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Effect of Planting Date, Variety and their Interaction on Seed Yield and its Components of Egyptian Clover (*Trifolium alexandrinum* L.)

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Abstract

This investigation was carried out to study the effect of temperature resulting from different planting dates during the stage of flowering and seed maturity and the effect of genotypes x environment interaction on seed yield and its components in some varieties of berseem clover (*Trifolium alexandrinum* L.). A set of six varieties (Serw-1, Gemmeiza 1, Giza-6, Sakha-4, Helally and Local variety) were sown on four planting dates (September 15th, October 15th, November 15th and December 15th) in randomized complete block design with four replicates for each planting date. Three cuts were taken after 70, 110, 145 days from sowing. The experiments were conducted during 2014/2015 and 2015/2016 seasons in experimental farm of Assiut University. Results revealed that the highest number of seeds/ inflorescence (48.1), seed setting (72.8%), 1000 seed weight (3.21 g) and seed yield (1.25 kg/plot) were obtained from sowing on the 15th September. This result due to seed production is taking place during the period from April to the Mid-June.

The Sakha-4 variety outyielded (1.07 kg/plot) other tested varieties. On the other hand, the local variety gave lowest yield (0.90 kg/plot) over all planting dates and seasons. The estimates of genotypic stability parameters (α and λ) for seed yield showed that variety Sakha-4 was the highest seed yielding, but exhibited less stability (instable) in seed yield. While Helally and Serw-1 varieties were more stable. On the other hand, local variety showed lower seed yield and instability.

Keywords: *Egyptian clover, Trifolium alexandrinum L., planting dates, stability, genotype x environment interaction, seed yield.*

Introduction

Egyptian clover (*Trifolium alexandrinum* L.) is the main and oldest cultivated winter forage leguminous crop in Egypt. It occupies about one third of the cultivated area with average of 1.63 million feddan (Feddan= 4200 m^2). With an estimated productivity of about 42.03 million tons of green fodder (B.A.S. 2016). Berseem clover is high nutritional quality for animal feed, also contributes to soil fertility and improved soil physical and chemical characteristics (Graves *et al*., 1996).

Current changes in the climatic conditions towards warming especially in Egypt are expected to prolong the summer season and shortens the winter season and extended in temperature during which berseem in 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 full season.

Seed yield of Egyptian clover is often reduced by high temperature at the reproductive period (Iannucci and Martiniello, 1998). Seed yield of Egyptian clover in Egypt was found to depend on several factor as weather conditions and insect activity during the period of blooming (Martiniello *et al*., 1999). Iannucci (2001) and Bakheit *et al*. (2012) found that high temperature during the growing season of berseem clover may affect the seasonal distribution of both forage and seed yields. Sowing berseem clover on the 15th of November gives more fresh forage and seed yields than sowing on the 1st December (Usmani-Khalil *et al*., 2001). El-Zanaty (2005) found significant differences between varieties in seed yield and seed production of berseem was affected by sowing date, number of cuttings and mainly by the date of the last cut. Furthermore, Bakheit *et al*. (2012) reported that the highest number of florets and seeds/head, seed setting, 1000-seed weight and seed yield were obtained from sowing on the first of October than first of November and December.

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). 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 two improve crop management which might favour seed production as an economically competitive enterprise. Under the changes in climatic conditions, it is important issue to determine the stability of the Egyptian clover varieties. The genotypic stability as estimated by Tai (1971) is a fit analysis to propose the stability of these varieties performance.

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

Materials and Methods

This study was carried out at the Agricultural Experimental Farm, Assiut University, Egypt, during 2014/2015 and 2015/2016 seasons to study the effect of temperature conditions resulting from different planting dates on seed yield and its components on six berseem varieties. Treatments involved four planting dates (15th September, 15th October 15th November and 15th December). The soil type was clay and characterized as sand (26%), silt (24%), clay (50%), soil pH (7.8), organic mater (1.6%), total N (0.1%) and $CaCO_3$ (1.2%). The randomized complete block design with four replicates was used for each planting date in both seasons. The berseem varieties i.e. Serw-1, Gemmeza-1, Giza-6, Sakha-4, Helally and Local variety were distribution randomly on the plots in each replicate. Plot size was 10.5 m^2. Berseem seeds for each variety were sown by hand at the rate of 6.0 g/m^2. Phosphorus was applied at level of 37.5 g/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 70, 40 and 35 days intervals, respectively. The plants were left for flowering and seed production in the Mid of February, March, April and May for the four planting dates, respectively in the two seasons.

