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ECOPHYSIOLOGICAL AND BIOCHEMICAL GENETIC STUDIES ON SOME *Vicia faba* L. GENOTYPES

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Trials were conducted in pots to investigate the response of the three faba bean genotypes, G716, L3 and L8 to three water regime treatments, and to study some biochemical genetic markers associated with drought tolerance. The obtained results showed that L3 genotype has the highest growth parameter, yield and yield component under the three irrigation intervals. The highest seed index value (103.6 g) was recorded in L3 genotype under weekly irrigation. Total carbohydrate and protein content of the three genotypes were significantly decreased with increase water deficit. The protein content of L3 genotype was higher than the other two genotypes. Total soluble sugars, proline and total free amino acids content were significantly increased with increasing irrigation intervals. The L3 genotype had the highest values of total soluble sugars and proline content. The water use efficiency points of the L3 genotype demonstrated its economic value. Both G716 and L8 genotypes were more affected by water deficit than L3. SDS-PAGE of total soluble protein of harvested beans revealed 18 monomorphic bands and two polymorphic bands which were recorded as unique bands for L8 genotype under irrigation every 3 weeks. The Electrophoretic patterns of acid phosphatase isozymes (Acph) showed that band No.5 was as unique band of the tolerant L3 genotype when all genotypes irrigation every three weeks. Band No. 4 was specific for L8 sensitive genotype. Band No. 3 a recorded as unique band for G 716 under weekly irrigated but disappeared under water stress. β-esterase isozyme patterns revealed a total of five bands. Band No. 5 was unique only in the L3 genotype when irrigated every week.

Keywords: faba bean, drought tolerance, pigments, amino acids, isozyme, electrophoresis.

Faba bean, *Vicia faba* L. is one of the most important pulse crop cultivated in Egypt due to the richness of seed protein content. Therefore, there is need to increase its production by expansion through newly reclaimed areas. The sensitivity of this crop to drought affects its extension in marginal lands. In arid regions, water is becoming limited factor in crop production, therefore this study focused on optimized crop production by limiting irrigation. Leport *et al.* (2006) investigated the effect of terminal drought on seed yield and its components in chick pea *(Cicer arietinum* L.*)*. Early stress affected biomass and seed yield. Ashraf and Ibram (2005) concluded that under water stress in leguminous species, free amino acids, proline and glycine betaine were increased. Oweis *et al.* (2005) examined a local variety (faba bean) under different levels of supplemental irrigation (SI), full SI, 2/3 SI, 1/3 SI and rainfed. Their results indicated that a 2/3 SI level provided the optimum water use efficiency for both seed yield and biomass. Ahmed (2004) studied the effect of irrigation intervals on productivity and seed quality of faba bean, the study showed that the increasing of irrigation intervals resulted in the greatest value of 100 seed weight and protein percent. Musallam *et al.* (2004 a and b) studied the yield, yield components and chemical composition of faba `bean under rain-fed and irrigation conditions; the yield more than double with irrigation. The highest carbohydrate and protein content were recorded under rainfed condition. Podlesny (2001) studied the effect of drought on the development and yield of two varieties of *Vicia faba* minor. Plants were grown in pots under 70, 50 and 30% of field water capacity. The soil water shortage contributed to a considerable impairment in the developmental and yield of both varieties. The traditional variety (Nodwislanski) was more drought tolerant than the newly released Tim variety. El- Far (2001) studied the response of different cultivars of faba bean to drought. The results showed that irrigation at all stages resulted the highest yield and biomass. The highest protein content was found under drought. Abd El-Hameed (1996) and Gendy *et al.* (1995) reported that total faba dry weight, leaf area index and yield increased with increasing the number of irrigations, while percent protein was the highest with decreasing irrigation.

The aim of this investigation is to develop genotypes exhibit high yielding under limited-water environments and to identify biochemical markers (SDS-PAGE of protein and isozyme profiles) associated with drought tolerance in faba bean genetic resources.

MATERIALS AND METHODS

Experiments were conducted in pots to investigate the response of three faba bean genotypes; G716, L3 and L8, to drought stress. The three genotypes were chosen from the faba bean program of the Plant Genetic

Resources Department, Desert Research Center, Cairo, Egypt.

The experiments were conducted in a greenhouse of the Botany Department, Women's College, Ain Shams University, Heliopolis, Cairo, during the 2003-2004 growing season. A split-plot design with six replications was used; six seeds were sown and kept moist in a pot of 30 cm diameter and 40 cm depth which contained 9 kg of soil. The irrigation regimes were applied after 21 days from sowing as follows: 1) plants irrigated every seven days, 2) plants irrigated every 14 days, and 3) plants irrigated every 21 days. Plants were harvested at 40 days, 70 days, 110 days, and 150 days.

