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MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY AND RESPONSE OF EGYPTIAN FABA BEAN TO HEAT AND DROUGHT STRESSES

M. A. Belal[(1)], Samah M. M. Eldemery[(2)], Y. A. Khidr[(1)] and K. F. Abdellatif[(1)*]
[(1)] **Plant Biotech. Dept., GEBRI, USC, Egypt,**
[(2)] **Mol. Biol. Dept., GEBRI, USC, Egypt,**
[*]**Corresponding Author: kamal2004gr@yahoo.com**

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ABSTRACT: ***Four Egyptian faba bean (Vicia faba L.) varieties ("Giza843", "Giza716", "Misr1" and "Sakha1") have been evaluated under heat and drought stresses using seventeen morphological traits and total protein content. Analysis of variance of the morphological trait revealed highly significant differences among treatments and genotypes for all studied traits and the interaction between genotypes and treatment was also significant. Both varieties "Giza716" and "Giza843" gave the highest means for most of the morphological traits. The control treatment surpass all the other treatments in the most of the morphological traits. The lowest significant means were obtained from the heat with drought treatment while the effect of heat on the morphological traits was less than the effect of drought stress. According to the cluster analysis of both morphological and biochemical data, the varieties "Giza716" and "Giza843" were clustered together on one group where the varieties "Misr1" and "Sakha1" were clustered in another group. A protein band of molecular weight of about 76 kDa was noticed in the protein pattern of the variety "Giza716" (heat with drought treatment) and of about 100 kDa was obtained in the protein pattern of the variety "Giza843" (heat with drought treatment). The morphological and biochemical parameters along with susceptibility test revealed that "Giza716" and "Giza843" appeared to be tolerant for drought and heat. However "Misr1" and "Sakha1" varieties appeared to be susceptible.***

Key words: ***Faba bean genotypes, Morphological traits, Heat, Drought, Stress, total protein analysis***

INTRODUCTION

Faba bean (*Vicia faba* L.) is a worldwide important legume crop, a diploid species with 2n=12 chromosomes and belong to the family of *Fabaceae* (*leguminosae*), subfamily of *Papilionoideae*, tribe of *Viceae*.

Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals or high salinity and biotic stresses such as insects, broomrape, fungi, bacteria etc. cause several effects to faba bean at morphological, molecular and biochemical levels. Faba bean is more sensitive to drought than some other grain legumes including common bean, pea and chickpea (McDonald and Paulsen 1997; Amede and Schubert 2003). The reduction in faba bean seed yield was positively related to the amount of water reduction and reach up to 50% of seed yield (Musallam *et al*., 2004; Ouda *et al.,* 2010; Ammar *et al.,* 2014 and Afiah *et al*., 2016). All of them stated that drought had negative effects on faba bean yield and its components. In addition, Abid *et al*., (2017) reported that drought stress reduced plant growth and affected physiological parameters whereas the plant response to drought stress significantly varied in the studied faba bean cultivars.

Faba bean plants bear heat stress when subjected to supra-optimal

temperatures or stressed by drought, mostly at the end of the growing season Stoddard *et al*. (2006). The reproductive stage of faba bean is also sensitive to heat stress (Patrick and Stoddard, 2010). Siddiqui *et al*. (2015) studied the effects of various levels of temperature (control, mild, and modest) on plant height (ph), fresh weight (fw), dry weight (dw) and leaf area in ten faba been genotypes and they found that the variety "C5" was the most heat stress tolerant whereas the "Espan" variety was the most heat stress sensitive genotypes.

The combined effect of both heat and drought stresses induce significant alterations in plant biochemistry and metabolism (Apel and Hirt, 2004). The possible responses under environmental stresses may cause membrane injuries, protein degradation; enzyme inactivation and induce oxidative stress (Zlatev and Lidon 2012). To study these alterations, Sodium Dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to differentiate between cultivars of faba bean exposed to environmental stress (Stegmann *et al*., 1980; Shaddad & El-Tayeb, 1990; Abdellatif *et al.,* 2012, Zakaria *et al.*, 2015 and Eldemery *et al.*, 2016).

The aim of this research was to evaluate the ability of Egyptian faba bean genotypes to tolerate the heat and/or drought stresses at both morphological and biochemical levels and determine specific protein bands related to tolerant genotypes.

MATERIAL AND METHODS

The present work was carried out at the Plant Molecular biology Laboratory, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat City, Egypt, during the period of 2015 to 2018.

Plant material

Four Egyptian faba bean (*Vicia faba* L.) varieties obtained from Sakha Agricultural Research station, Agriculture Research Center, Egypt have been used in this study. Their pedigrees are presented in Table (1).

Field Experiment

The field experiment has been carried out during growing seasons 2016-2017 and 2017-2018. The experiments were performed at the farm of Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt. Seeds of each variety were planted in six bags, every bag contained two seeds. Four treatments have been applied for the cultivated varieties. The first treatment was the (control) which cultivated at 15 November and was irrigated each two days. The second treatment was the (drought) that cultivated at 15 November and was irrigated one a week. The third treatment was the (heat) which cultivated at 15 January and was irrigated each two days. The fourth treatment was the (drought with heat) that cultivated at 15 January and was irrigated once a week. Seventeen morphological characteristics were measured during all the period of plants growth according to literature (Terzopoulos and Bebeli 2008; Leht, 2009; El-Absawy *et al*., 2012 and Abdellatif *et al*., 2012).