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

1- Number of inflorescences/plant (as the average of ten plants).

2- Number of seeds/inflorescences (as the average of 500 inflorescences (heads).

3- Seed setting percent (seeds per inflorescences/number florets x 100).

4- Seed index (1000 seed weight g (as average of five samples each of 1000 seeds weight).

5- Seed yield (kg/plot) determined from each plot, plants had harvested at seed maturity stage, where manually threshed.

Temperature data during the study period including maximum and minimum daily temperature measured from planting date to mean date of physiological maturity in each season and planting data (Table 1). The total growing degree days (GDD), (base = 7°C) was calculated according to Saeed and Francis (1984) as follows: Total growing degree days (GDD) =

Σ[((Maximum + Minimum temperature)/2)-7]

7= Zero growth point from sowing to maturity, (Table 2).

Separate as well as combined analysis 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 L.S.D. test at 5% level.

Stability analysis was computed according to Tai (1971), where he suggested partitioning the genotype x environment interaction (GE) effect of the i^{th} genotypes into two components (α_i and λ_i). These estimates (α_i and λ_i) were computed for each variety under four planting dates in two seasons (8 environments) to compare the relative stability of varieties. The parameter α_i measures the linear response to the environmental effect and λ_i parameter measures the deviation from linear response in terms of the magnitude of the error variance. The two components are defined as genotypic stability parameters.

Table 1. Summary of the daily temperature during the two studying seasons.

Month	Average temperature				Relative humidity				Photoperiod	
	Maximum		Minimum		Maximum		Minimum			
	2014/ 2015	2015/ 2016	2014/ 2015	2015/ 2016	2014/ 2015	2015/ 2016	2014/ 2015	2015/ 2016	2014/ 2015	2015/ 2016
15-30 September	38.1	39.4	21.5	21.3	54.5	56.4	16.21	16.4	12:09:28 pm	12:09:52 pm
1-15 October	33.9	36.6	18.3	20.6	59.4	73.8	17.4	22.73	11:45:28 am	11:45:52 am
16-31 October	32.2	33.5	15.8	19.0	59.6	77.8	19.12	26.2	11:21:41 am	11:22:11 am
1-15 November	30.1	28.3	14.0	14.5	67.66	84.3	20.6	33.6	10:59:56 am	11:00:16 am
16-30 November	26.0	27.5	11.7	12.0	72.8	84.0	29.6	29.2	10:42:36 am	10:42:52 am
1-15 December	26.0	22.7	10.7	8.3	68.1	86.8	26.0	33.0	10:30:56 am	10:31:00 am
16-31 December	23.1	21.6	8.4	6.9	73.6	90.2	28.7	31.3	10:27:11 am	10:27:08 am
1-15 January	18.3	21.5	4.5	6.2	71.0	84.1	29.0	28.7	10:32:00 am	10:31:52 am
16-31 January	24.5	19.7	7.6	4.6	64.7	85.5	19.2	29.8	10:45:07 am	10:44:56 am
1-15 February	23.5	32.7	7.3	5.9	58.9	85.1	16.5	25.9	11:04:16 am	11:03:52 am
16-28 or 29 February	23.6	27.6	7.9	9.6	66.0	73.0	22.6	17.5	11:24:37 am	11:23:00 am
1-15 March	27.6	29.6	11.6	12.9	62.8	64.0	18.2	17.66	11:46:48 am	11:47:36 am
16-31 March	30.3	28.2	12.8	11.7	55.3	67.3	13.6	18.2	12:11:26 pm	12:12:41 pm
1-15 April	28.1	34.3	12.3	15.3	45.3	63.1	13.4	10.1	12:36:16 pm	12:37:28 pm
16-30 April	34.9	27.6	14.6	16.9	39.7	59.6	7.8	8.4	12:58:00 pm	12:59:05 pm
1-15 May	34.6	38.1	18.1	19.7	49.5	47.4	13.1	9.1	13:20:00 pm	13:21:00 pm
16-31 May	39.8	38.0	22.0	19.7	44.6	55.5	12.3	10.0	13:35:55 pm	13:38:04 pm
1-15 June	37.8	44.1	21.5	24.0	60.3	41.6	16.6	6.6	13:48:24 pm	13:48:44 pm
16-30 June	38.0	41.5	22.4	24.8	63.1	59.6	15.4	12.6	13:46:19 pm	13:46:30 pm
1-15 July	38.1	39.6	23.0	24.1	72.2	58.4	17.4	16.6	13:47:44 pm	13:47:16 pm
16-31 July	41.3	38.7	23.5	23.5	51.4	65.9	9.5	16.0	13:36:04 pm	13:35:08 pm

Source: Meteorological authority, Assiut, Egypt.

