

Drought Stress Tolerance of Faba Bean as Studied by Morphological Traits and Seed Storage Protein Pattern

Kamal Fouad Abdellatif¹, El Sayed A. El Absawy² & Asmaa M. Zakaria²

¹ Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Minofiya University, Egypt

² Bioinformatics Department, Genetic Engineering and Biotechnology Research Institute, Minofiya University, Egypt

Correspondence: Kamal Fouad Abdellatif, Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Minofiya University, Egypt. E-mail: kamal2004gr@yahoo.com

Received: April 5, 2012 Accepted: May 18, 2012 Online Published: August 10, 2012

doi:10.5539/jps.v1n2p47

URL: <http://dx.doi.org/10.5539/jps.v1n2p47>

Abstract

Eight economical Egyptian faba bean (*Vicia faba* L.) varieties had been evaluated under different drought stress levels and their seed storage protein content patterns under drought stress conditions had been studied and compared with pattern under normal conditions. Analysis of variance of the morphological trait revealed highly significant differences among both treatments and genotypes for the most of studied traits. The susceptibility test for drought tolerance revealed that the variety 'Giza 3' showed the highest statistical significant susceptibility to drought stress and could be considered as susceptible variety for drought stress, while the variety 'Giza 843' was more tolerant to drought stress. Although 'Giza 3' variety gave the highest mean across all genotypes for the most of the morphological traits, it was the most drought susceptible variety. On the other hand, 'Giza843' gave medium mean across the genotypes but it was the most drought tolerant variety. Negative correlation between the morphological traits and the drought tolerance in faba bean. Using SDS-PAGE analysis, optical differences were obtained between the varieties 'Giza 843' (drought tolerant) and 'Giza 3' (drought susceptible) in their protein patterns. Many protein bands were obtained in the protein pattern of the variety 'Giza 843' that were not obtained in the protein pattern of the variety 'Giza 3' while some protein bands were observed in the protein pattern of the variety 'Giza 3' that were not present in the protein pattern of the variety 'Giza 843'. Significant association could be observed between morphological traits and biochemical markers.

Keywords: *Vicia faba* L., drought stress, morphological traits, SDS-PAGE, seed storage protein

1. Introduction

Faba bean (*Vicia faba* L.) is a major leguminous crop that grown in Egypt; it is an important source of protein for human and animal consumption and it plays a role in the crop rotation. However, the total production of this crop is still insufficient to cover the local consumption. From the above-mentioned facts, there is a great need to overcome this gap between local production and demand by expansion through reclaimed areas which represent the most hope of cultivated lands in increasing our agricultural production and subsequently in overcoming the deficiency in food requirements, as well as, increasing the vertical production through introduction of new varieties with high yield potential.

Drought is an important environmental factor, which induces significant alterations in plant physiology and biochemistry. Some plants exhibit a number of physiological adaptations that allow them to tolerate water stress conditions. The degree of adaptation to the decrease in water potential caused by drought may vary considerably between species (Savé et al., 1995) and also within species (Parker & Pallardy, 1985). Faba bean is more sensitive to drought than some other seed legumes including common bean, pea and chickpea (McDonald & Paulsen, 1997; Amede & Schubert, 2003). Although genotypic differences in the response of faba bean to drought have been documented (Heringa et al., 1984; Grzesiak et al., 1997; Abdelmula et al., 1999; Amede et al., 1999; Link et al., 1999), the physiological processes associated with drought tolerance are less understood than for other crop species.

Faba bean mainly grown for its high protein content (30 % on average). It is an important source of proteins for

humans and animals. Faba bean popularity has increased recently as its high yield makes it attractive to producers while its high protein content and low-priced makes it attractive to consumers (Pala et al., 2000). Electrophoresis techniques offer an exceptional opportunity to study the substructure difference in protein among different genotypes. Nevertheless, SDS-PAGE was used to differentiate between cultivars of *V. faba* (Stegmann et al., 1980) and to identify inbred lines (Gates & Boulter, 1979).

It is useful for the plant breeder to determine the genetic relationships among the genotypes of the available breeding material. The relationship between genotypes, according to Schut et al. (1997), is usually based on three sources of information: (1) geographic information about the origin of the genotypes, (2) pedigree information, and (3) information about plant characteristics. The objectives of this study were to evaluate eight economic Egyptian faba bean (*Vicia faba* L.) varieties under different levels of drought stress to study their response and behavior under different drought stress levels and study of seed storage protein content pattern of the eight faba bean varieties under drought stress conditions and compare them with pattern under normal conditions.