Table 1: Faba bean varieties, origin, and pedigree used in the study.

No	Varity	Origin	Pedigree
1	Giza843	Egypt	Cross 461 x cross561
2	Giza716	Egypt	(83/453/503x83/824/461)

3	Misr1	Egypt	(123A/45/76XG.3) ×(62/1570/66×G.2)×(Romi×Habashi)
4	Sakha1	Egypt	(85/283/620x88/724/716)

Biochemical Marker analysis

Sodium Dodecyle Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique was used to study the protein banding patterns of four tested varieties of (*Vicia faba* L.). Total protein was extracted according to the method of (Laemmli, 1970). Leaves of faba bean samples collected from the treated plants (control, drought, heat and heat with drought) were used for the total protein extraction. The protein patterns of different treatments of faba bean were compared using SDS-PAGE method to identify the protein bands associated with drought and heat stress in faba bean.

Data Scoring and Statistical Analysis

The morphological traits of faba bean have been evaluated in a completely randomized design (CRD) with five repetitions for each treatment. Data from morphological experiment were subjected to analysis of variance (ANOVA) to determine variation among the varieties using JMP® 7.0 software (Sall *et al.*, 2007).

The means were compared by Least Significant Difference (LSD) of the treatments at 5% probability level. The two-way multivariate hierarchical cluster analysis has been done also using JMP® 7.0 software. An equation was used to calculate the sensitivity of the varieties to heat and drought stress: Susceptibility coefficient =∑ (Treatment $_{mean}$ - Control $_{mean}$). The susceptibility to the whole environmental stresses has been significantly tested using LSD values in JMP® 7.0 software.

Protein pattern was photographed with digital camera and the photos were handled with Adobe Photoshop 9 (CS2) software in order to adjust the contrast and the brightness. The Protein bands were scored as ''1'' and ''0'', whereas ''1'' stands for the presence and ''0'' stands for the absence of bands. Specific bands have been determined for specific cultivars. Cluster analysis has been made for the collected data of protein pattern bands of different stresses along with the control. A dendrogram was generated from the similarity matrix using the unweighted pair group method with arithmetic in NTSYSpc 2.0 software (Rohlf, 2000).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) of the morphological traits

Analysis of variance of the morphological traits was carried out in order to detect the significant differences among the genotypes; treatments and the interaction between genotypes and treatments for all the morphological traits. The data revealed highly significant differences among the genotypes; treatments and the interaction between genotypes and treatments for all the morphological traits (Table 2).

Our results are in agreement with different results obtained on faba bean. Alghamdi, (2009) found significant variance in drought stress tolerance among the genotypes of faba bean. Moreover, Ouji *et al*., (2011) determined genetic variability in nine Tunisian faba bean populations belonging to three botanical classes (Var. minor, var. equina and var. major) using twenty-seven agro-morphological traits. They noted significant differences between populations for most agro-morphological traits in four main groups. Furthermore, Abdellatif *et al*., (2012) evaluated eight economical Egyptian faba beans varieties

Table 2: Analysis of variance of 17 morphological traits of faba bean in response to both drought and heat stresses.

Source of Variation	DF	Leaves number (L)	Leaflets number (LL)	LL/L	Leaflets width (cm)	Days to flowering	Total number of Flowers	First pod height (mm)	Pods number/ plant	Seeds number /Pod
Genotype	3	1523.9**	20205.9**	1.136**	0.721**	12.74**	263.86**	93.19**	22.34**	2.98**
Treatment	3	2847.3**	71570.0**	7.414**	11.889**	243.29**	13116.49**	188.61**	120.17**	10.78**
Genotype*Treatment	9	214.0**	5637.3**	1.524**	1.987**	36.33**	1028.32**	69.17**	33.43**	1.74**

Source of Variation	DF	Seeds number /plant	Seeds weight /pod (g)	Seeds weight /plant (g)	100 seeds weight (g)	Number of branches	Number of nodes	Plant height (cm)	Dry plant weight(g)
Genotype	3	289.62**	914.22**	162.75**	1426.05**	8.071**	1428.94**	799.46**	11.20**
Treatment	3	1523.18**	862.78**	1121.46**	9353.40**	7.456**	2977.05**	3994.15**	612.44**
Genotype*Treatment	9	191.73**	2836.96**	130.50**	3523.84**	0.333**	213. 50**	219.53**	76.06**

**Highly significant differences

results showed highly significant differences among all treatments and genotypes in most of studied traits and revealed a negative correlation between the morphological traits and the drought tolerance. Abid *et al*., (2017) found

significant difference in morphological responses of 11 faba bean cultivars to drought stress.

Least Significant Differences (LSD) values among faba bean genotypes

LSD values of faba bean varieties illustrated significant differences among genotypes concerning drought and/or heat stresses in all measured morphological traits (Table 3). LSD showed the highest significant mean in total of seven traits (leaflets width, pods number/ plant, seeds number/pod, seeds number/plant, seeds weight/pod, seeds weight/plant and dry plant weight) and the lowest significant mean in one trait (100 seeds weight) in the variety "Giza843". In addition, the highest significant mean in total of seven traits (leaves number (L), leaflets number (LL), days to flowering, first pod height, number of branches, number of nodes and plant height) and the lowest significant mean in one trait (leaflets width) were recorded in the variety "Giza716". Furthermore, the variety "Misr1" revealed the highest significant mean in only one trait (100 seeds weight) and the lowest significant mean in 10 traits (leaves number, leaflet number, (LL/L), total number of flowers, first pod height, seeds number/pod, seeds weight/pod, number of branches, number of nodes and plant height). Moreover, the variety "Sakha1" exhibited the highest mean in two traits (LL/L and total number of flowers) and the lowest significant mean in six traits (days to flowering, pods number/ plant, seeds number/plant, seeds weight/pod, seeds weight/plant and plant weight (Table 3).