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

Planting date	Forage yield from planting date until third cut		Seed yield after third cut until seed maturity	
	2014/2015	2015/2016	2014/2015	2015/2016
15th September	1930	1960	2152	2231
15th October	1625	1692	2037	2048
15th November	1562	1625	1880	1445
15th December	1767	1860	1565	1368

Results and Discussion

I- Performance of varieties under different planting dates:

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

The combined analysis of variance of the studied traits are presented in Table 3. The results showed significant differences among each of planting dates and varieties for all traits except varieties for seed setting. Also, significant differences among growth seasons were obtained for all studied traits except number of seeds/inflorescence. The planting dates x varieties interactions were also significant for all studied traits. Moreover, the planting dates x seasons interaction were also significant for all studied traits except seed setting and seed yield/plot (Table 3). This results may be due to the large differences in climatic conditions prevailing in these planting dates. The presences of these interactions suggested a differential response of the varieties to varied planting dates. Similar results were obtained by Medeiros *et al*. (1995), Iannucci and Martiniello (1998), El-Zanaty (2005), Ranjbar (2007) and Bakheit *et al*. (2012).

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 third cut (last cut) of the first planting date, the growth was during Mid February to last week of March but pollination, fertilization and maturity of seeds occurred during the period from April to second week of June. In the second planting date, growth of the seeds was during Mid March to last week of April, while pollination, fertilization and maturity of seed occurred during May until third week of June. For the fourth planting date, vegetative growth until seed maturity took place from Mid May until third and first week of July in first and second seasons, respectively.

Table 3. Combined analysis of variance for seed yield traits of Egyptian clover varieties under different planting dates over two seasons.

Source of variation	d.f	Mean squares for				
		Number of inflorescences/ plant	Number of seeds/ inflorescences	Seed setting %	1000-seed weight (g)	Seed yield (kg/plot)
		1387.8**	29.5	1195.1**	17.0**	26.53**
Reps/year	**6**	6.67	49.9	45.7	0.054	0.045
Planting date (D)	**3**	1623.9**	1480.0**	3314.2**	0.867**	2.20**
Y x D	**3**	2429.5**	182.5**	143.1	0.880**	0.068
Error (a)	**18**	12.08	34.56	55.4	0.083	0.040
Varieties (V)	**5**	190.47**	103.4*	51.8	0.284**	0.145**
Y x V	**5**	231.26**	21.3	53.8	0.038	0.006
D x V	**15**	69.47**	70.9*	114.5**	0.139*	0.092**
Y x D x V	**15**	191.0**	56.5	59.8	0.226**	0.054**
Error (b)	**120**	14.19	40.5	36.9	0.066	0.018

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

Results in Table 4 show that the first planting date recorded significantly higher average number of seeds/inflorescence, heavier 1000-seed weight, greater seed set and seed yield than other planting dates over seasons. These traits recorded 48.1, 3.21g, 72.8%, and 1.25 kg/plot, respectively in first planting date compared to 35.7, 2.90 (g), 53.1%, and 0.73 kg/plot in the fourth planting date averaged over two seasons, respectively. While, the number of inflorescence/plant trait recorded 43.7 in the second planting date and significantly higher than the other planting dates. These results are in agreement with those reported by Iannucci (2001), Usmani Khail *et al*. (2001), El-Zanaty (2005) and Bakheit *et al*. (2012).

These results suggest that the first planting date was the most suitable time for flowering and pollination which coincide with the activity of pollinators by honey bees which play a great role in increasing seed setting by tripping the flowers. With this respect, Medeiros *et al*. (1995), Iannucci and Martiniell (1998) and Bakheit *et al*. (2012) reported that high temperature during flowering probably limit insect pollination and enhance physiological losses of pollinated flowers.

Comparisons between varieties (Table 4) showed that the Sakha-4 variety significantly exceeded the general mean in number of inflorescence/plant and seed yield/plot. Also, Sakha-4 among all varieties gave the highest values of all studied traits except seed index. While, local variety gave lowest values for these traits. These results are in line with those reported by El-Zanaty (2005) and Bakheit *et al*. (2012).