Growth Criteria

Randomized five replicates were taken from each treatment for determination of plant height, total leaf area, and fresh and dry weight per plant. Yield and fresh and dry biomass of the plants were determined at the end of the experiment. Water use efficiency was also determined.

Biochemical Characterization

Photosynthetic pigments were estimated according to the protocol described by Metzner *et al.* (1965). Total carbohydrates were extracted according to Chaplin and Kennedy (1994), and the composition was determined according to Dubios *et al.* (1956). The extraction of total soluble carbohydrate was done as previously described (A.O.A.C, 1984). Total soluble sugar content was determined according to Dubios *et al.* (1956). Total free amino acids content was carried out according to A.O.A.C (1984) and Rosein (1957). Proline content was determined according to Bates *et al.* (1973). Total nitrogen was determined according to Peach and Tracey (1956), and the total crude protein content was calculated according to A.OA.C (1975). Total soluble protein was estimated using the method of Bradford (1976).

Electrophoretic Analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 10% acrylamide slab gels following the system of Laemmli (1970). Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted using three isozyme systems. The isozymes examined were acid phosphatase (Acph) and α- and β-esterase (α - and β-Est). These isozymes were separated in 10% polyacrylamide gels according to Stegemann et al. (1985).

Statistical Analysis

The data collected from the growing season were subjected to the ordinary analysis of variance of the split-plot design on individual plant means basis. The effect of blocks and genotypes were assumed to be fixed, as outlined by Snedecor and Cochran (1982).Treatment means were compared using the new least significant differences (new LSD) test as described by Waller and Duncan (1969).

RESULTS AND DISCUSSION

Plants were grown in alkaline, loamy sand soil. Characteristics of the soil are as follows: sand 78.8%, silt 8.0%, clay 13.2%, organic matter 0.81 %, pH 7.7, and EC 0.42. The soil moisture content before irrigation was: irrigation weekly, 9.3%; irrigation every two weeks, 7.5%; and irrigation every three weeks, 5.1%.

Growth Parameters

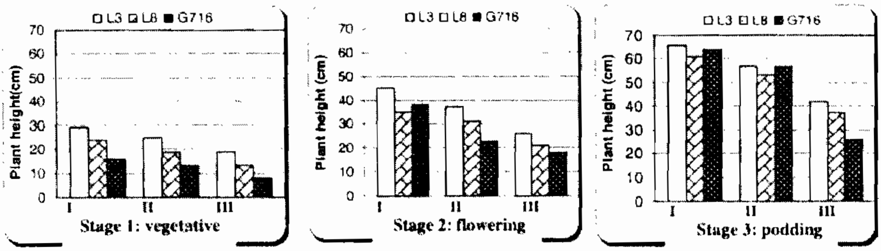
The highest values of plant height, fresh and dry weight, leaf area/plant and fresh and dry entire plant biomass for all genotypes were recorded in plants irrigated every week. L3 genotype showed the highest values in the three stages (vegetative, flowering and podding stage) under the three irrigation regimes (Figs. I-6). These results are in agreement with Leport *et al.* (2006) and Ashraf and Ibram (2005) who concluded that water deficit resulted in a significant reduction in total biomass. Anyia and Herzog (2004) reported that under water deficit, leaf area was sharply reduced due to a combination of leaf growth reduction and abscission. In addition, Abd El-Khalek and Afiah (2004) found that plant height was insignificantly associated with seed yield/plant under rain-fed conditions of the northwestern coast of Egypt. L8 and G716 genotypes were more affected by water stress. The results showed significant differences in growth parameters due to the variation in irrigation intervals.

Yield and Yield Components

Number of pods/plant, number of seeds/plant and seed index (wt. of 100 seeds) significantly increased by increasing water supply, with L3 showing the highest increases (Fig. 7). These results are coordinate with those of Soliman and Soliman (2002) and Ahmed (2004) who reported that decreasing irrigation intervals increased the number of pods/plant and number of seeds/plant. Also, El-Far (1994) demonstrated that decreasing irrigation intervals increased seed index.

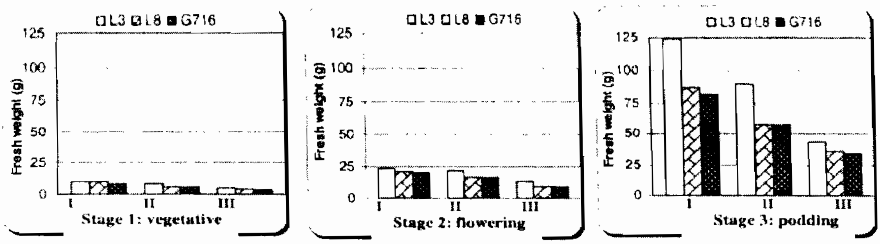
Photosynthetic Pigments

In each of the three genotypes, chlorophyll a and b and carotenoid contents increased in plants irrigated every two weeks, while irrigation every three weeks resulted in a decrease in pigment content. The L3 genotype had the highest value for all photosynthetic pigment content when compared with the other genotypes (Table 1). Our results were comparable with those reported by Ashraf (1994) and Garg *et al.* (1998).