2. Material and Methods

2.1 Plant Material and Field Experiment

The present work was carried out at the Plant Molecular biology Laboratory, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minoufiya University, Egypt, during the period of 2009-2011. Eight Egyptian faba bean (*Vicia faba* L.) varieties have been used for field experiment and the biochemical analysis, all of the varieties have known pedigrees (Table 1) and were kindly obtained from the Agricultural Research Center, Field Crop Research Institute, (FCRI), Giza, Egypt.

The field experiment was carried out at the farm of Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minoufiya University, Egypt. Seeds of each variety were planted in four rows (three meters in length) in completely randomized design (CRD). The rows were planted with an additional empty row between the planted rows. Four irrigation treatments have been applied out for the cultivated varieties. The first treatment was the control and was irrigated by dropping day by day. The second treatment was irrigated by dropping one time each week. The third treatment was irrigated by dropping one time each two weeks, while the last treatment did not irrigated after germination until the seed harvesting date.

Eleven morphological characteristics were measured during all the period of plant growth according to literature (Singh et al., 1991; Terzopoulos et al., 2008). These traits included number of leaves (L), number of leaflets (LL), the percentage between the them (LL/L), plant height (cm), number of legumes, number of days to flowering, plant weight (g), number of seeds per plant, the percentage of fertilized flowers, total number of flowers and number of branches. Five measurements had been taken for each trait and then the average of each trait was calculated to be used for statistical analysis.

Table 1. Faba bean varieties, origin, and pedigree used for molecular marker analyses and morphological traits

NO	Variety	Origin	Pedigree
1	Giza 3	EGYPT	G1*NA 29
2	Sakha 1	EGYPT	(85/283/620x88/724/716)
3	Giza 716	EGYPT	(83/453/503x83/824/461)
4	Giza 843	FCRI ¹	Cross 461 x cross561
5	Nubaria1	ESPAIN	By individual selection from Rina Blanka
6	Misr 1	FCRI ¹	(123A/45/76XG3)×(62/1570/66×G.2)×(Romi×Habashi)
7	Sakha 2	FCRI ¹	Reina Blanka x461/845/83
8	Sakha 3	EGYPT	By individual selection from G716

¹ FCRI = Field Crop Research Institute, Agricultural Research Center, Giza, Egypt.

2.2 Seed Storage Protein

Sodium Dodecyle Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique was used to study the protein banding patterns of eight varieties of (*Vicia faba* L.). Seeds of stressed faba bean varieties (collected from

the treatment which was irrigated only one time after germination in the field experiment) were used for the total seed storage protein extraction as well as the seeds of the normal treatment of the varieties according to the method of Laemmli, (1970). The protein patterns of stressed and non stressed faba bean seeds were compared.

2.3 Data Analysis

The faba bean varieties had been evaluated in completely randomized design (CRD) experiment. Data from morphological experiment were subjected to analysis of variance (ANOVA) using SPSS 14 (Statistical Package for the Social Sciences), Snedecor and Cochran (1967). The means were compared by the Student's Least Significant Difference (LSD) value of the irrigation treatments and the genotypes at 5% probability level. An equation was used to calculate the susceptibility of the varieties to drought stress as following: Susceptibility coefficient = $\sum (\text{Treatment}_{\text{mean}} - \text{Control}_{\text{mean}})$

The susceptibility result was tested using the Student's LSD values.

Protein gels were photographed with digital camera and handled with Adobe Photoshop 9 (CS2) software in order to adjust the contrast and the brightness then gel was scored as 0/1 for the absence/presence of bands, respectively. Specific bands have been determined for specific varieties and correlation between the morphological traits and the biochemical markers has been made according to the specific protein bands.

3. Results and Discussion

3.1 Analysis of Variance (ANOVA) of the Morphological Traits

Analysis of variance of the morphological trait was carried out in order to detect the significant differences among the genotypes for all the morphological traits (Table 2). The data revealed highly significant differences among the treatments for all the studied traits except for days to flowering trait. Moreover, all traits revealed highly significant differences among genotypes except of the leaves number (L) trait in which no significant differences were obtained. Similarly, the interaction between genotypes and treatments was significant for all traits except for the percentage (LL / L) and days to flowering traits (Table 2).