Table 4. Mean number of inflorescences/plant, number of seeds/ inflorescence, seed setting %, 1000 seed weight and seed yield for the six varieties under each planting date over two Seasons.

Planting date Variety	15th Sept.	15th Oct.	15th Nov.	15th Dec.	Mean	15th Sept.	15th Oct.	15th Nov.	15th Dec.	Mean
	Number of inflorescences/plant					**Number of seeds/inflorescence**				
Serw 1	31.5	45.6	30.7	36.5	36.1	49.1	46.0	42.8	33.6	42.8
Gemmeiza -1	28.5	44.9	40.2	33.2	36.7	49.4	46.3	41.4	35.1	43.1
Giza 6	26.5	39.1	48.2	29.9	35.9	43.2	46.7	41.1	39.5	42.6
Sakha-4	33.4	52.4	52.1	32.1	42.5	52.1	52.4	39.2	36.6	45.1
Helally	35.8	41.4	40.0	32.5	37.4	46.0	44.8	43.6	35.6	42.5
Local variety	35.5	39.1	36.5	33.7	36.2	48.8	39.1	36.3	33.6	39.5
Mean	31.8	43.7	41.3	34.1	37.7	48.1	45.8	41.2	35.7	42.7
LSD 5%	**D**= 1.9	**V**= 1.86		**DxV**= 3.71		**D**= 2.52	**V**= 3.14		**DxV**= 6.27	
	VxY= 2.62	**DxY**= 2.11		**VxDxY**= 5.25		**VxY**= N.S	**DxY**= 3.57		**VxDxY**= N.S.	
	Seed setting %					**1000-seed weight (g)**				
Serw 1	73.1	68.9	60.9	54.1	64.3	3.15	3.30	2.96	2.81	3.06
Gemmeiza -1	75.5	65.9	66.2	52.6	65.1	3.28	3.11	3.11	2.95	3.11
Giza 6	66.9	69.6	62.7	54.5	63.4	3.00	3.03	2.95	2.89	2.97
Sakha-4	74.5	73.8	60.5	53.1	65.5	3.17	2.97	2.94	2.77	2.96
Helally	69.5	65.8	66.7	52.2	63.6	3.05	3.08	2.98	2.86	2.99
Local variety	77.4	59.9	57.5	52.3	61.8	3.64	3.05	2.97	3.14	3.20
Mean	72.8	67.3	62.4	53.1	63.9	3.21	3.09	2.98	2.90	3.04
LSD 5%	**D**= 3.19	**V**= N.S.		**DxV**= 5.98		**D**= 0.124	**V**= 0.127		**DxV**= 0.253	
	VxY= N.S.	**DxY**= N.S.		**VxDxY**= N.S.		**VxY**= N.S.	**DxY**= 0.175		**VxDxY**= 0.358	
	Seed yield/plot (gk)									
Serw 1	1.10	1.00	1.02	0.75	0.97					
Gemmeiza -1	1.33	1.09	1.08	0.65	1.04					
Giza 6	1.07	0.99	1.06	0.71	0.96					
Sakha-4	1.49	1.03	1.02	0.77	1.08					
Helally	1.36	1.12	1.08	0.66	1.06					
Local variety	1.15	0.78	0.86	0.82	0.90					
Mean	1.25	1.00	1.02	0.73	1.00					
LSD 5%	**D**= 0.09	**V**= 0.07		**DxV**= 0.13						
	VxY= N.S	**DxY**= N.S		**VxDxY**= 0.19						

II- Stability analysis

Analysis of variance across varieties and environments indicated that the environments, varieties, and varieties x environments (GE) interactions were highly significant for all studied traits except for varieties in seed setting % (Table 5). The environment mean square was significant indicating that the four planting dates in two seasons provided a sufficient range of environments, and hence the validating of environmental requirements suggested by Tai (1971) were fulfilled. The results are in broad agreement with earlier findings that linear regression forms a predominant portion of genotype x environment interactions in Egyptian clover (Bakheit, 1985; Khatri *et al*., 1991; Bakheit and El-Hinnawy, 1993; Abdel Galil *et al*., 2007 and Bakheit *et al*., 2012).

Table 5. The Joint analysis of variance over environments for number of inflorescences/plant, seed setting, 1000-seed weight, and seed yield for six Egyptian clover varieties.