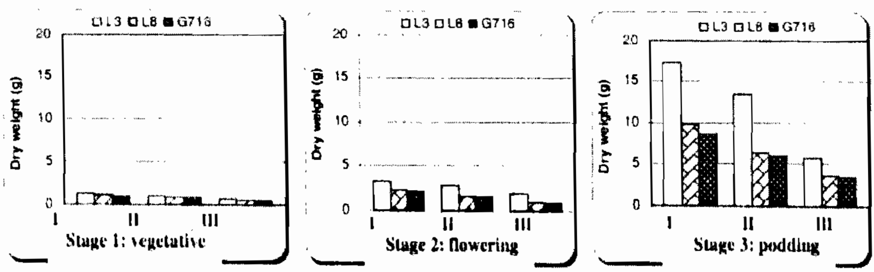
LSD at 5% → 0.85 LSD at 5% → 0.64 LSD at 5% → 0.78

Fig. (1). Plant height of 3 *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



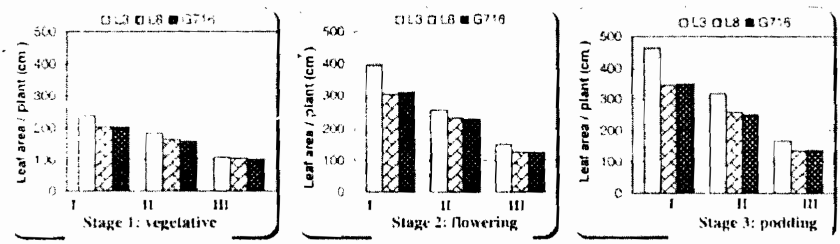
LSD at 5% → 0.67 LSD at 5% → 0.52 LSD at 5% →0.73

Fig. (2). Fresh weight of 3 *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



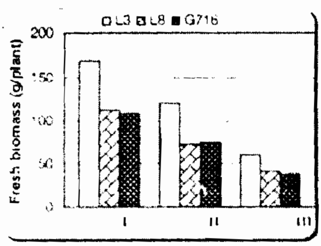
LSD at 5% → 0.003 LSD at 5% → 0.011 LSD at 5% → 0.014

Fig. (3). Dry weight of 3 *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



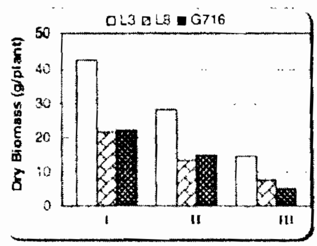
LSD at 5% → 0. 30 LSD at 5% → 0.37 LSD at 5% → 0.51

Fig. (4). Leaf area of 3 *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



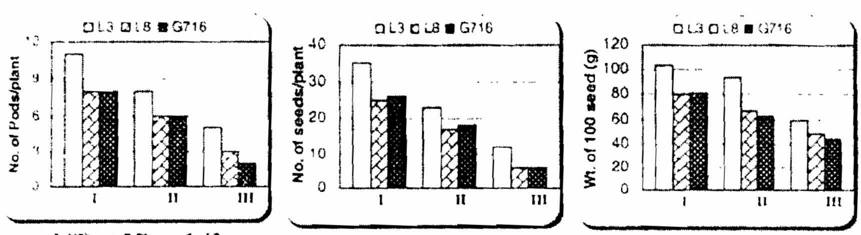
LSD at 5% → 21.57

Fig. (5). Fresh biomass of 3 *Vicia faba* genotypes as affected by different irrigation regime (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



LSD at 5% → 821

Fig. (6). Dry biomass of 3 *Vicia faba* genotypes as affected by different irrigation regime (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



LSD at 5% → 1.43 LSD at 5% → 4.2 LSD at 5% → 9.8

Fig. (7). Yield and yield components a- No. of pods per plant, b- No. of seeds per plant and c- Seed index (weight of 100 seeds) of three *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).

TABLE (1). Photosynthetic Pigments content (mg/g fresh wt.) in leaves of three *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).

