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### Selection for Frequent Cutting Tolerance among Egyptian Ecotypes of Barseem Clover "*Trifolium alexandrinum*, L."

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#### ABSTRACT

The main objective of the recent study was to trace barseem clover landraces with potential resistance to frequent cutting stress. 200 ecotypes of multi-cut barseem clover were collected from ten governorates. Ecotypes were divided to ten random sets each of 20 ecotypes. For each set ecotypes, the most tolerance ecotypes to very frequent cutting regime were identified during two successive cycles. Commonly, the first cycle of selection gave a cumulative realized gain over cuttings in green forage yield over the base population reached 9.09, 76.38 and 46.62% when evaluated under infrequent, frequent and very frequent cutting regimes, respectively. Meanwhile, the second cycle of selection gave a substantial realized gain in green forage yield relative to cycle one reached 108.6, 94.52 and 180.56% when evaluated under infrequent, frequent and very frequent cutting. Cumulative realized gain over cuttings in related to the average of the evaluated check varieties were -23.14, 104.2 and 177.6 when evaluated under infrequent, frequent and very frequent cutting regimes. Meanwhile, the second cycle of selection (C2) gave a cumulative realized gain over cuttings relative to the average of the first cycle (C1) reached 67.32, 218.6 and 367.1% when evaluated under infrequent, frequent and very frequent cutting regimes. It was evident that the realized gain in green forage yield from selection for frequent cutting tolerance among barseem ecotypes was more obvious after the second cycle of selection. Also, the superiority of selected cycles (C1 and C2) was clearly shown over the studied check varieties.

**Keywords:** barseem clover, selection, frequent cutting tolerance, green forage yield, realized gain.



#### INTRODUCTION

Barseem clover is the principal forage crop in Egypt, as well as, many other Mediterranean countries. Egyptian farmers rely on barseem as a base feed for animals, because of animal's high rate of ingestion even when mixed with another feed or forage of higher dry matter content and less palatability. That animal's behavior is mostly reflected on higher animal's returns. The positive role of barseem as a legume forage when fed to animals goes to the levels of poly unsaturated fatty acid in milk or meat (Wu *et al.*, 1997, and Fraser *et al.*, 2004), along with low levels of Fecal methane (Dewhurst *et al.*, 2009). Besides, the role of barseem as a nitrogen fixer that maintain the basic principle of sustainable agriculture (Heichel and Henjm, 1991 and Morris and Greene, 2001). This role is accomplished by barseem through increasing the efficiency of nitrogen use and reduce competition for soil nitrate and transfer nitrogen from roots to soil to become available for other species that share the space and follow barseem in rotation (Temperton, 2007).

Breeding barseem clover for frequent cutting tolerance can be defined as breeding to achieve acceptable balance between persistence, quality, yield and animal's safety (Sewell *et al.*, 2011). Identifying a specific plant type then applying selection program is essential to reach a frequent-cutting tolerant population (Annicchiarico *et al.*, 2010). Moreover, it is well known that selection for one trait or many traits related to frequent-cutting tolerance might lead to reduction of productivity and loss or other quality characters (Smith *et al.*, 2000). In white clover "*Trifolium repens*, L." Frequent-cutting tolerant genotype had morphological character correlated with stolon diameter, branching potentiality, leaf size and root structure (Ayres *et al.*,

1996; Jahufer *et al.*, 2002, Sanderson *et al.*, 2003, Annicchiarico and Piano, 2004, Bouton *et al.*, 2005 and Jahufer *et al.*, 2008). Stolon and roots morphology affect white clover persistence through influencing water and nutrients use.

Breeding for frequent-cutting tolerance is a type of breeding for special type of adaption (Annicchiarico *et al.*, 2010). Consequently, such programs must use genotypes that developed through specially improved adaptive mechanisms. In other words, must depend on ecotypic selection, which might be most efficient, since, legumes are more sensitive to stress conditions in comparison to grasses. Another reason for the former assumption, is that all newly developed barseem cultivars were bred under optimum conditions for productivity. There are many successful examples for the use of ecotypic selection to improve persistence and adaptability in forage legume species. Among them, Finne, 2002 for frost-tolerance, Spencer *et al.*, 1980. For phosphorus-deficient tolerance, Mitev and Goranova, 2008, for Aluminum and magnesium high levels tolerance, Ayres *et al.*, 1996, for drought tolerance, Radovic *et al.*, 2008, and Piano and Annicchiarico, 1995, for persistence related to high productivity and feeding value (Goranova and Mihovsky, 2005, and Vuckovic *et al.*, 2007). Lusic *et al.*, 2004 for persistence, Naydenova, 2008, for high productivity in late-season growth.

Depending on the aforementioned studies, it is possible to say, that high degree of intrapopulation variability and dominant crosspollination lead to rapid genetic draft in population's ecotypes, whether under normal or artificial selection (Collins, *et al.*, 2002). Such variability in genotypes adaptability to Frequent-cutting tolerance might be used and

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transferred to commercial cultural germplasm through traditional breeding methods (Boller, et.al, 2012).

The recently cultivated ecotypes of barseem clover in Egypt is the genotypic result of environment × the method of use interaction that dominated at long history of cultivation in identified environments under certain agriculture practices, which is responsible for what is known today as barseem clover ecotypes. The main objectives of the recent study were; 1) identifying ecotypes of barseem clover that has adaptive responses to frequent-cutting tolerance, 2) Improving frequent-cutting tolerance in Egyptian barseem clover gene-pool by selection, ending in a new population of adaptive response

## MATERIALS AND METHODS

### "Ecotypes evaluation"

200 ecotypes of multi- cut barseem clover were collected from ten governorates (Assuit, Sohag, El-Minya, EL-Fayoum, Giza, Qalyubia, Monufia, Kafr EL- Sheikh, EL-Bahira and EL- Sharkia) covering different environments of Egypt. Farmers' fields and local markets in each governorate supported ten distinguished ecotypes.

In winter season of the first year (2016/2017), ecotypes (200) were divided to ten random sets each of 20 ecotypes. Each set was sown in a replicated trial of three replicates. Each replicate

**Table 1. Cutting frequency levels.**

Cutting frequency level	1 <sup>st</sup> Cutting	2 <sup>nd</sup> Cutting	3 <sup>rd</sup> Cutting	4 <sup>th</sup> Cutting	5 <sup>th</sup> Cutting	6 <sup>th</sup> Cutting	7 <sup>th</sup> Cutting
Infrequent	50	45	30	30	30	-	-
Frequent	50	40	25	25	25	20	-
Very frequent	40	35	25	25	20	20	20

Trails of the ten sets were laid-out in a rectangular form, when possible.

### Nucleus of first selected cycle:

At seed ripening, selected ecotypes were harvest separately. Equal seed weights representing each ecotype (total of 40 ecotypes) were mixed to produce composite I seed. Remaining seeds of each selected ecotype were used for second cycle of selection.

### 2017/2018 season:

Selected ecotypes (40 ecotype) were evaluated in two sets each of 20 ecotypes. Each set was evaluated in a split-plot design with cutting frequency regimes in main-plots and ecotypes in sub-plots. Three replicates were used. Each sub-plot was four rows of four meter long and 0.2 m apart. Seeding rate was 13 kg faddan<sup>-1</sup>. The same cutting Shadwell as in the first season was applied (Table 1). Data were recorded for plant height, green and dry forage yields, crown diameter, number of tillers and leaves percentage similar to what indicated in the first cycle.

### Identifying elite ecotypes:

Depending on green forage yield analysis for each set, the best 20% producing ecotypes under very frequent cutting regime were identified (four ecotypes). Unselected ecotypes were uprooted before flowering. Vigorous plants in each selected ecotype's plot were selected, whereas, other plants with small crown size were uprooted. Muslin cloth covering a surrounding post (cadge) was used to isolate the two sets of selected plants. A heave of honeybee was placed inside the muslin cadge to permit random matting and avoid assortative matting.

### Nucleus of the second selected cycle:

Seeds of selected ecotypes were harvested separately. Equal seed weight representing each ecotype (total of eight ecotypes) was mixed to produce composite II seed.

was divided to three main plots. Each main plot was randomly subjected to one of three cutting frequency levels, i.e.; a) Infrequent; where, first cutting was taken after 50 days, second cutting after 45 days, third fourth and fifth cuttings after 30 days each, b) Frequent, where, first cutting was taken after 50 days, second cutting after 40 days, third, fourth and fifth cuttings after 25 days each then sixth cutting after 20 days and C) Very frequent, where, first cutting was taken after 40 days, second cutting after 35 days, third and fourth cuttings after 25 days each, and fifth, sixth and seventh cuttings after 20 days each. The sub-plots randomly received the assigned ecotypes. Each sub- plot comprised four rows of four meters long and 0.2 meter apart. Seeding rate was 13 kg faddan<sup>-1</sup>. Table 1 summarize the cutting frequency levels.

### Identifying elite ecotypes:

Depending on data analysis for each set ecotypes, the most tolerance ecotypes to very frequent cutting regime (High green forage yield), were identified. Unselected ecotypes were uprooted prior to flowering. Vigorous plants in each selected ecotype's plot were selected, whereas, other plants of small crown size were uprooted. Adequate irrigation along with honeybee heaves was maintained in flowering field to permit random cross-pollination and good seed setting.

### 2018/2019 season

During the winter season of 2018/2019, seeds of composite I, composite II, seed mixture of base population and three commercial cultivars namely; Giza 6, Serrow and Hellaly Were evaluated. Split-plot design with three replicates was used. Main plots were devoted to cutting frequency regimes, whereas, sub-plots represented the evaluated populations. Plot size was six rows, four meters long and 0.2 m a part. Response to selection was traced by recording Green forage yield. Data collected was analyzed as a split-plot according to Cochran and Cox (1957).

## RESULTS AND DISCUSSION

The recent study was designed to select landraces of barseem clover "*Trifolium alexandrinum*, L." that is adapted to (withstand) frequent cutting regimes. Two cycles of selection were practiced. The selected populations were evaluated in terms of green and dry forage yields under infrequent, frequent and very frequent cutting regimes. Three check-verities were included, namely; Giza6, Serrow and Hellaly.

Table 2 showed mean squares of green forage yield for the evaluated barseem populations as affected by cutting, cutting regime and their interactions. The obtained yield from different cuttings was significantly ( $p \geq 0.01$ ) different. Cutting regimes, significantly ( $p \geq 0.01$ ) affected green yield. The evaluated populations were significantly ( $p \geq 0.01$ ) different with regard to the produced green forage. Also, the interaction between cutting and cutting regime (C×R) was significant ( $p \geq 0.01$ ), indicating that, the obtained green forage yield with each cutting varied depending upon the practiced cutting regime. In the meantime, the tested barseem population (base, selected and checks) gave significantly ( $p \geq 0.01$ ) different green yield among the harvested cuttings (significant C×P

interaction). Evaluated populations gave significantly ( $p \geq 0.01$ ) different green yield with different cutting regimes (significant R×P interaction). The magnitude of green forage yield produced by each evaluated population significantly ( $p \geq 0.05$ ) varied in rank or magnitude from other evaluated populations, when exposed to different cutting regime in each separate cutting (significant cutting × Regime × population interaction).

**Table 2. Mean squares of green forage yield for barseem populations as affected by cutting regime and their interactions, analyzed over cuttings.**

Source of variance	d.f	Mean Squares
Regimes (R)	2	16704253.31**
Rep / regimes	14	638169.16
Cutting (C)	6	94126189.91**
C × R	12	52485378.59**
Error	28	744608.57
Population (P)	5	13175938.19**
C × P	30	1790705.24**
R × P	10	1756389.73**
C × R × P	60	712499.92*
Error	210	511496.53

Means of green forage yield (kg plot<sup>-1</sup>) for the evaluated populations under different cutting regimes were presented in Table 3. Under the infrequent cutting regime, the first selected population gave significantly lower green yield

than the base population during the first three cutting (2.57 vs. 3.05, 4.27 vs. 5.55, 6.28 vs. 6.61 kg plot<sup>-1</sup>) for the former and the latter during the three successive cuttings, respectively. By the fourth and fifth cutting, the obtained green yield from the first selected cycle significantly surpassed the base population (6.29 vs. 4.80 and 5.37 vs. 4.42 kg plot<sup>-1</sup>, respectively). The second cycle of selection scored significantly higher green yield in all studied cuttings. That yield was insignificantly different from that scored by the checks in cutting two and three under frequent cutting regime. The significant superiority of selected cycles over the base population, had not realized until the second cutting (4.47, 5.38 and 5.31 kg plot<sup>-1</sup> for base, first selected and second selected populations, respectively), then, continued until the 6<sup>th</sup> cutting. The significant superiority over the average of check varieties was only scored by the third cutting, and then continued to the 6<sup>th</sup> cutting.

The significant superiority of the first selected population in green forage yield (kg plot<sup>-1</sup>) under the very frequent cutting regime over the base population, had not realized until the fourth cutting. Also, it was shown in the 6<sup>th</sup> and 7<sup>th</sup> cuttings (4.89, 3.98 and 4.61 vs. 4.46, 3.16 and 3.89 kg plot<sup>-1</sup> for the fourth, sixth and the seventh cuttings of the first selected (C<sub>1</sub>) and the base population, respectively).