These results indicate that "Giza716" and "Giza843" varieties displayed the highest significant means of the most the morphological traits (13 out of 17 traits) including the yield and its components (Table 3). On the other hand, "Sakha1" and "Misr1" varieties manifested the lowest significant means. This might be an indicator for drought and/or heat tolerance of "Giza716" and "Giza843" varieties. Our results are similar to the results obtained by Link *et al*., (1999) who found significant variance in drought stress tolerance among the genotypes of faba bean. Likewise, Abdelmula and Abuanja (2007) evaluated the performance of 22 faba bean genotypes under heat stress and stated that the genetic variability, yield stability and correlations among yield, yield components and other vegetative traits were significantly different among genotypes for most of the agronomic characters.

Least Significant Differences (LSD) values among the treatments means

The significant differences among means of the four treatments (control, drought, heat and drought with heat) were investigated and presented in Table (4). According to the LSD values, the control treatment exhibited the highest significant means in 13 out of 17 traits (leaves number, leaflets number, LL/L, total number of flowers, pods number/ plant, seeds number/pod, seeds number/plant, seeds weight/pod, seeds weight/plant, 100 seeds weight, number of nodes, plant height and dry plant weight) and the lowest significant mean in only one trait (first pod height). While, drought treatment revealed the highest significant means in only one trait (days to flowering) and the lowest significant

mean in two traits (leaflets width and number of branches). Whereas, heat

Table 3: LSD Student's values among faba bean genotypes in response to both drought and heat stresses.

Genotype	Leaves number (L)	Leaflets number (LL)	(LL/L)	Leaflets width (cm)	Days to flowering	Total number of Flowers	First pod height (mm)	Pods number/ plant	Seeds number /Pod
Giza843	34.45 B	118.2 B	3.36 C	3.38 A	48.05 B	36.07 C	18.47 C	5.20 A	2.40 A
Giza716	44.4 A	158.85 A	3.46 B	2.99 D	48.30 A	36.10 B	21.20 A	3.90 B	2.25 B
Misr1	25.95 D	88.97 D	3.09 D	3.14 B	47.55 C	29.92 D	17.05 D	3.45 C	1.70 D
sakha1	29.77 C	112.86 C	3.61 A	3.04 C	46.65 D	37.23 A	20.82 B	2.95 D	1.75 C

Genotype	Seeds number /plant	Seeds weight/pod (g)	Seeds weight/plant (g)	100 seeds weight (g)	Number of branches	Number of nodes	Plant height (cm)	Dry plant weight (g)
Giza843	13.65 A	1.67 A	10.22 A	51.66 D	3.05 B	34.25 B	63.40 B	12.47 A
Giza716	9.55 B	1.46 B	6.49 B	63.75 B	3.37 A	44.85 A	63.92 A	12.08 B
Misr1	7.35 C	1.29 C	5.97 C	70.25 A	2.15 D	26.90 D	53.57 D	11.23 C
sakha1	5.60 D	1.19 D	3.98 D	61.25 C	2.33 C	30.75 C	53.77 C	11.03 D

Levels not connected by same letter are significantly different.

treatment displayed the highest significant means in three traits (leaflets width, first pod height, and number of branches) and the lowest significant means in two traits (days to flowering and 100 seeds weight). On the other hand, heat with drought treatment revealed the lowest significant means in 12 traits (leaves number (L), leaflets number (LL), LL/L, total number of flowers, pods number/ plant, seeds number/pod, seeds number/plant, seeds weight/pod, seeds weight/plant, number of nodes, plant height and dry plant weight, Table 4).

Thus, it can be stated that control treatment surpasses the other treatments in most of the morphological traits. On the others hand, the combined treatment of drought with heat stresses showed the lowest significant means in most of the morphological traits. In addition, the effect of heat stress on the morphological traits is less than the drought stress effect (Table 4). Our results are similar to the results obtained by Al-Suhaibani (2009) who assessed the effect of water regimes on faba bean cultivar "Giza 957" at four different growth stages encompassing its entire life cycle. The results manifested that water deficit significantly influenced seed yield and quality of faba bean. Furthermore, Siddiqui *et al*., (2015) estimated the morphological traits (plant height (PH), fresh weight (FW) and dry weight (DW), leaf area) of different faba bean genotypes under different levels of drought stresses. The results revealed that drought stress reduced all growth parameters.