Source of variation	d.f	Mean squares for			
		Number of inflorescences/ plant	Seed setting %	1000-seed weight (g)	Seed yield (kg/plot)
Environment (E)	7	1935.43**	1652.67**	3.179**	4.76**
Rep./Envir.	24	10.73	52.99	0.075	0.041
Varieties (V)	5	190.47**	51.80	0.284**	0.145**
V x E	35	190.89**	82.44**	0.162**	0.063**
Error	120	14.19	36.97	0.066	0.018

** Significant at 0.01 levels of probability.

According to Tai's theory the variety by environment interaction is partitioned into two components: α which measures the linear response to environmental effect and λ which measures the deviation from the linear response. The genotypic stability parameters were determined ($\overline{X}$, α and λ) in all varieties for number of inflorescences/plant, number of seeds/inflorescence, seed setting, 1000 seed weight and seed yield (Table 6).

Table 6. Average performance over eight environments ($\overline{X}$) and stability parameters (α, λ) of six varieties for seed yield and its components.

	Number of inflorescences/ plant			Seed setting %			1000-seed weight (g)			Seed yield (kg/plot)		
	$\overline{X}$	α	λ	$\overline{X}$	α	λ	$\overline{X}$	α	λ	$\overline{X}$	α	λ
Serw 1	36.1	-0.14	18.08*	64.3	-0.03	0.74	3.06	-0.09	1.55	0.968	-0.04	1.80
Gemmeiza -1	36.7	-0.10	3.18*	65.1	0.13	1.57	3.11	-0.17	1.55	1.038	0.03	2.68*
Giza 6	35.9	0.28	10.27*	63.4	-0.32	1.44	2.97	-0.14	0.90	0.958	-0.02	3.91*
Sakha-4	42.5	0.59	6.47*	65.5	0.17	2.18*	2.96	0.01	0.59	1.078	0.08	5.27*
Helally	37.4	-0.10	12.60*	63.6	-0.09	1.31	2.99	0.04	0.76	1.055	0.04	1.64
Local variety	36.2	-0.53	9.69*	61.8	0.14	4.67*	3.20	0.36	6.50*	0.903	-0.10	3.96*

* λ value greater than Fa value derived from E table with n_1= 6, n_2= 120 and a= 0.05
n_1= Number of environment-2 n_2= Degree of Freedom for Error a= Level of probability.