**Table 3. Means, of green forage yield for barseem population under different cutting regimes.**

Regime	Population	Means							
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	
I Infrequent	Base population (C <sub>0</sub> )	3.05	5.55	6.60	4.80	4.42			
	Selected population (C <sub>1</sub> )	2.57	4.27	6.28	6.30	5.37			
	Selected population (C <sub>2</sub> )	3.69	5.97	6.68	6.83	5.92			
				Checks					
II Frequent	Giza 6	3.96	5.88	6.81	5.60	5.52			
	Serrow	3.04	5.64	6.17	5.23	3.95			
	Hellaly	3.13	6.06	6.43	5.14	4.85			
	Average	3.37	5.86	6.47	5.32	4.77			
III Very Frequent	Base population (C <sub>0</sub> )	4.04	4.67	5.31	4.24	3.83	3.83		
	Selected population (C <sub>1</sub> )	2.74	5.38	5.76	5.49	4.88	4.89		
	Selected population (C <sub>2</sub> )	3.44	5.31	5.52	5.85	6.55	6.55		
				Checks					
I Infrequent	Giza 6	3.59	4.94	6.32	4.05	4.02	4.02		
	Serrow	4.06	5.77	48.8	3.84	3.90	3.90		
	Hellaly	3.42	4.90	4.17	3.65	2.87	2.87		
	Average	3.69	5.20	5.13	3.85	3.4	3.4		
II Frequent	Base population (C <sub>0</sub> )	2.19	2.26	2.67	4.46	3.58	3.16	3.89	
	Selected population (C <sub>1</sub> )	2.12	2.30	2.50	4.89	3.62	3.98	4.61	
	Selected population (C <sub>2</sub> )	3.11	2.75	3.39	5.18	4.26	5.04	5.85	
				Checks					
III Very Frequent	Giza 6	2.43	2.92	3.40	4.00	3.57	3.41	3.38	
	Serrow	2.36	2.96	2.30	2.93	2.77	1.91	2.91	
	Hellaly	2.92	2.50	1.53	2.13	2.77	2.05	2.98	
	Average	2.57	2.80	2.41	3.02	3.04	2.46	3.09	
L.S.D interaction			0.31						

Realized gain % in green forage yield due to selection of frequent cutting tolerant ecotypes of barseem clover, relative to base population (C<sub>0</sub>) and first selection population (C<sub>1</sub>) were illustrated in Table (4). The first cycle of selection for very-frequent cutting tolerance, when evaluated under infrequent cutting regime showed a reduction in green yield reached -15.71, -22.99 and -4.93% in the first, second and third cuttings, respectively. Whereas, an improvement in green forage productivity amounted to 31.19 and 21.53% of the base population was realized by the fourth and fifth

cuttings. In contrary, the second selected population (C<sub>2</sub>) showed a positive superiority over the first selected population reached 43.73, 39.81, 6.37, 8.56 and 10.21 for the five successive cuttings, respectively.

Frequent cutting regime, showed reduction in realized green forage yield gain of (C<sub>1</sub>) relative to the base population reached -32.26% in the first cutting. Whereas, a positive gain was scored during the following five cuttings (15.23, 8.36, 29.53, 27.76 and 27.76% for cuttings from the second to the sixth, respectively). Second selected cycle (C<sub>2</sub>) showed a

positive realized gain in green forage yield relative to the first selected cycle in all cuttings, but, the second and the third that showed negative response (25.57, -1.25, -4.21, 6.63, 33.89 and 33.89% for the six successive cuttings, respectively).

Evaluation of selected cycles under very frequent cutting showed a positive realized gain in green forage yield

of (C<sub>1</sub>) over the base population, except for, the first and the third cuttings (-3.28, 1.59, -6.46, 9.52, 1.06, 25.87 and 18.32% for the seven cuttings, respectively). Meanwhile, the second selected cycle (C<sub>2</sub>) scored positive realized gain throughout the seven cuttings (46.40, 20.58, 35.86, 5.89, 17.62, 26.48 and 27.23% for the seven cuttings, respectively).

**Table 4. Realized gain (%) relative to base population (C<sub>0</sub>) and first cycle of selection (C<sub>1</sub>) of green forage yield for barseem population under different cutting regimes.**

Regime	Population	Realized gain % Relative to													
		C <sub>0</sub>							C <sub>1</sub>						
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>
I Infrequent	Base population (C <sub>0</sub> )														
	Selected population (C <sub>1</sub> )	-15.71	-22.99	-4.93	31.19	21.53									
	Selected population (C <sub>2</sub> )								43.73	39.81	6.37	8.56	10.12		
II Frequent	Base population (C <sub>0</sub> )														
	Selected population (C <sub>1</sub> )	-32.26	15.23	8.36	29.53	27.76	27.76								
	Selected population (C <sub>2</sub> )								25.57	-1.25	-4.21	6.63	33.89	33.89	
III Very Frequent	Base population (C <sub>0</sub> )														
	Selected population (C <sub>1</sub> )	-3.28	1.59	-6.46	9.52	1.06	25.87	18.32							
	Selected population (C <sub>2</sub> )								46.90	20.58	35.86	5.89	17.62	26.48	27.23

Table 5, showed the realized gain relative to the average of check varieties due to selection for frequent-cutting tolerance among ecotypes of barseem clover. The first selected population (C<sub>1</sub>), exhibited superiority with positive gain over the average of tested check, under any of the studied cutting regimes, except for, the first three cuttings of infrequent cutting regime, the first cutting of the frequent

cutting regime and the first and the second cuttings of the very frequent regime. While, the second cycle of selection (C<sub>2</sub>), showed positive realized gains over the average of the check varieties, irrespective of the used cutting-frequency regime, except for the first cutting under frequent cutting regime and the second cutting under the very frequent regime.

**Table 5. Realized gain (%) relative to the average of checks of green forage yield for barseem population under different cutting regimes.**

Regime	Population	Realized gain % Relative to Checks													
		C <sub>1</sub>							C <sub>2</sub>						
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>
I Infrequent	Base population (C <sub>0</sub> )														
	Selected population (C <sub>1</sub> )	-23.81	-27.13	-2.94	18.16	12.58									
	Selected population (C <sub>2</sub> )								9.95	1.88	3.25	28.27	23.97		
II Frequent	Base population (C <sub>0</sub> )														
	Selected population (C <sub>1</sub> )	-25.79	3.47	12.35	42.61	35.79	35.79								
	Selected population (C <sub>2</sub> )								-6.82	2.08	7.62	52.07	81.83	81.83	
III Very Frequent	Base population (C <sub>0</sub> )														
	Selected population (C <sub>1</sub> )	-0.17	-17.74	3.49	61.68	19.29	62.14	48.91							
	Selected population (C <sub>2</sub> )								21.05	-0.81	40.61	71.22	40.51	105.1	89.46

Commonly, the first cycle of selection for frequent-cutting tolerance gave a cumulative realized gain over cuttings in green forage yield over the base population reached 9.09, 76.38 and 46.62% when evaluated under infrequent, frequent and very frequent cutting regimes, respectively. Meanwhile, the second cycle of selection (C<sub>2</sub>) for frequent cutting tolerance gave a substantial realized gain in green forage yield relative to cycle one (C<sub>1</sub>) reached 108.6, 94.52 and 180.56% when evaluated under infrequent, frequent and very frequent cutting.

Cumulative realized gain over cuttings in green forage yield related to the average of the evaluated check varieties were -23.14, 104.2 and 177.6 when evaluated under infrequent, frequent and very frequent cutting regimes. Meanwhile, the second cycle of selection (C<sub>2</sub>) gave a cumulative realized gain over cuttings in green forage yield relative to the average of the first cycle (C<sub>1</sub>) reached 67.32, 218.6 and 367.1% when evaluated under infrequent, frequent and very frequent cutting regimes.

It was evident that the realized gain in green forage yield from selection for frequent cutting tolerance among barseem ecotypes, was more obvious after the second cycle

of selection proposing that successive cycles of selection might result in better gains. Also, the superiority of selected cycles (C<sub>1</sub> and C<sub>2</sub>) was clearly shown over the studied check varieties. Meanwhile, the advantage of selection for frequent cutting tolerance was sounder and more illustrative, when improved cycles were evaluated under very frequent cutting regime.

Breeding barseem clover for frequent cutting tolerance can be defined as breeding to achieve acceptable balance between persistence, quality, yield and animal's safety (Sewell *et al.*, 2011). Identifying a specific plant type then applying selection program is essential to reach a frequent-cutting tolerant population (Annicchiarico *et al.*, 2010). Breeding for frequent-cutting tolerance depending on single character is though insufficient. Since, frequent-cutting tolerance is considered as a complex character that includes many morphological and physiological traits, along with their interactions with environment (Katepa-Mupondwa, *et al.*, 2002). Moreover, it is well know that selection for one trait or many traits related to frequent-cutting tolerance might lead to reduction of productivity and loss or other quality characters (Smith *et al.*, 2000). In white clover "*Trifolium repens*, L.