LSD values of interaction between genotypes and treatments

The interaction between genotypes and treatment was significant in all the investigated morphological traits (Table 2). The highest significant mean (59.4) obtained from the variety "Giza716" with the control treatment, while the lowest significant mean (13.8) was observed on variety "Misr1" with the combined treatment (heat with drought) in the number of leaves trait (Table 5). In the number of leaflets the highest significant mean (252.8) obtained from the variety "Giza716" with control treatment, however, the slightest significant mean (31.9) was observed on variety "Misr1" with the combined treatment (heat with drought). For LL/L, the highest significant mean (4.23) obtained from the variety "Sakha1" in control treatment whereas, the lowest significant mean (2.31) was observed on variety "Misr1" with the combined treatment (heat with drought). The highest significant mean (4.30) obtained from the variety "Giza843" with heat treatment while the smallest significant mean (2.28) was observed on variety "Giza716" with drought treatment in the leaflets width. For days to flowering trait, the highest significant mean (53.0) obtained from the variety "Giza716" in control treatment whereas the lowest significant mean (42.4) was observed on variety "Giza716" in heat treatment. For total number of flowers trait, the highest significant mean (81.2) obtained from the variety "Sakha1" in control treatment while the lowest significant mean (42.4) was observed on variety "Misr1" in heat with drought treatment. For first pod height trait, the highest significant mean (25.0) obtained from the variety "Sakha1" in heat with drought treatment while the least significant mean was (13.2) noted on variety "Misr1" in control treatment. For pods number/ plant, the highest significant mean (9.2) obtained from the variety "Giza843" in control treatment while the lowest significant mean (1.0) was recorded on variety "Misr1" in heat with drought treatment. For seeds number/pod, the highest significant mean (3.0) obtained from the variety "Giza843"

Table 4: LSD Student's values among drought and heat stresses treatments of faba bean genotypes.

Treatment	Leaves number (L)	Leaflets number (LL)	LL/L	Leaflets width (cm)	Days to flowering	Total number of Flowers	First pod height (mm)	Pods number/ plant	Seeds number /Pod
Control	47.05 A	191.8 A	4.05 A	2.59 C	50.25 B	68.40 A	15.52 D	7.15 A	2.9 A
Drought	27.50C	98.1 C	3.42 B	2.49 D	50.50 A	32.65 B	19.15 C	3.30 B	2.2 B
Heat	37.50B	126.2 B	3.36 C	3.88 A	44.45 D	21.92 C	21.75 A	2.92 C	1.5 C
Heat+Drought	22.52 D	62.|8 D	2.69 D	3.60 B	45.35 C	16.35 D	21.12 B	2.12 D	1.5 D

Treatment	Seeds number /plant	Seeds weight /pod (g)	Seeds weight /plant (g)	100 seeds weight (g)	Number of branches	Number of nodes	Plant height (cm)	Dry plant weight (g)
Control	20.70 A	2.31 A	16.56 A	80.00 A	3.05 B	47.35 A	72.50 A	18.93 A
Drought	7.55 B	1.70 B	5.83 B	77.25 B	1.95 D	27.45 C	53.50 C	9.89 C
Heat	4.40 C	0.88 C	2.58 C	41.41 D	3.20 A	39.12 B	65.37 B	10.80 B
Heat+Drought	3.50 D	0.72 D	1.69 D	48.25 C	2.71 C	22.82 D	43.29 D	7.205 D

Levels not connected by same letter are significantly different.

Table 5: LSD Student's values of the interaction between faba bean genotypes and drought and heat stresses treatments.

Interaction	Leaves number (L)	Leaflets number (LL)	LL/L	Leaflets width (cm)	Days to flowering	Total number of Flowers	First pod height (mm)	Pods number / plant	Seeds number /Pod
Giza843,Control	43.0 D	166.0 C	3.87 E	2.58 L	50.0 C	61.0 D	16.8 L	9.2 A	3.0 A
Giza843,Drought	31.4 J	102.8 J	3.26 I	2.46 M	51.0 B	29.2 I	15.6 M	6.2 D	2.6 D
Giza843,Heat	42.0 F	140.0 E	3.33 H	4.30 A	45.8 F	35.4 F	22.5 E	2.9 G	2.0 G
Giza843,Heat+Drought	21.4 L	64.0 N	2.99 M	4.20 B	45.4 H	18.7 L	19.0 I	2.5 J	2.0 G
Giza716,Control	59.4 A	252.8 A	4.20 B	2.30 O	53.0 A	66.6 B	17.0 K	7.0 C	3.0 A
Giza716,Drought	37.6 G	136.6 G	3.57 G	2.28 P	51.0 B	32.8 G	22.8 D	2.6 I	2.0 F
Giza716,Heat	44.0 B	136.0 H	3.10 K	4.00 C	42.4 K	24.5 J	23.5 B	3.0 F	2.0 G
Giza716,Heat+Drought	36.6 H	110.0 I	3.00 L	3.40 F	46.8 E	20.5 K	21.5 F	3.0 F	2.0 G
Misr1,Control	42.4 E	165.0 D	3.91 D	2.84 J	50.0 C	64.8 C	13.2 O	8.2 B	2.6 C
Misr1,Drought	19.6 M	68.2 M	2.92 N	2.34 N	50.0 C	31.0 H	18.0 J	1.8 L	2.2 E
Misr1,Heat	28.0 K	90.8 K	3.24 J	3.90 D	45.6 G	13.0 O	18.0 J	2.8 H	1.0 I
Misr1,Heat+Drought	13.8 O	31.9 P	2.31 P	3.50 E	44.6 I	10.9 P	19.0 H	1.0 M	1.0 I
sakha1,Control	43.4 C	183.4 B	4.23 A	2.66 K	48.0 D	81.2 A	15.1 N	4.2 E	2.98 B
sakha1,Drought	21.4 L	84.8 L	3.94 C	2.88 I	50.0 C	37.6 E	20.2 G	2.6 I	2.0 H
sakha1,Heat	36.0 I	138.0 F	3.80 F	3.34 G	44.0 J	14.8 N	23.0 C	3.0 F	1.0 J
sakha1,Heat+Drought	18.3 N	45.2 O	2.47 O	3.30 H	44.6 I	15.3 M	25.0 A	2.0 K	1.0 K

Table 5: Cont.