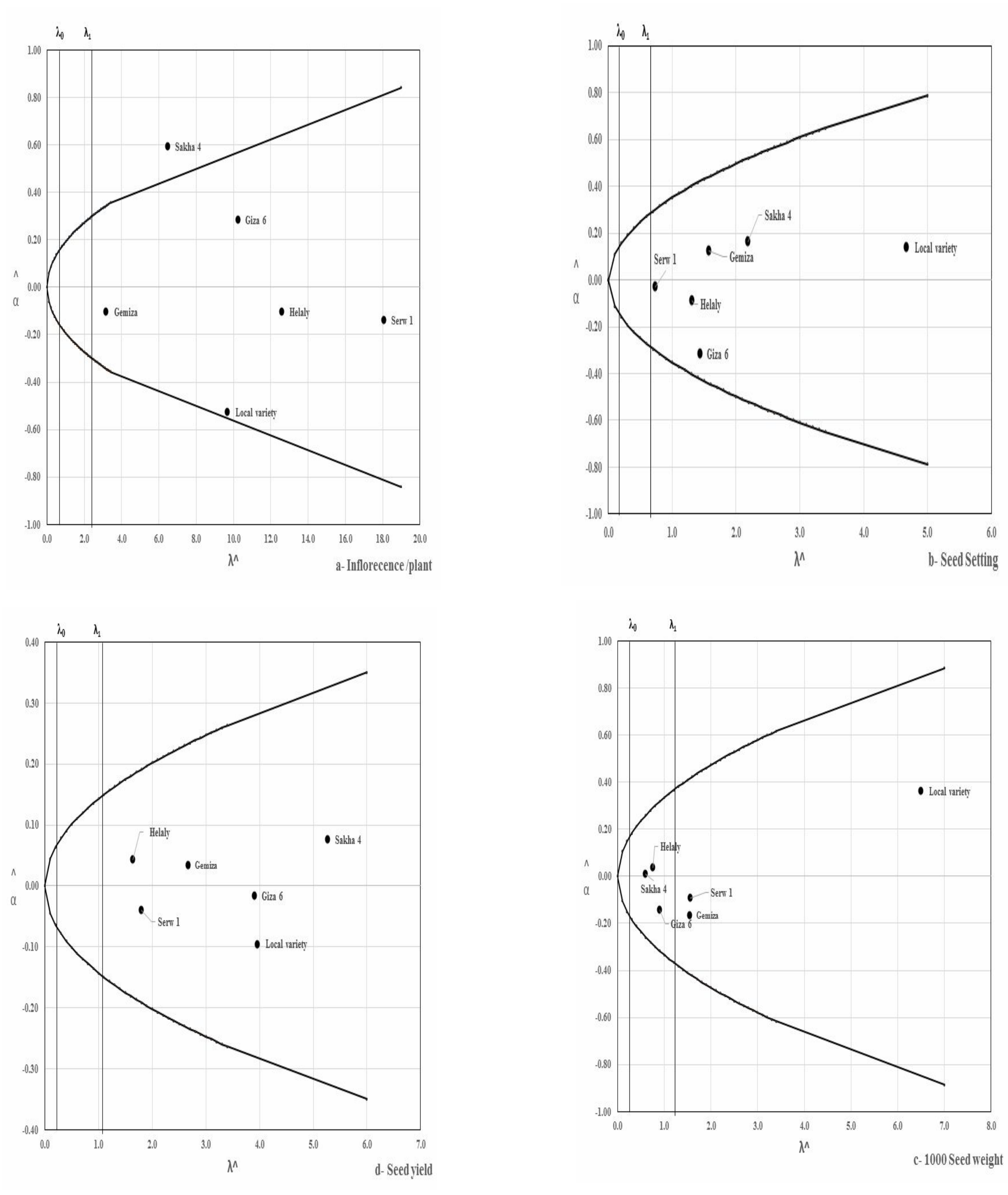

Fig. 1. F_1 distribution of estimates of genotypes stability parameter (α, λ) for number of inflorescent/plant, seed setting, 1000 seed weight and seed yield/plot of six Egyptian clover varieties.

According to this method, the values (α= -1, λ= 1) refer to perfect stability, while a genotype that had only average stability might have an estimate of α= 0.0 and λ= 1. The all varieties under study except Serw-1 variety had significant values for seed yield. Therefore, they were considered to be unstable. On the other hand, the local variety had significant values for all studied traits and considered to be unstable.

Also, the average stability region involved all varieties except local variety for seed setting and 1000-seed weight, but for seed yield, the average stability resion involved only Helally and Serw-1 varieties (Table 6 and Fig. 1).

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تأثير ميعاد الزراعة والصنف والتفاعل بينهما علي المحصول البذري ومكوناته في البرسيم المصري

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الملخص:

أجري هذا البحث لدراسة تأثير درجات الحرارة الناتجة عن اختلاف مواعيد الزراعة خلال مراحل التزهير ونضج البذور وتأثير تفاعل التراكيب الوراثية والبيئة علي المحصول البذر ومكوناته في بعض أصناف البرسيم المصري. تم زراعة ست أصناف (سرو-١، جميزة-١، جيزة-٦، سخا-٤، هلالي ، صنف محلي) في أربعة مواعيد زراعة (١٥ سبتمبر ، ١٥ أكتوبر، ١٥ نوفمبر، ١٥ ديسمبر) في تصميم القطاعات الكاملة العشوائية (RCBD) باستخدام أربع مكررات في كل ميعاد زراعة. وتم أخذ ثلاث حشات علي فترات (٧٠ ، ١١٠ ، ١٤٥ يوم من الزراعة) وقد نفذت التجربة خلال موسمي ٢٠١٤/٢٠١٥ ، ٢٠١٥/٢٠١٦ في المزرعة البحثية لكلية الزراعة – جامعة أسيوط.

أظهرت النتائج أن ميعاد الزراعة في ١٥ سبتمبر الذي كان إنتاج البذور فيه خلال الفترة من أبريل حتي منتصف يونيه أعطي أعلي عدد البذور في النورة (٤٨,١) ونسبة العقد (٧٢,٨%) ووزن الـ ١٠٠٠ بذرة (٣,٢١ جرام) والمحصول البذري (١,٢٥ كجم/للقطعة).

وعند تقدير الثبات الوراثي أظهر الصنف سخا-٤ أعلي محصول بذري إلا أنه كان أقل ثباتاً لنفس الصفة بينما كانت الأصناف هلالي وسرو-١ أكثر ثباتاً، وفي المقابل كان الصنف المحلي أقل محصولاً وغير ثابت.