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The Influence of Temperature, Genotype and Genotype x Temperature Interaction on Seed Yield of Berseem Clover (*Trifolium alexandrinum* L.)

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ABSTRACT
This study was carried out to evaluate the effect of temperature during the stage of flowering and seed maturity and the effect of genotype x environment interaction on seed yield and its components in recently developed varieties of berseem (*Trifolium alexandrinum* L.). Five varieties (Giza 15, Giza 6, Assiut population, Sakha 3 and Serw 1) were sown on three sowing dates (1st October, 1st November and 1st December) in a split plots in randomized complete block design. Three cuts were taken after (75, 115, 150 days from sowing). The experiment was conducted during 2006/2007 and 2007/2008 seasons. Results revealed that the highest number of florets (55.9) and seeds/head (40.9), seed set, (77.8%), 1000-seed weight (3.38 g) and seed yield (1.26 t ha^{-1}) were obtained from sowing on the first of October and seed production taking place during the period from April to the end of May. The Giza 15 variety out yielded (1.10 t ha^{-1}) other tested varieties followed by Giza 6 (1.07 t ha^{-1}) and Assiut population (0.99 t ha^{-1}) over both seasons. The estimates of phenotypic stability parameters (bi and S2di) for seed yield showed that the highest seed yielding varieties Giza 15 and Giza 6 exhibited less instabihty in seed yield while the Assiut population was more stable.

Key words: Berseem, *Trifolium alexandrinum*, sowing dates, stability, seed setting, genotype x temperature interaction

INTRODUCTION
Berseem clover (*Trifolium alexandrinum* L.) is the most important winter forage crop in Egypt. Berseem clover is high nutritional quality for animal feed. Berseem also contributes to soil fertility and improved soil physical characteristics (Graves *et al*., 1996). Berseem forage is superior to grasses in protein and mineral contents (Laghari *et al*., 2000).

Berseem clover is a major seed export crop in Egypt. Annual exports of berseem seed have increased to more than 12000 tons in 2004 (El-Nahrawy, 2005). Berseem is also the main honey producing crop. Berseem was introduced to the state of California where it is favoured for its multiple advantages in comparison with other forage crops (El-Nahrawy, 2005).

Current changes in the climatic conditions towards warming especially in Egypt are expected to prolong the summer season and shortens the winter season during which berseem is grown. Thus, it was thought desirable to change the planting date of berseem clover to avoid the high temperature effects at the beginning of the fall season; a practice which was studied by few workers.

Seed yield of berseem clover is often reduced by high temperature at the reproductive period (Iannucci and Martiniello, 1998). Seed yield of berseem in Egypt was found to depend on several factors as weather conditions and insect activity during the period of blooming (Martiniello *et al.*, 1999). Iannucci (2001) found that high temperature during the growing season of berseem clover may affect the seasonal distribution of forage and seed yield. Sowing berseem clover on the 15th of November gives more fresh forage and seed yields than sowing on the 1st December (Usmani Khail *et al.*, 2001). El-Zanaty (2005) also found that seed production of berseem was affected by sowing date, number of cuttings but mainly by the date of the last cut. She also reported significant differences between varieties in seed yield.

Variation in weather conditions at various stages of plant development may affect the differential response of genotypes to environments. Identification of weather variables associated with the genotype x environment interaction is thus important in understanding the nature and patterns of these interactions (Saeed and Francis, 1984). Therefore, it is important to determine how the temperature affects seed yield components and define the nature of their associations with seed yield in berseem clover. Such information may be used to plan efficient breeding programs to develop more productive cultivars or to improve crop management which might favuor seed production as an economically competitive enterprise.

Very little information is available in Egypt regarding the influence of temperature and the effect of genotype x environment interaction on seed yield production of berseem.

This study was undertaken to determine the influence of temperature conditions resulting from different sowing dates on the growth and flowering on seed yield and its components and the stability of seed yield of berseem varieties when tested in different environments.

MATERIALS AND METHODS

This study was carried out at the Agriculture Experimental Farm, South Valley University, Qena, Egypt, during 2006/2007 and 2007/2008 seasons, to study the effect of temperature on seed yield and its components on five berseem varieties. Treatments involved three sowing dates (1st of October, 1st November and 1st December) and five berseem varieties. The soil type was sandy loam (sand was 74%, silt was 16.6%, clay was 9.4%, soil pH was 8.12, organic matter was 0.35%, total N was 0.04%, P was 9.4 ppm, K was 0.19% and calcium carbonate was 13.6%.

A split plot design in randomized complete block design with three replicates was used in both seasons. Sowing dates occupied the main plots and berseem varieties (1-Giza 15, 2-Giza 6, 3-Assiut population, 4-Sakha 3 and 5-Serw-1) were placed in the subplots. Sub-plot size was 10.5 m^2 with 15 rows 3.5 m long, 20 cm apart, with 70 plants per row. Berseem seed were sown by hand at the rate of 6.0 gm m^{-2}. Phosphorus was applied at level of 37.5 g P_2O_5/plot in the form of calcium super phosphate (P_2O_5 15.5%) before seeding. All cultural practices were maintained at optimum level for maximum berseem productivity. Three cuts were taken from each planting date at 75, 115 and 150 days after sowing at 75, 40 and 35 day intervals, respectively. The crop was left for flowering and seed production in the first week of March, April and May for the three planting dates, respectively.

The following traits were recorded at the time of seed harvesting:

- Number of florets/head (as the average of 500 heads)
- Seed number/head (as the average of 500 heads)
- Percent seed setting [(seeds per head/number of florets per head)×100]

Table 1: Weather data from October to July during the period of berseem growth in 2006/2007 and 2007/2008 seasons.

	Average temperature				Relative humidity (%)			
	Maximum		Miuimum		Maximum		Minimum	
Month	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008	2006/2007	2007/2008
October	34.2	35.5	19.9	20.8	50.1	50.1	17.1	15.8
November	25.8	29.0	12.2	13.5	70.7	54.9	26.3	18.3
December	22.0	23.5	8.3	9.4	70.0	63.4	27.7	20.4
January	22.3	19.8	7.1	7.6	73.4	66.9	31.4	22.9
February	24.3	23.4	9.6	8.7	57.2	63.0	22.9	19.6
March	28.2	31.9	13.2	16.0	49.6	43.6	13.1	8.6
April	33.2	35.7	18.3	19.2	37.5	34.9	8.7	6.4
May	38.2	37.1	23.2	22.5	31.6	26.9	8.8	4.5
June	40.5	41.5	25.9	25.9	30.3	28.0	6.8	6.1
July	40.9	40.3	26.0	26.1	41.9	36.4	16.6	5.8

Source: Meteorological authority, Qena, Egypt

Table 2: Total growing degree days (GDD) for each planting date and season at Qena where Egyptian clover trials were conducted.

	Total growing degree days (GDD)		
	2006/2007	2007/2008	
1st October	3229	3460	30th May
1st November	3190	3402	21st June
1st December	3170	3352	5th July

- Seed index (1000 seed weight), g
- Seed yield obtained by threshing seed head from each plot and seed weight per plot was transformed to seed yield ha^{-1}

Climatic data during the study period including minimum and maximum daily temperature and daily relative humidity measured from planting date to mean date of physiological maturity in each season and planting date (Table 1). The total growing degree days GDD, (base = 8°C) was calculated according to Saeed and Francis (1984) as follows:

Total growing degree days = [(Maximum + Minimum temperature)/2 − 8 (Zero vegetation point)] (Table 2).

Separate as well as combined analyses of variance were performed for the data over the two seasons according to Gomez and Gomez (1984) whenever the homogeneity of variances between seasons was detected. Means were compared using LSD test at 5% level.

Stability analysis was carried out and the phenotypic stability parameters; regression coefficients (b_i) and mean square deviations from regression (S^2d_i) were computed for each genotype using the model described by Eberhart and Russell (1966). This model provides the means of partitioning the genotype-environment interaction of each variety into two parts: 1) the variation due to the response of the variety to varying environmental indexes (b_i) and (2) the unexplainable deviations from the regression on the environmental index (S^2d_i). Based on this model the desired variety would have a high mean, a regression coefficient ($b_i = 1$) and a deviation from regression as small as possible ($S^2d_i = 0$). Hence, the definition of a stable variety will be one with $b = 1$ and $S^2d = 0$.

RESULTS AND DISCUSSION

The planting dates used to evaluate the varieties performance in this study provided a range of variation in seasonal climate (Table 1). The climatic conditions and the total Growing Degree Days (GDD) were different during the two growing seasons (Table 2).

Separate as well as combined analyses of variance of the studied traits are presented in Table 3 and 4, respectively. The results revealed significant differences between planting dates and among varieties for all traits. The planting dates x varieties interactions were also significant for all studied traits except number of seeds/head in the first season and 1000-seed weight in the second season and in the combined analysis over seasons (Table 3 and 4). The varieties and varieties x years interactions were also significant for all studied traits except 1000-seed weight (Table 4). The interaction between varieties, years and planting dates were also significant for all studied traits. This may be due to the large differences in climatic conditions prevailing in these planting dates. The presence of these interactions suggested a differential response of varieties to varied planting dates. Similar results were obtained by Medeiros *et al.* (1995), Iannucci and Martiniello (1998), El-Zanaty (2005) and Ranjbar (2007).

Table 3: Mean squares from ANOVA for number of florets/head, number of seeds/head, %seed set, 1000-seed weight and seed yield for each season

		Mean squares									
		2006/2007					2007/2008				
		No. of florets/head	No. of seeds/head	Seed set (%)	1000-seed weight (g)	Seed yield (t ha^{-1})	No. of florets	No. of seeds/head	Seed set (%)	1000-seed weight (g)	Seed yield (t ha^{-1})
Reps	2	104.70	38.16	55.40	0.62*	0.317*	303.24*	45.53	43.96	1.28*	0.312*
Dates(D)	2	735.06*	2548.97**	5099.00**	3.04**	1.107**	847.23**	602.68**	1853.67**	1.74*	1.124**
Error (a)	4	104.03	29.76	9.90	0.06	0.025	46.33	19.60	19.38	0.12	0.040
Varieties(V)	4	151.62**	52.36**	266.32**	0.44**	0.106**	106.03**	370.64**	1301.55**	0.47**	0.132**
D x V	8	56.80*	20.95	240.32**	0.22**	0.053**	130.39**	37.81**	213.16**	0.10	0.038**
Error (b)	24	23.95	10.69	6.70	0.05	0.010	18.61	6.42	9.26	0.09	0.004

*,** Significant at 0.05 and 0.01 probability levels, respectively

Table 4: Mean squares from ANOVA for number of florets/head, number of seeds/head, seed set%, 1000-seed weight and seed yield over seasons

		Mean squares				
		No. of florets/head	No. of seeds/head	Seed set (%)	1000-seed weight (g)	Seed yield (t ha^{-1})
Years (Y)	1	268.4	39.3	1094.2**	0.06	0.03
Reps/year	4	204.0	50.5	49.7	0.95	0.32
Planting date (D)	2	11549.0**	2804.4**	6543.1**	4.60*	2.19**
Y x D	2	33.0	347.2**	409.6**	0.17	0.04
Error (a)	8	75.2	24.1	14.6	0.09	0.03
Varieties (V)	4	177.4**	247.0**	826.5**	0.73**	0.17**
Y xV	4	80.3*	176.0**	741.4**	0.18	0.07**
D x V	8	137.9**	36.4**	306.9**	0.12	0.06**
Y x D x V	8	49.3*	22.4*	146.6**	0.18*	0.04**
Error (b)	48	21.3	9.4	8.0	0.07	0.01

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Temperature conditions during growth, flowering, pollination and seed maturity for each planting date were quite different and this had a significant impact on the results. For the last cut (third cut) of the first planting date, the growth was during March but pollination, fertilization and maturity of seeds occurred during the period from April to end of May. In the second planting date, growth of the seed crop was during April while pollination, fertilization and maturity of seed occurred during May until the third week of June. For the third planting date, vegetative growth until seed maturity took place from May until first week of July.

Data in Table 5 show that the first planting date recorded significantly higher average number of florets and seeds/head, heavier 1000 seed weight and greater seed set and seed yield than other planting dates over seasons. These traits recorded, 55.9, 40.9, 77.8%, 3.38 g and 1.26 t ha^{-1}, respectively, in first planting date compared to 41.6, 22.3, 49.5%, 2.62 g and 0.71 t ha^{-1}, respectively in the third planting date averaged over seasons. These results are in agreement with those reported by Iannucci (2001), Usmani Khail *et al.* (2001) and El-Zanaty (2005).