Interaction	Seeds number/plant	Seeds weight/pod (g)	Seeds weight/plant (g)	100 seeds weight (g)	Number of branches	Number of nodes	Plant height (cm)	Dry plant weight (g)
Giza843,Control	27.6 A	2.39 B	22.08 A	80.0 B	3.4 D	42.6 D	75.4 B	20.44 B
Giza843,Drought	16.2 D	2.03 E	12.64 D	78.0 D	2.4 G	32.6 J	63.0 G	11.00 G
Giza843,Heat	5.8 G	1.30 I	3.77 G	6 5.0H	3.6 B	40.0 F	68.0 E	11.24 F
Giza843,Heat+Drought	5.0 I	0.96 L	2.40 L	48.0 M	2.8 E	21.8 M	47.2 M	7.22 N
Giza716,Control	21.0 C	2.25 C	15.75 C	75.0 E	3.6 B	61.0 A	76.4 A	15.46 D
Giza716,Drought	5.2 H	1.46 H	3.80 F	73.0 F	2.6 F	34.4 I	55.8 J	10.50 H
Giza716,Heat	6.0 F	1.14 J	3.42 I	57.0 I	3.8 A	48.0 B	73.0 C	13.00 E
Giza716,Heat+Drought	6.0 F	1.00 K	3.00 K	50.0 L	3.5 C	36.0 H	50.5 K	9.36 K
Misr1,Control	21.6 B	2.21 D	18.36 B	85.0 A	2.6 F	42.4 E	73.0 D	21.22 A
Misr1,Drought	4.0 K	1.87 F	3.40 J	85.0 A	1.2 K	20.8 N	45.2 N	7.75 M
Misr1,Heat	2.8 M	0.57 M	1.60 M	57.0 I	2.6 F	29.9 K	60.5 H	10.36 I
Misr1,Heat+Drought	1.0 O	0.54 N	0.54 P	54.0 J	2.2 I	14.5 P	35.6 P	5.61 P
sakha1,Control	12.6 E	2.40 A	10.08 E	80.0 C	2.6 F	43.4 C	65.2 F	18.60 C
sakha1,Drought	4.8 J	1.46 G	3.51 H	73.0 G	1.6 J	22.0 L	50.0 L	10.32 J
sakha1,Heat	3.0 L	.0.51 O	1.53 N	51.0 K	2.8 E	38.6 G	60.0 I	8.60 L
sakha1,Heat+Drought	2.0 N	0.41 P	0.82 O	41.0 N	2.3 H	19.0 O	39.9 O	6.63 O

Levels not connected by same letter are significantly different.

treatment. For seeds number/plant, the highest significant mean (27.6) obtained from the variety "Giza843" in control treatment while the lowest significant mean (1.0) was observed on variety "Misr1" in heat with drought treatment. For seeds weight/pod the highest significant mean (2.40) obtained from the variety "Sakha1" in heat treatment whereas the lowest significant mean (0.41) was scored for variety "Sakha1" in heat with drought treatment. For seeds weight/plant, the highest significant mean (22.08) obtained from the variety "Giza843" in control treatment while the lowest significant mean (0.54) was scored for variety "Misr1" in heat with drought treatment. For 100 seeds weight, the highest significant mean (85.0) obtained from the variety "Misr1" in control treatment and from "Misr1" in drought treatment while the lowest significant mean (41.0) was observed on variety "Sakha1" in heat with drought treatment. For number of branches, the highest significant mean (3.8) was scored for the variety "Giza716" in heat treatment while the lowest significant mean (1.2) was observed on variety "Misr1" in drought treatment. For number of nodes, the highest significant mean (61.0) was scored for the variety "Giza716" in control treatment; whereas the lowest significant mean (14.5) was marked on variety "Misr1" in heat with drought treatment. For plant height, the highest significant mean (76.4) was scored for the variety "Giza716" in control treatment while the lowest significant mean (5.61) was observed on variety "Misr1" in heat with drought treatment. For dry plant weight, the highest significant mean (21.22) obtained from the variety "Misr1" in control treatment while the lowest significant mean (5.61) was observed for the variety "Misr1" in heat with drought treatment.

It can be concluded that the control treatment in both varieties "Giza716" and "Giza843" exhibited the highest significant means in most of the morphological traits (10 out of 17). On the other hand, the heat with drought treatment in the variety "Misr1" displayed the lowest significant means in most of the morphological traits 10 out of 17, (Table 5). Thus, it can be summarized that the results of the interaction support the results of the main effects (genotypes and treatments separately). Our results are similar to those of Afiah *et al*., (2016) in their study on the response of five divergent faba bean genotypes namely ("NBL Mar 3", "NBL 5", "L 3", "Nubariya1" and "Misr1") to water stress on some morphological characteristics. They found wide range of differences among all genotypes in seed yield /plant and "NBL 5" was the most drought tolerant genotype while "Misr1" was the most sensitive one.

Susceptibility test

Determination of heat and drought tolerance of the faba bean varieties were investigated by application of drought and or heat treatments. The variety which exhibited stable results across both the different irrigation and heat treatments was considered as drought and heat tolerant variety and the variety which displayed unstable or variable results through the different treatments was considered as drought and heat susceptible variety according to (Cattivelli *et al*., 2008; Khan *et al*., 2010; Abdellatif *et al.,* 2012, El-Absawy *et al.*, 2012). An equation was used according to the previous references to detect the susceptibility of tested varieties to drought and heat stresses as following.

Susceptibility coefficient = ∑ (Treatment mean - Control mean).

The Significant differences according to this equation were tested using LSD, the variety "Misr1" showed the highest significant susceptibility value to drought

stress for the number of leaves (-65), number of pods (-16), seeds weight/plant (-49.54), plant height (-1.79), number of branching (-77.7), number of nodes (-117.64) and seeds number/plant (-57) (Table 6). Thus, this variety could be considered as susceptible variety for drought and heat stress. On the other hand, the variety "Giza 843" was the most significant tolerant to drought and heat in the number of leaves (-34), leaflet number (-191.2), number of flowers (-99.69), first pod height (6.7), seeds weight/pod (-2.88), 100 seeds weight (-39), dry plant weight (-33.4) and seeds number/plant. Thus, this variety could be considered as tolerant variety for drought and heat stress (Table 6).

Morphological cluster analysis

For morphological traits, the two-way hierarchical cluster analysis divided (in the first way of clustering) the *Vicia faba* varieties into two clusters, the first cluster contained "Giza843" and "Giza716" varieties (the environmental stress tolerant varieties), while the second cluster consisted of "Misr1" and "Sakha1" (susceptible varieties) (Figure 1). For morphological traits (in the second way of clustering), the cluster analysis divided the morphological traits into three cluster. The first cluster was divided into two sub clusters; the first sub cluster consisted of leaves number, number of nodes and leaflets number traits, while the second sub cluster contained days to flowering, seeds number/pods, dry plant weight, plant height and number of branching traits. The second cluster was divided into two sub clusters, the first sub cluster consisted of leaflets width traits, while the second sub cluster consisted of pods number, seeds number /plant and seeds weight /plant traits. The third cluster was divided into two sub cluster, the first sub cluster contained LL/L, numbers of flowers, first pods height and seeds weight /pods traits, while the second sub cluster contained 100 seeds weight trait (Figure 1).

It seems that the environmental stress tolerant varieties ("Giza843" and "Giza716") were aggregated in one cluster while the environmental susceptible varieties ("Misr1" and "Sakha1") were clustered together. These results are in harmony with those found with Abdellatif *et al.,* (2012) and El-Absawy *et al.,* (2012).

Table 6 : Estimation of Susceptibility of four faba bean varieties under heat and/or drought stresses using morphological traits.

Genotype	Leave number (L)	Leaflet number (LL)	LL/ L	Leaflet width	Days to flowering	number of Flowers	First pod height	Pods number	Seeds number /pods
Giza843	-34A	-191.2A	-2.03B	3.22D	-7.8B	-99.69A	6.7A	-16C	-3.6B
Giza716	-60C	-375.8D	-2.93D	2.6C	-18.8D	-122B	16.8C	-12.4B	-3A
Misr1	-65D	-304.1C	-1.34A	1.31A	-9.79C	-139.5C	15.4B	-19D	-3.6B
sakha1	-54.4B	-282.2B	-2.48C	1.54B	-5.39A	-175.9D	22.9D	-5A	-4.94C

Genotype	Seeds number /plant	Seeds weight /pod (g)	Seeds weight /plant (g)	100seeds weight(g)	Number of branching	Number of nodes	Plant height (cm)	Dry plant weight (g)
Giza843	-22.6A	-2.88A	-47.43C	-39A	-48B	-31.86C	-1.4C	-33.4A
Giza716	-49.8C	-3.15B	-37.03B	-45B	-49.9C	-13.52A	-0.9A	-63.99D
Misr1	-57D	-3.65C	-49.54D	-59C	-77.7D	-117.64D	-1.79D	-62C
sakha1	-28B	-4.82D	-24.38A	-75D	-45.7A	-30.25B	-1.3B	-50.6B

Values connected with the same letter are not significantly different at 0.05 probability level