Our results are similar to the results of EL-Harty et al. (2008) when they studied heterosis and genetic analysis of yield and some characteristics in faba bean (*Vicia faba* L.) using six faba bean genotypes ('Aquadolce', 'Nubaria 1', 'Giza 716', 'Sakha 3', 'Giza 429' and 'Triple white'). They studied combining ability and genetic components for yield and its components: plant height, number of branches, number of pods, number of seeds, seed yield /plant and 100-seed weight. Their analysis of variance indicated highly significant differences among the entries for all characters. Ouji et al. (2011), determined genetic variability in nine Tunisian faba bean (*Vicia faba* L.) populations which belonging to three botanical classes (Var. minor, var. equina and var. major) using twenty seven agro-morphological traits. Analysis of variance, correlation coefficients was performed. Significant differences between populations were noted for most agro morphological traits in four main groups.

3.2 Least Significant Differences (LSD)

The ANOVA illustrated that highly significant differences among the genotypes were obtained. LSD means differences showed that the variety 'Giza 3' gave the highest significant mean in the most of the measured traits while the lowest significant mean was obtained from the variety 'Misr 1' for the most of the measured traits (Table 4). The mean of the variety 'Giza 843' was placed at a medium level for the most of the morphological traits. These means represented the ability of yield productivity and growth rate of each variety. However, the highest value of means does not mean the ability to tolerate the drought stress. The susceptibility test proved that the variety 'Giza 3' was the most drought susceptible variety while the variety 'Giza 843' was the most tolerant variety (Table 4).

The means of the four irrigation treatments (e.g. normal irrigation, irrigation each one week, irrigation each two weeks and irrigation only one time at the flowering stage) were compared to study the significant differences among the treatments, (Table 3). According to the LSD, the results showed that the treatments "irrigation each two weeks" and "irrigation each one week" were not significantly different from the control for the following traits: leaflets number (LL), the percentage LL / L, number of legumes and the percentage (%) of fertilized flowers; (Table 3). The treatment "irrigation only one time" gave the least response to stress for all the studied traits and was significantly different from all other treatments. The control treatment "irrigation day by day" gave the best significant response only for the trait number of branches, while it was not significantly differed from the treatment "irrigation each one week" for the traits plant weight (g) and number of seeds per plant. The treatments "irrigation each one week" and "irrigation each two weeks" significantly surpassed the control in the traits leaves number and plant height (cm), while the treatment "irrigation each one week" significantly surpassed all the other treatments including the control in the total number of flowers trait. No significant

differences were obtained among the treatments for the number of days to flowering trait; this trait did not affected with drought stress condition. This trait could be considered as morphological marker for drought stress tolerance in faba bean and it could be proposed to be controlled by low gene number and has high percentage of heritability, (Table 3). The traits of leaves number, plant height (cm), total number of flowers, plant weight (gm) and number of branches were significantly differences among the treatments. These traits were affected by the drought stress condition and could be considered as morphological marker for drought stress susceptible in faba bean. Thus, this trait is proposed to be controlled by high gene number and has percentage of heritability lower than the trait of days to flowering and consequently affected by the environmental stresses.

Link et al. (1999) studied genotypic variation for drought tolerance in *Vicia faba*. Four sets with 10-19 faba bean genotypes each were evaluated in multilocal field trials between 1992 and 1996. Stress occurred as natural drought in one experiment and as artificial terminal drought in three experiments. Artificial drought was induced by rain shelters, the control treatment was irrigated. Tolerance was assessed as the ratio of yield under drought (Yd) to well watered yield (Yw). Highly significant variances between genotypes occurred heritability of tolerance was $0.51 < h^2 < 0.88$. Exotic (North African, Latin American) genotypes were more tolerant than adapted material. Correlations between Yw and Yd were $0.77^{**} < r < 0.97^{**}$ and variance of Yd was less than one- third of the variance of Yw. Relative reduction of plant height due to drought was a promising trait to improve drought tolerance indirectly in two sets.

These results are in agreement with these obtained by Khalafallah et al. (2008); where they studied drought tolerance of seven varieties of faba bean (*Vicia faba* L). One of the varieties was Egyptian origin and the other six varieties were provided by ICARDA. Faba bean plants were grown under three different irrigation water intervals, 5, 10 and 15 days. Control plants showed higher growth and yield than plants subjected to water. Expose the faba bean varieties (1, 2, 4, 5, 6 and 7) to water stress leads to significant increase in photosynthetic pigments after 45 days from sowing, this effect inversed after 90 days.

Emam et al. (2010) studied water Stress Effects on Two Common Bean Cultivars with Contrasting Growth Habits There were four water stress levels (100, 75, 50 and 25% of field capacity by weight). The results showed that plant height, number of leaves, leaf area, number of pods, pod dry weight and total dry weight of both cultivars responded significantly to water stress conditions. Water stress also reduced stem height and reduced leaf area. Furthermore, it reduced pod dry weight in both cultivars and in 50 and 25% water stress levels, all plant pods of both cultivars were aborted.

3.3 Drought Tolerance of Faba Bean Varieties

The drought tolerance of the faba bean varieties were studied by application of different irrigation treatments. The variety which gave stable results across the different irrigation treatments was considered as drought tolerant variety and the variety which gave unstable or variable results through the different drought stress treatments was considered as drought susceptible variety according to (Cattivelli et al., 2008; Khan et al., 2010). According to the previous role an equation was calculated to estimate the susceptibility of the varieties to drought stress. Susceptibility coefficient = $\sum ((\text{Treatment}_{\text{mean}} - \text{Control}_{\text{mean}}))$.

Table 2. Analysis of variance for faba bean morphological traits under study analysis

Source	DF	Leaflets No.(LL)	Leaves No.(L)	LL / L	Plant height (cm)	Total No. flowers	No. of legumes	% Fert. flowers	Days to flowering	Seeds No.	Plant weight (g)	branches No.
Treatment(T)	3	9355**	119.26**	5.09**	12680.5**	5381.79**	210.51**	416.10**	22.45 ^{N.S}	3979.67**	3163.71**	25.78**
Genotype(G)	7	1151.7**	20.38 ^{N.S}	1.00**	558.7**	369.16**	65.04**	297.05**	64.64**	83.06**	103.38**	1.27**
T*G	21	981.8**	30.91**	0.36 ^{N.S}	165.1**	140.18**	23.32**	108.63**	41.87 ^{N.S}	161.85**	92.49**	3.95**

**indicate significance at the 0.01 level of probability, N.S: not significant.

Table 3. Effect of irrigation treatment on traits under study of faba bean

Irrigation Treatment	Leaflets No.(LL)	Leaves No.(L)	LL / L	Plant height(cm)	Total No. f lowers	No. of legumes	% Fert. flowers	Days to f lowering	Seeds No.	Plant weight(g)	branches No.
Control	115.33 a	22.83 b	5.1 a	64.45 b	52.58 b	6.75 a	13.39 a	50.7 a	28.35 a	23.58 a	3.25 a
Each one Week	122.83 a	23.83 a b	5.16 a	73.73 a	63.83 a	6.98 a	11.00 a	52.23 a	25.95 a	23.57 a	2.83 b
Each Two weeks	120.4 a	24.58 a	4.90 a	70.05 a	52.9 b	6.2 a	12.16 a	52.3 a	19.63b	18.80 b	2.5 b
Only one time	89.58 b	20.6 c	4.38 b	34.66 c	35.73 c	2.1 b	6.34 b	51.48 a	6.1 c	4.78 c	1.38 c

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

Table 4. LSD means comparisons of eight faba bean genotypes evaluated under different irrigation treatments

Genotype	Leaflets No.(LL)	Leaves No.(L)	LL / L	Plant height(cm)	Total No. flowers	No. of legumes	% Fert. flowers	Days to flowering	Seeds No.	Plant weight (g)	branches No.
Giza 3	127.15 a	25.05a	5.06ab	73a	50.5ab	8.6a	16.57a	51.15b	22.35a	17.69abc	2.35b
Sakha 1	105.15 c	22.6 a	4.65cd	60.1 bc	47.95b	4.25c	8.92bc	55.8a	20.2abc	17.06bc	2.95a
Sakha 2	118.15 ab	22.7 a	5.22a	55.35c	48.3b	4.05c	8.14c	51.75b	21.75ab	19.51ab	2.25b
Misr 1	104.9 c	23.1 a	4.53d	60.4 b	46.05b	7.9a	16.08a	51.2b	18bc	13.43d	2.8a
Giza 716	107.55 c	22.6 a	4.79bcd	57.7 bc	55.55a	4.25c	7.69c	50.9b	22.6a	20.01a	2.45b
Nubaria1	109.6 bc	21.95 a	5.00ab	60.8b	56.95a	4.35c	7.75c	51.95b	18.55bc	20.33a	2.35b
Sakha 3	114.4 bc	23.65 a	4.87bc	58.3 bc	48.6b	4.7c	9.95bc	50.95b	17.35c	17.69abc	2.35b
Giza 843	109.35 bc	22 a	4.97ab	60.05 bc	56.15a	5.95b	10.71b	49.7b	19.25abc	17.06bc	2.95a