These results suggest that the first planting date was the most suitable time for flowering and pollination which coincide with the activity of pollinators (honey bees) which play a great role in increasing seed setting by tripping the flowers. These results are in line with those reported by Dobrenzo *et al.* (1965) in alfalfa who found that the time required for flowering initiation decreased as the average minimum temperature increased. Furthermore, Medeiros *et al.* (1995), Iannucci and Martiniello (1998) reported that high temperatures during flowering probably limit insect pollination and enhance physiological losses of pollinated flowers and increase embryo abortion. Thus the first planting date is more favourable for both vegetative growth and seed yield. This is probably because, to increasing the duration of spring growth which allows the plant to accumulate more starch in its roots and vegetative organs. Also, nitrogen reserves that are subsequently mobilized to a greater extent just after cutting allowing a longer regrowth interval and a greater opportunity to re-accumulate vegetative storage proteins and starch reserves. Similar results were reported by Avice *et al.* (1997).

Comparisons between varieties (Table 5) showed that the variety "Assiut population" significantly exceeded the general mean in number of florets/head. With respect to number of seeds/head and seed set only the varieties Giza 15 and Assiut population significantly exceeded the general mean. However, the variety Giza 6 produced significantly heavier seeds than the general mean while the varieties Giza 15 and Giza 6 significantly out yielded the general mean over seasons.

The obtained results also revealed that the Giza 15 variety significantly exceeded the other varieties in number of seeds/head, seed set% and seed yield.

STABILITY ANALYSIS

The analysis of variance for the stability of number of florets and seeds/head, seed set, 1000-seed weight and seed yield, for the five varieties under six environments, according to Eberhart and Russell (1966) is given in Table 6. Mean squares due to varieties and environments were significant for all studied traits. This reveals that there is variability among varieties as well as environments under study. Significant mean squares due to environments plus genotypes x environments interaction reveal that the genotypes interacted considerably with environmental conditions. In addition the significance of the genotype x environment interaction indicated that the planting date had major effects on the relative genotypic potential for number of florets/head,

Table 5: Mean number of florets/head, number of seeds/head, %seed set, 1000-seed weight and seed yield for five varieties under each planting over seasons

	No. of florets/head				No. of seeds/head				Seed set (%)				1000-seed weight (g)				Seed yield (t ha^{-1})			
	1st Oct.	1st Nov.	1st Dec.	Mean	1st Oct.	1st Nov.	1st Dec.	Mean	1st Oct.	1st Nov.	1st Dec.	Mean	1st Oct.	1st Nov.	1st Dec.	Mean	1st Oct.	1st Nov.	1st Dec.	Mean
1-Giza 15	54.7	48.0	45.6	49.4	42.6	38.9	29.7	37.1	80.1	76.1	62.8	73.0	3.25	3.12	2.79	3.05	1.26	1.11	0.94	1.10
2-Giza 6	49.8	49.6	45.6	48.3	40.1	39.5	23.6	34.5	81.9	77.4	39.0	66.1	3.50	3.29	2.90	3.23	1.28	1.16	0.78	1.07
3 Assiut pop.	59.0	58.1	44.6	53.9	44.4	40.4	22.4	35.7	81.7	80.4	54.0	72.0	3.00	2.92	2.20	2.71	1.31	1.07	0.59	0.99
4-Sakha 3	62.5	45.0	36.8	48.1	39.6	30.4	18.7	29.6	70.2	67.7	51.0	62.9	3.58	3.29	2.50	3.12	1.34	0.94	0.63	0.97
5-Serw-1	53.7	46.7	35.4	45.3	37.6	32.0	17.0	28.9	75.2	53.2	41.2	56.5	3.57	3.13	2.70	3.13	1.13	0.85	0.63	0.87
Mean	55.9	49.5	41.6	49.0	40.9	36.3	22.3	33.1	77.8	70.9	49.5	66.1	3.38	3.15	2.62	3.05	1.26	1.03	0.71	1.00
LSD 5% for																				
Planting dates (D)		5.2				2.9				2.3				0.18				0.11		
Varieties (V)		3.1				2.1				1.9				0.17				0.06		
D x V		5.4				3.6				3.3				N.S.				0.10		

Table 6: The joint regression analysis of variance over envirouments for number of florets/head, number of seed/head, seed set (%), 1000-seed weight and seed yield for five varieties