"Frequent-cutting tolerant genotype had morphological character correlated with stolon diameter, branching potentiality, leaf size and root structure (Ayres *et.al*,1996; Jahufer *et.al*, 2002, Sanderson *et.al*,2003, Annicchiarico and Piano, 2004, Bouton *et.al*,2005 and Jahufer *et.al*,2008). Stolon and roots morphology affect white clover persistence through influencing water and nutrients use. Taylor (2008) described legume forage persistence (continuous productivity irrespective of frequent cutting) as a result of interaction between adaptive potentialities of genotype and stress condition that result in a level of stress tolerance. So, all breeding effects to acquire tolerance to stresses (climatic, edaphic or biotic) are trials to reach plant potentiality to reach tolerance and persistence. Many researchers attempted to reach that, among them Tayloy,2008 on red clover "*Trifolium pretense*, L". Annicchiarico, 1997 and Widdup and Barret, 2011 on white clover "*Trifolium repens*,L." and Smith *et.al*, 2000 and Bouton, 2012 on alfalfa "*Medicago sativa*, L."

Breeding for frequent-cutting tolerance is a type of breeding for special type of adaption (Annicchiarico *et.al*, 2010). Consequently, such programs must use genotypes that developed through specially improved adaptive mechanisms. In other words, must depend on ecotypic selection, which might be most efficient, since, legumes are more sensitive to stress conditions in comparison to grasses. Another reason for the former assumption is that all newly developed barseem cultivars were bred under optimum conditions for productivity. There are many successful examples for the use of ecotypic selection to improve persistence and adaptability in forage legume species. Among then, Finne, 2002 for frost-tolerance, Spencer *et.al*, 1980. For phosphorus-deficient tolerance, Mitev and Goranova, 2008, for Aluminum and Magnesium high levels tolerance, Ayres *et.al*, 1996, for drought tolerance, Radovic *et.al*, 2008, and Piano and Annicchiarico, 1995, for persistence related to high productivity and feeding value (Goronova and Mihovsky, 2005, and Vuckovic *et.al*, 2007). Lusic *et.al*, 2004 for persistence, Naydenova, 2008, for high productivity in late-season growth.

Depending on the aforementioned studies, it is possible to say, that high degree of intrapopulation variability and dominant crosspollination lead to rapid genetic draft in population's ecotypes, whether under normal or artificial selection (Collins, *et.al*, 2002). Such variability in genotypes adaptability to Frequent-cutting tolerance might be used and transferred to commercial cultural germplasm through traditional breeding methods (Boller, *et.al*, 2012). The recent results might be discussed considering previous studies on breeding for improved green forage yield of barseem clover. Abou El-Shawareb (1971), studied the efficiency of mass and recurrent selection breeding methods on Miskawy variety of barseem. The gain in yield obtained by the modified mass selection technique after one cycle were 11.6, 19.8, 16.7 and 17.7 % over the original population for three successive cuts and total yield. He concluded that, both mass and modified mass selection methods might be efficient in improving the yield of barseem clover. El-Nahrawy (1980), studied variation in productivity of farmers seed lots of Miskawy barseem. Selection of the top lots from original seed was expected to be fruitful approach to the improvement of green forage yield by selection. Omara and Hussein (1982), evaluated