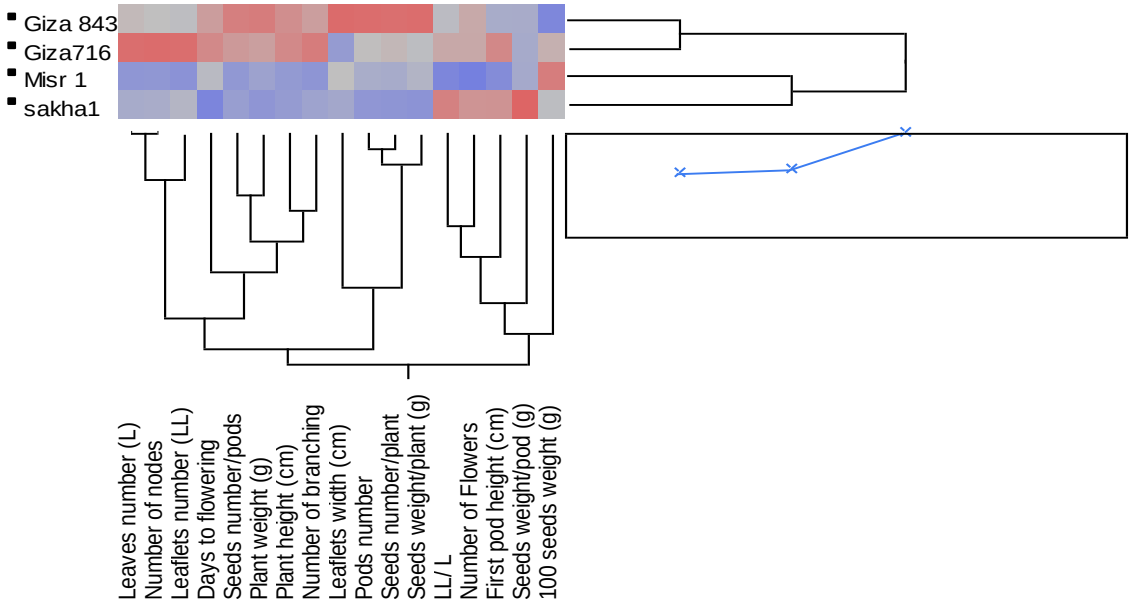

Figure 1: Two-way hierarchical cluster analysis of faba bean varieties and morphological traits under different levels of heat and drought stresses.

Biochemical analysis

SDS-PAGE technique was used to study the protein banding patterns of four varieties of (*Vicia faba* L.) under drought and heat stresses. According to the protein analysis, a total of twenty-four bands were observed from the total protein pattern overall the cultivars. Some bands were identified that may be correlated with drought and/or heat tolerant in faba bean; whereas a band at about molecular weight of 78 kDa has been noticed at the protein pattern of "Giza716" variety under heat and drought treatment (Figure 2). Another band at about molecular weight of 100 kDa has been noticed from the total protein pattern of "Giza843" variety under heat and drought treatment. Those two bands were not found in the pattern of the other two varieties (Figure 2). These findings may support that both varieties "Giza843" and "Giza716" accumulate proteins with different molecular weight in response to the environmental stresses which reflects their tolerance in different ways to that stresses. On the other hand, the varieties "Misr1" and "Sakha1" protein patterns have no changes which may reflects their negative to the environmental stresses. Similar results were reported in barely by Elrabey *et al.,* (2009) and in wheat by Elsawy *et al.,* (2015).

Cluster analysis of protein data

The results of cluster analysis of protein data for total treatment showed that the studied faba bean cultivars were divided into two clusters. The first cluster consisted of "Giza843"and "Giza716". The second cluster contained "Misr1" and "Sakha1" (Figure 3).

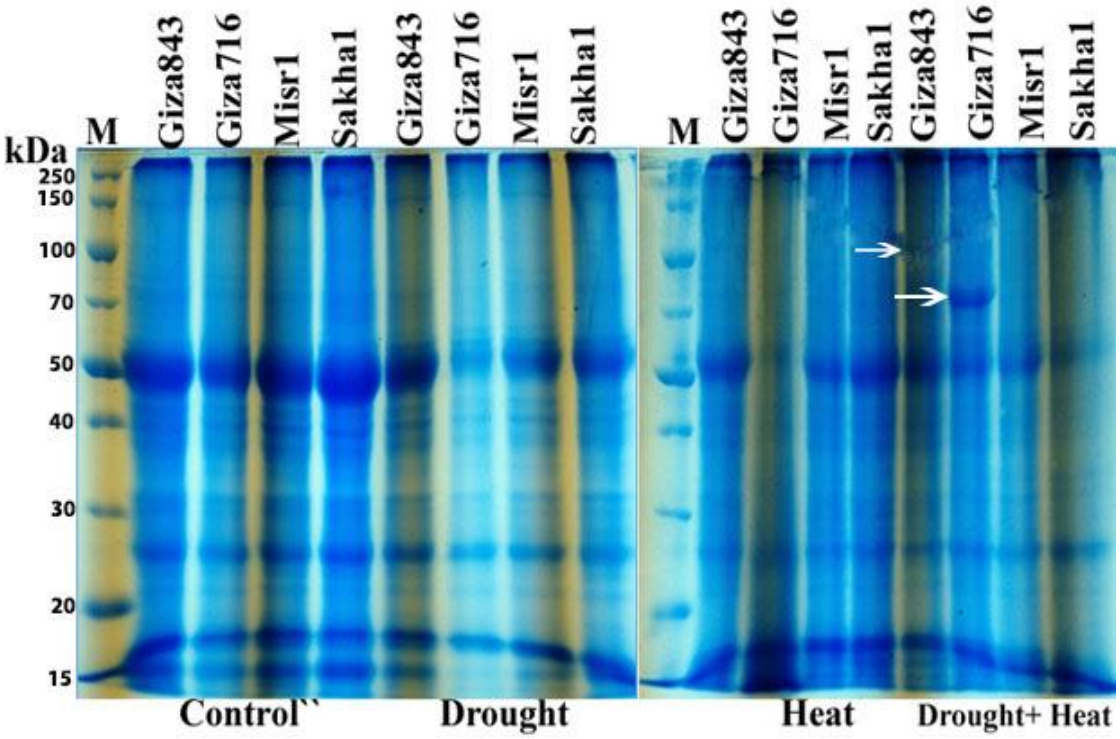

Figure 2: SDS-PAGE protein pattern of four drought and heat stressed faba bean varieties separated on 15% SDS-PAGE.