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

Table 5. Estimation of Susceptibility of eight faba bean varieties under drought stress condition using eleven morphological traits

Genotype	Leaflets No.(LL)	Leaves No.(L)	LL / L	Plant height(cm)	Total No. flowers	No. of legumes	% Fert. flowers	Days to flowering	Seeds No.	Plant weight (g)	branches No.
Giza 3	-81.8 g	-5.4 f	-2.16 h	-67.2 h	13.2 g	-19.2 h	-43.15 h	7.8 c	-61 g	-41.57 h	1.4 a
Sakha 1	-49.8 f	-12 h	0.19 b	-16.4 e	-16.2 f	-2.2 e	-1.992 c	20.8 a	-93.2 h	-21.2 d	-1.8 d
Sakha 2	49.4 a	12.4 a	-0.62 e	3 c	28.4 a	1.8 c	-5.76 e	-1.8 f	-47.4 e	-31.84 f	-8.6 h
Misr 1	-93.2 h	-10 g	-1.95 f	-27.2 f	-3.8 d	-16.4 g	-37.12 g	2.4 e	-31.2 d	-23.61 e	-3.2 f
Giza 716	-20.2 e	4 d	-1.99 g	-45.2 g	-37.8 h	-9.4 f	-11.99 f	10.8 b	-48 f	-34.22 g	-1.4 c
Nubaria1	12 d	2.2 e	-0.28 c	-1.6 d	7.8 c	-1 d	-2.54 d	-9.8 h	-22.6 c	-17.95 c	-7.4 g
Sakha 3	31.2 c	5.8 c	-0.42 d	14 b	-21.6 g	2 b	8.74 a	7 d	-18.6 b	-17.01 b	-2.8 e
Giza 843	47 b	7.2 b	0.47 a	21 a	-12.2 e	4.6 a	8.66 b	-6 g	1 a	-1.24 a	-0.6 b

According to this equation, the variety 'Giza 3' showed the highest susceptibility value to drought stress for the leaflets number LL (-81.8), plant height (-67.2), percentage (%) of fertilized flowers (-43.152), plant weight (-41.57), number of legumes (-19.2) and the percentage LL / L (-2.156) traits (Table 5). Thus, this variety could be considered as susceptible variety for drought stress (Table 5). On the other hand, The variety 'Giza 843' was more tolerant to drought in the leaflets number (LL), (47), plant height (21), percentage(%) of fertilized flowers (8.6) and number of legumes traits (4.6); while 'Sakha 2' variety was more tolerant in leaflets number (LL), (49.4), leaves number (L), (12.4) and total number of flowers(28.4) traits. Thus these two varieties could be considered as tolerant varieties for drought stress. Moreover, the variety 'Sakha 1' was the most susceptible variety in the number of seeds per plant (-93.2) and leaves number (L) (-12) traits. The variety 'Sakha 2' was the most susceptible variety in the number of branches (-8.6) trait, the variety 'Misr 1' was the most susceptible variety in leaflets number (-93.2) (LL) trait while 'Giza 716' variety was the most susceptible variety in the trait total number of flowers (-37.8). 'Nubaria 1' gave the least response for the trait days to flowering (-9.8) (Table 5) and 'Sakha 3' in trait number of seeds (-18.6). Thus, it can be said that the variety 'Giza 3' is the most susceptible Vicia faba variety in comparing with other varieties. On the other hand, 'Giza 843' variety is the most tolerant variety in comparing to the other varieties.

Many studies have been carried out on the drought tolerance in faba bean varieties, (Schmidhalter & Oertli, 1991; El-Tayeb & Hassanein, 2000; Schütz et al., 2002). They studied the effect of the drought on seed germination and stated that the most common symptom of water stress injury is the decrease in seed germination and the inhibition of growth, which is reflected in a reduction in the dry matter yield. Faba bean is more sensitive to drought than some other seed legumes. In addition, Bayuelo-Jimenez et al. (2002) found that the germination capacity was higher in *Phaseolus filiformis* (more drought-resistant) than in *Phaseolus Vulgare* (more drought-sensitive) under water stress. EL-Tayeb (2006) detected which cultivar of *Vicia faba* could germinate and sustain growth under water stress conditions, and detected the physiological mechanisms underlying the differential tolerance of two *Vicia faba* cultivars to drought. The changes in growth, photosynthetic pigment, pigments, relative water content (RWC), membrane stability index (MSI). They found that “Giza 40” showed the highest germination capacity and “Giza 667” the lowest. Drought caused a greater decrease in “Giza 667” than in “Giza 40”, indicating that “Giza 40” was more tolerant of low soil water content.