two cycle of mass selection for forage yield in Miskawy Egyptian clover. They found a significant response to mass selection after the first cycle of selection which were 20.58% and 5.11 % over the base population mean in the two successive yield trails. Also; no further response had obtained after the second cycle of selection. Bakheit (1985), reported that, the realized gains of the first and second cycle of mass selection in Egyptian clover for fresh forage yield were 8.43% and 10.7% of the original population, respectively. Also, he found that, family selection was more rewarding that mass selection and produced a response of 15.48% of the unselected base family mean after one cycle of selection. Bakheit and Mahdy (1988), studied the efficiency of one cycle of pedigree selection for forage yield among 34 Egyptian clover (Miskawy) accessions. The overall families mean (42.8 tons/fad.) surpassed significantly the base populations mean (37.5 tons/fad.). The observed gain from selection relative to the base populations mean and the check variety Giza-1 amounted to 14.14% and 13.77% respectively. These results reflect the efficiency of pedigree selection in improving fresh forage yield. Bakheit (1989a), studied the response of Egyptian clover forage yield to recurrent selection and synthetic varieties. Data showed that, the realized gains were 13.9 and 14.8% for fresh forage yield in the first and second cycle of recurrent selection over the base population, respectively. The first generation of the synthetic (syn.1.f<sub>1</sub>) showed an increase over parents of 3.7% in fresh forage yield relative to check variety Giza-1. Corresponding means in (syn.1.f<sub>2</sub>) were not different. Bakheit (1989b), applied two generations of modified mass and family selection of Egyptian clover *cv. Fahl*. The cycle 1 and cycle 2 of half-sib families and modified mass selection along with the base population family were evaluated for forage yield. The realized gains from modified mass selection were 6.03 and 9.31 for fresh forage yield in cycle 1 and cycle 2, respectively, over the base population, while the realized gain from family selection as a percentage of the base population mean amounted to 11.32% for fresh forage yield. Ahmed (1992), compared three selection methods regarding improving forage yield of *Miskawy* barseem over two seasons. The realized gain in green forage yield reached 27% over parents and check variety, meanwhile, the expected gain from selecting the highest 20% was about 18.98% indicating that selection based on half-sib families might be feasible with Egyptian clover. Maternal line selection with S<sub>1</sub> progeny as a mating units (M-L) s<sub>1</sub> gave the highest gain among the selection methods. The improvement obtained by two cycles of modified mass selection (C.M) c<sub>2</sub> was not significantly different from that obtained by maternal line selection with half-sib progeny as a mating unit (M-L) h.s. Bakheit and El-Nahrawy (1997), evaluated progress in forage yield improvement in alfalfa using two methods of breeding, namely recurrent selection and seed synthetic. They found that, realized gains over the base population were 17.7 and 25.2% for fresh forage yields in the first and second cycles of recurrent selection, respectively. Also, they reported that, population improvement through the accumulation of favorable alleles and elimination of deleterious alleles and increasing the level of heterozygosis would contribute to alfalfa varieties development. Ahmed (2000), compared single trait with multiple trait selection in

barseem clover. He reached that, selection for multiple traits was significantly much rewarding than single trait selection. A realized gain of 12.20 from index selection was obtained vs. 6.58 from single trait recurrent selection for total green forage yield. Awad (2001), studied the genetic variation and response to selection in fresh forage yield of five Egyptian clover populations. He reached that, each selected population out-yielded the base population in fresh forage yields. Ahmed (2006), studied the response to three methods of recurrent selection (half-sib family selection,  $S_1$  family selection and  $S_2$ -family selection) in khadarawi multi-cut barseem population. Selection based on protein yield resulted in significant changes in other studied characters. Fresh forage yield was significantly improved with all three methods of selection. The realized gain per cycle due to H.S reached 3.1 ton / faddan (7.14 and 5.66% relative to base population and the average of checks, respectively). A more realized correlated gain of 5.19 ton / fad (11.96 and 10.40%) resulted with  $S_1$  selection (relative to the base and the checks, respectively). Corresponding values for  $S_2$  selection were insignificantly different from  $S_1$  selection, were 5.75 ton / fad (13.25 and 11.68%, respectively). On per year basis the realized gain from  $S_1$  family selection was the highest as 1.73 ton / fad (3.99 and 3.47% of base population and checks, respectively). Tag El-Din (2006), studied genetic improvement in five local alfalfa varieties, through applying two cycles of phenotypic directional selection. Results indicated positive responses to selection in fresh forage yield per plant with an average of 7.57% of the base population mean. Abd El-Galil et al. (2007) evaluated yield potential, genetic variation, correlation and path coefficient for two newly developed synthetics, compared to three commercial varieties of alfalfa. Data revealed that, syn.2 had higher fresh forage yield due to high plant height, high number of tillers and leaf/stem ratio than Ismailia-94. Bakheit et al. (2007) studied the efficiency of modified mass selection and one cycle of family selection for highly seed yield in Egyptian clover cv. *Fahl*. The realized gains from  $C_1$  and  $C_2$  mass selection for fresh forage yield were 4.94 and 14.38%, respectively. Rajab (2010), studied response to selection within 14 populations that were selected from 71 populations of Egyptian clover. Selection was carried-out based on fresh forage yield. Two cycles of selection within the selected populations resulted in significant differences among the selected and the original populations. Mean of fresh yield for the selected seven populations ranged from 42.03 to 44.51 t fed<sup>-1</sup> compared to the check variety (38.01 t fed<sup>-1</sup>). As a result of selection, relative increases in fresh yield over the check average of 11.29-17.10% were obtained. Bakhiet et al. (2011), studied the effect of two different methods of selection (modified mass and family selection) for crown diameter on forage yield and quality of *Ismailia-91* alfalfa variety. The realized gains in fresh forage yield from mass selections were 14.94%, while gain from family selection was 17.24% over the base population.

## CONCLUSION

Evaluation and release of superior accessions is the prime goal of ecotypic selection. The evaluated accessions are mostly populations of plants grown for long time under prevailing conditions. Screening of accessions requires multiple of seasons. When breeding methodology is practiced

through a cyclic pattern, hence the term recurrent is used to describe breeding method. The process includes the base population from which superior individuals are identified and recombined to concentrate all desirable genes, resulting in a new population for the next cycle of selection. Repeating of cycles depends up on the level of desired response and the existence of variability.

While the success of recurrent selection depends upon the inheritance of selected character (s), gain from ecotypic depends essentially on the superiority of initial accessions and the success of screening protocol. Genetic improvement of original base accessions is doubtful unless, poor individuals were culled out during screening. The main objective of the recent study was to trace barseem clover "*Trifolium alexandrinum*, L" landraces with potential resistance to frequent cutting stress. Those might represent a base for generating anew synthetic suitable for continues commercial supply of green forage. That approach depends on inducing the transcription of genes that controls fast regrowth and efficient carbohydrate reserves at low levels of leaves area.

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## الانتخاب لتحمل الحش المتكرر بين الطرز البيئية المصرية من البرسيم المصري . " *Trifolium alexandrinum*, L " محمد عبدالستار أحمد<sup>1\*</sup> ، طارق كامل عبدالعزيز<sup>2</sup>، وليد محمد محمد الديبكي<sup>2</sup> وأسماء محمد سميرراضي<sup>1</sup>

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يعتبر البرسيم المصري محصول العلف الأساسي في مصر والعديد من دول حوض البحر الأبيض المتوسط. الهدف الأساسي من الدراسة الحالية هو تحديد الطرز البيئية من البرسيم المصري التي لها القدرة علي تحمل إجهاد الحش المتكرر. تم تجميع 200 طراز بيئي من البرسيم المصري متعدد الحشوات من عشرة محافظات تمثل البيئات المختلفة في مصر. قسمت الطرز البيئية إلي عشرة مجموعات عشوائية بكل منها عشرون طراز بيئي وزرعت كل مجموعة في تجربة بمكررات. واعتماداً علي تحليل نتائج كل مجموعة، تم انتخاب أكثر الطرز البيئية تحملاً للحش المتكرر ممثلة بارتفاع محصول العلف الأخضر وكذلك لدورتان. اعطت الدورة الأولى من الانتخاب استجابة محققة تجميعية للحشوات المتتالية في محصول العلف الأخضر منسوبة إلي محصول دورة الأساس بلغت 9.09 و 76.38 و 46.62% وذلك عند تقييمها تحت نظم الحش الغير متكرر والمتكرر وشديد التكرار علي الترتيب. بينما نتج عن الدورة الثانية من الانتخاب، تحسن في محصول العلف الأخضر منسوباً إلي محصول الدورة الأولى من الانتخاب بلغ 108.6 و 94.52 و 180.56% عند تقييم الدورة الثانية تحت نظم الحش الغير متكرر والمتكرر وشديد التكرار علي الترتيب. وعند حساب التحسن المحقق في محصول العلف الأخضر من الدورة الأولى للانتخاب منسوباً إلي متوسط الأصناف الاختبارية المدروسة، فإن القيم بلغت 23.14 و 104.2 و 177.6% تحت نظم الحش الغير متكرر والمتكرر وشديد التكرار علي الترتيب. أما الدورة الثانية من الانتخاب فقد حققت تحسن في محصول العلف الأخضر بلغ 67.32 و 218.6 و 367.1% من متوسط الأصناف الاختبارية عند تقييمها تحت نظم الحش الثلاث علي الترتيب. ومن الواضح أن التحسن المحقق في محصول العلف الأخضر نتيجة للانتخاب بين الطرز البيئية للبرسيم المصري لتحمل الحش المتكرر كان أكثر وضوحاً بعد الدورة الثانية من الانتخاب بما يضمن زيادة التحسن مع تقدم دورات الانتخاب، كذلك كان من الواضح تفوق دورات الانتخاب علي متوسط الأصناف الاختبارية. يضاف لذلك بأن أفضل تحسن تم رصده تحت نظم الحش شديد التكرار.