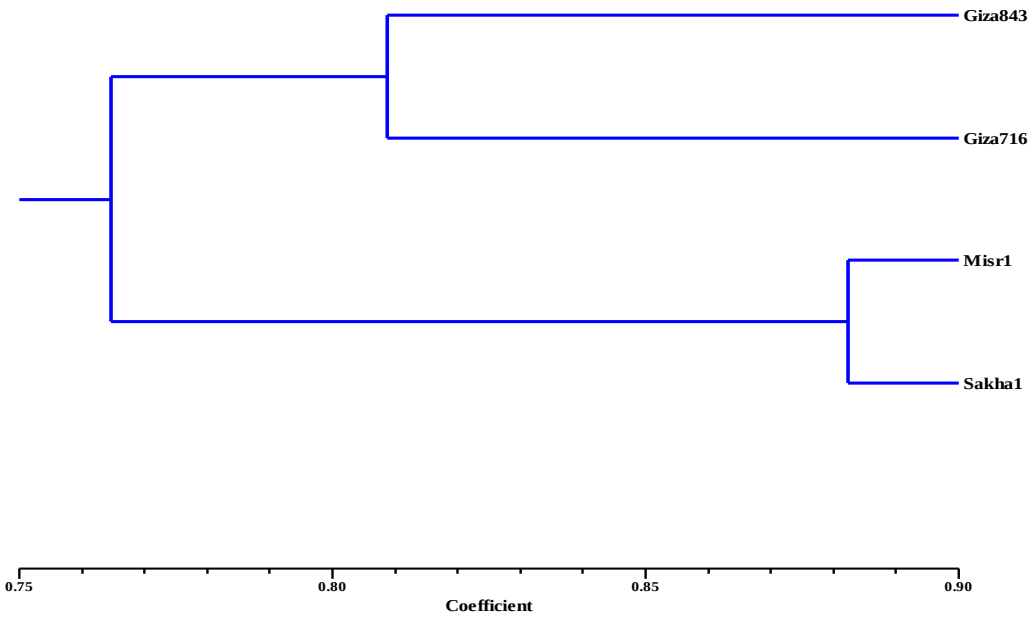

Figure 3: Dendrogram of total protein pattern in the four tested faba bean varieties.

These results support the results of morphological traits whereas they revealed that both varieties "Giza843"and "Giza716" are having protein patterns different from those of the varieties "Misr1" and "Sakha1". In addition, the latter are susceptible to both heat and drought stresses. Abdellatif *et al.,* (2012) found that the protein pattern of the variety "Giza843" (the drought tolerant variety) is different from the protein pattern of the variety "Giza3" (the drought susceptible variety).

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الاختلافات الظاهرية والكيموحيوية واستجابة الفول البلدي المصري للحرارة والجفاف

محمد ابوالعلا بلال[1] ، سماح محمد محمود الدميري [2] ، يحيي عبدالله خضر[1] ، كمال فؤاد عبداللطيف[1]

[1] قسم البيوتكنولوجيا النباتيه، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية – جامعه مدينه السادات – المنوفيه– مصر.

[2] قسم البيولوجيا الجزيئيه، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية – جامعه مدينه السادات – المنوفيه– مصر.

الملخص العربي

تم تقيم أربع أصناف من الفول البلدي تحت ظروف الجفاف والحرارة باستخدام 17 صفة مورفولوجية ومحتوي البرروتين الكلي. تحليل التبيان للصفات المورفولوجية اظهر اختلافات معنوية بين المعاملات والاصناف لكل الصفات المدروسة و التفاعل بين الاصناف والمعاملات كان ايضا معنوي. كلا من صنفي "جيزة716" و "جيزة "843" اعطي اعلي فرق معنوي لمعظم الصفات المدروسة ومعاملة الكنترول تفوقت علي باقي المعاملات. اقل فرق معنوي تم الحصول عليه من معاملة الحرارة مع الجفاف بينما تاثير الاجهاد الحراري علي الصفات المورفولوجية كان اقل من من تاثير اجهاد الجفاف. طبقا للتحليل العنقودي للبيانات المورفولوجية والبروتين صنف "جيزة "716" وصنف جيزة843" كانوا في مجموعة واحدة معا بينما صنف "مصر1" وصنف "سخا1" كانوا في مجموعة احري واحدة. تم الحصول علي حزمة بروتين عند وزن 78 كيلو دالتون في نمط بروتين صنف "جيزة716" (معاملة الحراة والجفاف) وعند وزن 100 كيلو دالتون لنمط بروتين صنف "جيزة843" (معاملة الحرارة والجفاف). اختبار الحساسية لتحمل الجفاف والحرارة ونتائج كلا من المورفولوجي والبيوكيميائي اشارت الي ان كلا من صنف "جيزة "716" وصنف جيزة 843" اصناف متحملة للجفاف والحرارة بينما صنف "مصر1" وصنف "سخا1" اصناف حساسة للجفاف والحرارة.

أسماء السادة المحكمين

أ.د/ رجاء عبدالعزيز عيسى كلية الزراعة – جامعة المنوفية

أ.د/ ميرفت إدوارد سوريـــال كلية الزراعة – جامعة المنوفية