Water regime	Cultivars	Stage 1: vegetative				Stage 2: flowering			
		Chl. a	Chl. b	Chl. a+b	Carote-noids	Chl. a	Chl. b	Chl. a+b	Carote-noids
I	L3	0.98	0.49	1.47	0.26	1.28	0.65	1.93	0.37
	L8	0.92	0.43	1.35	0.24	1.18	0.63	1.81	0.37
	G716	0.95	0.48	1.43	0.25	1.17	0.64	1.81	0.37
II	L3	1.12	0.56	1.68	0.27	1.31	0.71	2.02	0.38
	L8	0.95	0.48	1.43	0.26	1.22	0.66	1.88	0.38
	G716	0.99	0.51	1.50	0.26	1.20	0.67	1.87	0.37
1I	L3	0.93	0.43	1.36	0.27	0.98	0.62	1.60	0.38
	L8	0.88	0.43	1.31	0.26	0.97	0.54	1.51	0.37
	G716	0.92	0.47	1.39	0.26	1.11	0.59	1.70	0.37
New LSD at 5%		**0.031**	**0.024**	**0.050**	**0.018**	**0.013**	**0.021**	**0.030**	**0.011**

Chl.a = chlorophyll-a Chl.b = chlorophyll-b

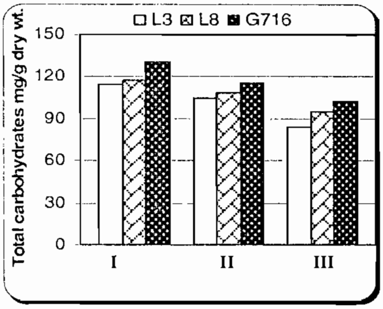
Metabolic Products

Total carbohydrates

Total carbohydrates of the three genotypes were significantly decreased with increasing water stress. Total carbohydrate content of the three genotypes decreased in the following order: G716 > L8 > L3 (Fig. 8). Abou-Bakr *et al.* (1993) and Abd El-Rahman *et al.* (1994) found that drought caused a reduction of total carbohydrates that may be due to decreased photosynthetic activity, resulting from lowered CO_2 diffusion due to stomatal closure, and/or lower chlorophyll content. Leport *et al.* (1999) and Ma *et al.* (2001) observed a reduction in the rate of leaf photosynthesis in chick pea under stress.

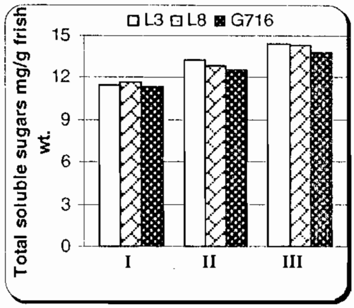
Total soluble sugars

L3 had the highest value of total soluble sugars of the three cultivars studied. Total soluble sugars increased with increasing intervals between irrigation (Fig. 9). Similar trends were observed by Sanchez *et al.* (1998) who reported that soluble sugars increased when plants were subjected to stress and the stimulation of sugar accumulation was proportional to osmotic adjustment. Bohnert *et al.* (1995) and Ingram and Bartels (1996) suggested that under water stress soluble sugars can function in two ways which are difficult to separate as osmotic agents and as osmoprotectors.



LSD at 5% → 4.52

Fig. (8). Total carbohydrates in leaves of 3 *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



LSD at 5% → 0.18

Fig. (9). Total soluble sugars in leaves of 3 *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).

Protein content

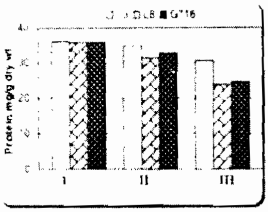
Protein represents the main component of beans. Protein content of the leaves of the three genotypes significantly decreased as irrigation intervals increased. Protein content of the L8 genotype significantly decreased with increasing irrigation intervals relative to L3 and G716 (Fig. 10). These results are in agreement with those of Irigoyen *et al.* (1992) who reported that proline accumulation and a general decline in protein synthesis occurred under water stress.

Proline content

Figure 11 shows that leaf proline content of all three faba bean genotypes significantly increased as a result of increasing irrigation intervals. These results are similar to those of Ozturk and Demir (2002), Hsu *et al.* (2003) and Kavi Kishore *et al.* (2005) who reported that proline accumulates in large quantities in response to stress. The L3 genotype had the highest proline content under severe water stress. Accumulation of proline under stress has been correlated with stress tolerance. In addition to its role as an osmolyte for osmotic adjustment, it may also a function as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995).

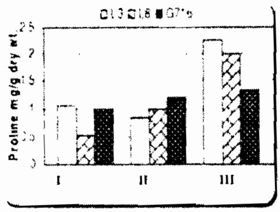
Total free amino acids

Total free amino acids (mg/g dry wt) significantly increased in the three genotypes as irrigation intervals increased. L8 had the highest values of total free amino acids under the three irrigation regimes (Fig.12). It has been reported that free amino acids contribute to osmotic adjustment. Free amino acids in plants growing under drought stress may result from protein hydrolysis.



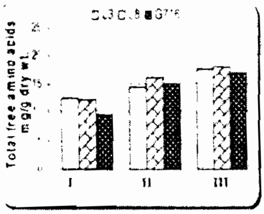
LSD at 5% → 0.53

Fig. (10). Protein content in leaves of three *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks)



LSD at 5% → 0.09

Fig. (11). