characterization revealed polymorphic differences between Io and selected inbred.

Bakheit *et al.* (2012) studied the influence of temperature, genotype and genotype×temperature interaction on seed yield of Egyptian clover (*Trifolium alexandrinum* L.). Five varieties of Egyptian clover were sown on three sowing dates (1st October, 1st November and 1st December). The sowing on the first of October produced the highest mean values of number of florets (5.9), seeds head^{-1} (40.9), seed set (77.8%), thousand seed weight (3.38 g) and seed yield (1.26 ton ha^{-1}). The estimates of phenotypic stability parameters (b_1 and S^2_d) for seed yield showed that the highest seed yielding varieties Giza 15 and 6 exhibited less instability in seed yield while the Assiut population (multifoliate strain) was more stable.

DEVELOPMENT OF A NEW MULTIFOLIATE STRAIN OF EGYPTIAN CLOVER (*TRIFOLIUM ALEXANDRINUM* L.) IN EGYPT

Cultivars of Egyptian clover (*Trifolium alexandrinum* L.) have trifoliate leaves. The term multifoliate is used to describe leaves consisting of more than three leaflets plants with large leaf area exhibit high rates of photosynthesis per plant and thus tend to be high yielding. Therefore, understanding factors influencing leaf area development of berseem clover may provide valuable insight into yield determining factors of this species. Increasing the number of leaflets per leaf may be one method of modifying plant leaf area with this respect. Bakheit (1996) research on berseem clover over ten years period resulted in the transfer of the multifohate gene(s) from a mutant plant of the mono-cut Fahl cultivar which possess such trait to the multi-cut trifoliate cultivar Meskawi by crossing between them. A multi-cut multifoliate strain was developed after nine generations of selection. The multifoliate strain surpassed the commercial cultivars i.e., Giza 6, 10, 15, Sakha 4 and Helaly by 36.7, 22.1, 24.8, 22.5, 29.9%, respectively in the first cut. Therefore, this multifoliate multi-cut strain could be useful for forage especially before sowing cotton or other early summer crops. In addition to that, it could be used as a genetic marker for berseem breeders. Also, Bakheit and El-Nehrawi (1997) and Bakheit (2003) re-evaluated the new multifoliate strain of Egyptian clover with eleven Egyptian varieties of berseem clover. They reported that, the new multifoliate strain gave the highest fresh and dry forage yield in the first cut. Also, the new multifoliate strain gave higher seasonal dry forage yield than six commercial varieties by range of 4.0-14.3%.

Bakheit *et al.* (2008) the new multifoliate strain was subject to field evaluation with seven Egyptian commercial cultivars. The multifohate strain gave significant higher fresh and dry forage yield than all other five commercial cultivars except Gommiza and mono-cut Fahl cultivars in the first cut.

Protein and RAPD markers were used to identify and assess the phylogenetic relationship between the new multifoliate strain and the other Egyptian commercial cultivar. A total of 31 protein bands were generated from the new multifoliate strain including five polymorphic bands. Seven protein bands disappeared in the new strain while they appeared at least in one or more of the commercial cultivars. The analysis of relationship between the new multifoliate strain and commercial cultivars showed genetic diversity. The protein cluster showed three main cluster groups while the new multifoliate strain took an independent position. The new multifoliate strain was very distinct and unique from all other commercial cultivars. Out of 13 bands generated from five primer four bands 402 bp (B7 primer), 505 and 610 bp (A12 primer) and 708 bp (C10 primer) could be used as positive marker for new multifoliate strain.

The multifoliate strain with another populations were evaluated in Italy by Martiniello *et al.* (1992), Iannucci *et al.* (1996), Martiniello and Iannucci (1998) and Iannucci (2001) for forage and seed yields, chemical component and variability.

CONCLUSION

From this review article the investigator recommend that using of synthetic variety methods as a recommended method for berssem breeding after evaluated genotypes for yield and combining ability.

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