3.4 Seed Storage Protein Pattern

According to the morphological results, the variety ‘Giza 843’ was the most variety tolerant to the drought stress while the variety ‘Giza 3’ was the most one susceptible to the drought stress (Table 5). So that, it can be considered that ‘Giza 843’ variety is tolerant to the drought stress and the variety ‘Giza 3’ is susceptible to the drought stress.

In order to find out biochemical markers associated with the above findings, SDS-PAGE for the total seed storage protein of all varieties (control and drought stress treated) had been performed. Using one-dimensional SDS-PAGE analysis, optical differences were obtained between the varieties ‘Giza 843’ (tolerant) and ‘Giza 3’ (susceptible) in their protein patterns. Many protein bands were obtained in the protein pattern of the variety ‘Giza 843’ that were not obtained in the protein pattern of the variety ‘Giza 3’ (i.e. appeared at molecular weight of 90, 80, 75, 50, 35, 29, 22, 21.5, 18 and 8.5 kDa, Figure 1). On the other hand, some protein bands were observed in the protein pattern of the variety ‘Giza 3’ that were not present in the protein pattern of the variety ‘Giza 843’ (i.e. at molecular weight of 60, 14 and 9 kD, Figure 1). The results also revealed that the drought tolerant and susceptible varieties of faba bean differed from each other in their protein patterns and each of them characterized by the presence of some specific protein bands. For example, the variety ‘Giza3’ characterized by presence of bands at molecular weight 60 and 14 kDa (Figure 1) which were appeared in the control pattern and disappeared in the stressed pattern. On the contrary, the variety ‘Giza 843’ was characterized by presence of bands at molecular weight of 90, 75, 35, 29, 21.5, 18 and 12 kDa (Figure 1). The above findings prove that the variety ‘Giza 843’ is drought stress tolerant variety while the variety ‘Giza 3’ is drought stress susceptible variety. Similar results could be obtained for the other varieties (e.g. tolerant varieties such as ‘Misr 1’, ‘Sakha 2’ and ‘Sakha 3’ varieties and susceptible varieties such as ‘Sakha 1’ variety). The most two discriminant bands could be noted at molecular weight of 60 kDa for the tolerant varieties and 14 kDa for the susceptible varieties (Figure 1). This is almost the first report in such way of research in faba bean. Robinson et al., (1990) suggested that the disappearance of polypeptides during stress were compensated by the increased synthesis of others. Moreover, under salt stress, despite the reduction in protein levels (Singla & Grover, 1994), the cells preferentially synthesized a few specific proteins that are termed stress proteins (Pureek et al., 1995).

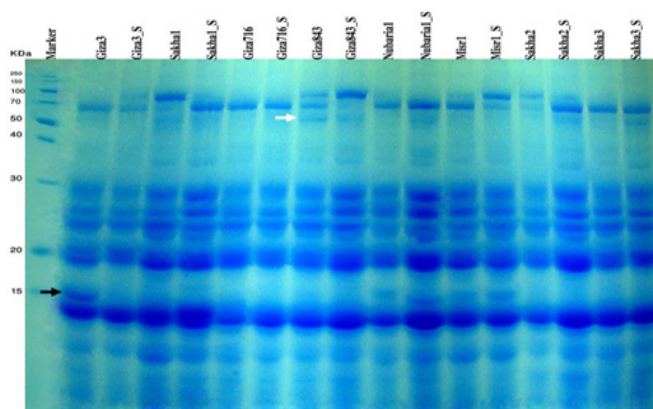


Figure 1. SDS-PAGE protein pattern of eight drought stressed faba bean varieties separated on 15% SDS-PAGE. The white arrow shows positive marker while the black arrow shows negative protein marker

Generally, it can be said that both morphological and biochemical markers were able to differentiate among the faba bean varieties and study the genetic diversity depending on their background regard the drought tolerance. Although 'Giza 3' variety gave the highest mean through the genotypes for the most of the morphological traits, it was the most drought susceptible variety. On the other hand, 'Giza843' gave medium mean across the genotypes but it was the most drought tolerant variety. Thus, it can be said that there is negative correlation between the yield and the drought tolerance in faba bean. It can be noted that biochemical markers (seed storage protein content) is efficient marker to study the genetic diversity in faba bean and significant association could be observed between morphological and biochemical markers. The variety 'Giza 843' seems to be genetically different from the other faba bean varieties and is considering being drought tolerant variety. On the other hand, 'Giza 3' variety could be consider as faba bean drought susceptible variety according to both types of markers that have been used in this study (i.e. morphological and biochemical markers).