		Mean squares				
		No. of florets/head	No. of seeds/head	Set set (%)	1000-seed weight (g)	Seed yield (t ha^{-1})
Varieties (V)	4	59.0*	82.4**	268.1*	0.26**	0.057**
Env. + (Varieties x Env.)	25	70.7*	100.2**	291.9**	0.16**	0.074**
Env. (Linear)	1	1163.4**	2114.1**	5111.1**	2.90**	1.507**
Varieties x Env. (Linear)	4	57.5*	11.6	91.7	0.10**	0.037**
Pooled deviation	20	18.7**	17.2**	91.0**	0.03	0.010**
Pooled error	48	5.7	2.3	2.1	0.02	0.002

Significant at 0.05 and 0.01 probability levels, respectively

Table 7: Average performance over six envirouments ($\bar{x}$) and stability parameters (bi, S^2d_i) of five berseem varieties for number of florets/head, number of seeds/head, seed set%, 1000- seed weight and seed yield.

	No. of florets/head			No. of seeds/head			Seed set (%)			1000-seed weight (g)			Seed yield (t ha^{-1})		
	$\bar{x}$	b_i	S^2d_i	$\bar{x}$	b_i	S^2d_i	$\bar{x}$	b_i	S^2d_i	$\bar{x}$	b_i	S^2d_i	$\bar{x}$	b_i	S^2d_i
1-Giza 15	49.4	-0.24	10.4	37.1	-0.25	13.7**	73.0	-0.39	30.6**	3.1	-0.53*	0.01	1.1	-0.49*	0.007*
2-Giza 6	48.3	-0.58	11.9	34.4	-0.06	25.9**	66.1	0.44	92.3**	3.3	-0.29	0.01	1.1	-0.10	0.014**
3-Assiut pop.	53.9	-0.10	33.2**	35.7	0.15	5.4	71.5	-0.09	78.5**	2.7	0.14	0.05*	1.0	0.31	0.003
4-Sakha 3	48.1	0.75*	16.9*	29.6	0.13	27.7**	62.9	-0.04	167.1**	3.1	0.52*	0.07*	1.0	0.38	0.023**
5-Serw-1	45.3	0.16	21.1*	28.9	0.03	13.5**	56.5	0.08	86.6**	3.1	0.18	0.03	0.9	-0.09	0.003
Mean	49.0	-	-	33.1	-	-	66.0	-	-	3.1	-	-	1.0	-	-
LSD 5%	1.6	-	-	1.0	-	-	1.0	-	-	0.1	-	-	0.03	-	-

*,** Significantly different from unity for (b_i) and from zero for (S^2d_i) at the 0.05 and 0.01 probability levels, respectively

1000 seed weight and seed yield. This means that for reliable evaluation of berseem clover for seed yield, it would be necessary to evaluate the genotypes with great emphasis on planting date.

The results of this study are in broad agreement with earlier findings indicating that linear regression forms a predominant portion of genotype x environment interaction in berseem (Bakheit, 1985; Bakheit and El-Hinnawy, 1993; Abdel-Galil *et al*., 2007). Consequently, stability performance should be carried out to identify the reaction and response of each genotype to environmental changes.

The phenotypic stability statistics; regression (b_i) and deviation from regression (S^2d_i) for the five varieties in six environments (2 seasons x 3 planting dates) are given in Table 7. Taking into account all three parameters ($\bar{x}$, b_i and S^2d_i), it is interesting to note that the variety Giza 15 which was highest in number of seeds/head and seed yield (Table 7) but it was unstable for both traits. However, the variety Assiut population was low in seed yield but was stable for this trait. The variety Giza 6 was highest in 1000-seed weight and was stable for this trait. Tai (1971) reported that high yielding ability genotypes are unstable over environments and genotypes possessing average stability were generally low in productivity. Results obtained in the present investigation clearly agree with these conclusions.

For the practical utility of these results, it is worth mentioning that it may be possible to select a high seed yielding genotype which shows relatively low level of instability such as Giza 15 as a source of high seed yielding genes to be crossed with genotypes of low stability, such as Serw-1 as sources for stability genes followed by selection for high seed yield and stability, since some investigators (Perkins and Jinks, 1968a, b; Finlay, 1971) reported that stability index is a heritable trait.

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