References

- Abdelmula, A. A., Link, W., Kittlitz, E. V., & Stelling, D. (1999). Heterosis and inheritance of drought tolerance in faba bean, *Vicia faba* L. *Plant Breed*, 118, 485-490. <http://dx.doi.org/10.1046/j.1439-0523.1999.00411.x>
- Amede, T., & Schubert, S. (2003). Mechanisms of drought resistance in seed legumes. I. Osmotic adjustment. *Ethiop. J. Sci*, 26, 37-46.
- Amede, T., Kittlitz, E. V., & Schubert, S. (1999). Differential drought responses of faba bean (*Vicia faba* L.) inbred lines. *J. Agron. Crop Sci*, 183, 35-45. <http://dx.doi.org/10.1046/j.1439-037x.1999.00310.x>
- Bayuelo-Jimenez, J. S., Craig, G., & Lynch, J. P. (2002). Salinity tolerance of Phaseolus species during germination and early seedling growth. *Crop. Sci.*, 42, 1584-1594. <http://dx.doi.org/10.2135/cropsci2002.1584>
- Cattivelli, L., Rizza, F., Badeck, f., Mazzucotelli, E., Mastrangelo, A. M., Francia, E., ... Stanca, A. M. (2008). Drought tolerance improvement in crop plants: An integrated view from breeding to genomics Field. *Crops. Research*, 105, 1-14. <http://dx.doi.org/10.1016/j.fcr.2007.07.004>
- EL-Harty, E. H., Shaaban, M., Omran, M. M., & Ragheb, S. B. (2008). Heterosis and genetic analysis of yield and some characters in faba bean (*Vicia faba* L). *Minia J. of Agric. Re*, 27(5), 897-913.
- EL-TAYEB, M. A. (2006). Differential responses of pigments, lipid Per-oxidation, organic solutes, catalase and per-oxidase activity in the leaves of two *Vicia faba* L. Cultivars to Drought. *International J. of agricultural & Biology*, 1, 116-122. <http://dx.doi.org/10.1556/AAgr.54.2006.1.3>
- El-Tayeb, M. A., & Hassanein, A. M. (2000). Germination, seedling growth, some organic solutes and peroxidase expression of different *Vicia faba* lines as influenced by water stress. *Acta Agron. Hung.*, 48, 11-20. <http://dx.doi.org/10.1556/AAgr.48.2000.1.2>
- Emam, Y., Shekoofa, A., Salehi, F., & Jalali, A. H. (2010). Water Stress Effects on Two Common Bean Cultivars with Contrasting Growth Habits. *American-Eurasian J. Agric. & Environ. Sci.*, 9(5), 495-499.
- Gates, P., & Boulter, D. (1979). The use of seed isoenzymes as an aid to the breeding of field bean (*Vicia faba* L.) *New Phytologist*, 83, 783-790. <http://dx.doi.org/10.1111/j.1469-8137.1979.tb02309.x>
- Grzesiak, S., Iijima, M., Kono, Y., & Yamauchi, A. (1997). Differences in drought tolerance between cultivars of field bean and field pea. A comparison of drought resistant and drought-sensitive cultivars. *Acta .Physiol. Plant*, 19, 349-357. <http://dx.doi.org/10.1007/s11738-997-0012-y>
- Heringa, R. J., Norel, A., & Post, J. (1984). Searching for tolerance to drought in *Vicia faba*. *FABIS Newslett*, 8, 13-14.
- Khalafallah, A. A., Tawfik, K. M., & Abd El-Gawad, Z. A. (2008). Tolerance of seven faba bean varieties to drought and salt stresses. *Research. J of Agriculture and Biological Sciences*, 4(2), 175-186.
- Khan, H. R., Paull, J. G., Siddique, K. H. M., & Stoddard, F. L. (2010). Faba bean breeding for drought-affected environments: A physiological and agronomic perspective. *Field Crops Res*, 115, 279-286. <http://dx.doi.org/10.1016/j.fcr.2009.09.003>
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. <http://dx.doi.org/10.1038/227680a0>
- Link, W., Abdelmula, A. A., Kittlitz, E. V., Bruns, S., Riemer, H., & Stelling, D. (1999). Genotypic variation for

- drought tolerance in *Vicia faba*. *Plant Breed*, 118, 477-483. <http://dx.doi.org/10.1046/j.1439-0523.1999.00412.x>
- McDonald, G. K., & Paulsen, G. M. (1997). High temperature effects on photosynthesis and water relations of seed legumes. *Plant Soil*, 196, 47-58. <http://dx.doi.org/10.1023/A:1004249200050>
- Ouji, A., Rouaissi, M., Abdellaoui, R., & El Gazzah, M. (2011). The use of reproductive vigor descriptors in studying genetic variability in nine Tunisian faba bean (*Vicia faba* L.) populations. *African J. of Biotechnology*, 10, 896-904. <http://dx.doi.org/10.1007/s13258-010-0070-1>
- Pala, M., Armstrong, E., & Johansen, C. (2000). Linking research marketing opportunities for pulses in the 21st century. In: Proc. of the 3rd Int. Food Legumes Res. Conference, 22-26.
- Parker, W. C., & Pallardy, S. G. (1985). Genotypic variation in tissue water relations of leaves and roots of black walnut (*Juglans nigra*) seedlings. *Physiol. Plant.*, 64, 105-110. <http://dx.doi.org/10.1111/j.1399-3054.1985.tb01219.x>
- Pureek, A., Singla, S., & Grover, A. (1995). Immunoligical evidence for accumulation of two high-molecular-weight (104 & 90 kDa) HSPs in response to different stress in rice and in response to high temperatures stress in diverse plant genera. *Plant Mol. Biol.*, 29, 293-301. <http://dx.doi.org/10.1007/BF00043653>
- Robinson, N. L., Tanaka, C. K., & Hurkman, W. J. (1990). Time dependent changes in polypeptide translatable mRNA levels caused by NaCl in barley roots. *Physiol. Plant.*, 78, 128-34. <http://dx.doi.org/10.1034/j.1399-3054.1990.780121.x>
- Savé, R., Biel, C., Domingo, R., Ruiz-Sanchez, M., C., & Torrecillas, A. (1995). Some physiological and morphological characteristics of citrus plants for drought resistance. *Plant Sci.*, 110, 167-172. [http://dx.doi.org/10.1016/0168-9452\(95\)04202-6](http://dx.doi.org/10.1016/0168-9452(95)04202-6)
- Schmidhalter, U., & Oertli, J. J. (1991). Germination and seedling growth of carrots under salinity and moisture stress. *Plant Soil*, 132, 243-251. <http://dx.doi.org/10.1007/BF00014785>
- Schut, J. W., Qi, X., & Stam, P. (1997). Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. *Theor. Appl. Genet.*, 95, 1161-1168. <http://dx.doi.org/10.1007/s001220050677>
- Schütz, W., Milberg, P., & Lamont, B. (2002). Germination requirements and seedling responses to water availability and soil type in four eucalypt species. *Acta Oecol.*, 23, 23-30. [http://dx.doi.org/10.1016/S1146-609X\(01\)01130-4](http://dx.doi.org/10.1016/S1146-609X(01)01130-4)
- Singh, S. P., Gutierrez, J. A., Molina, A., Urrea, C., & Gepts, P. (1991) Genetic diversity in cultivated common bean: II. Marker-Based Analysis of Morphological and Agronomic Traits. *Crop Sci*, 31, 23-29. <http://dx.doi.org/10.2135/cropsci1991.0011183X003100010005x>
- Singla, S. L., & Grover, A. (1994). Detection and quantification of a rapidly accumulating and predominant 104 KDa heat shock polypeptide in rice. *Plant Sci.*, 97, 23-30. [http://dx.doi.org/10.1016/0168-9452\(94\)90103-1](http://dx.doi.org/10.1016/0168-9452(94)90103-1)
- Snedecor, G. W., & Cochran, W. G. (1967). Statistical methods. The 6th ed. IOWA State college press, Amer. IOWA, USA.
- Stegmann, H. W., Shehata, A. E. T., & Hamza, M. (1980). Broad bean proteins (*Vicia faba* L.) Electrophoresis studies on seeds of some German and Egyptian cultivars. *Zeitschrift für Acker-Und Pflanzenbau. J. Agronomy*, 6, 446-454.
- Terzopoulos, P. J., Kalitsikes, P. J., & Bebeli, P. J. (2008). Determining the sources of heterogeneity in Greek faba bean local populations. *Field Crops Res*, 105, 124-130. <http://dx.doi.org/10.1016/j.fcr.2007.08.006>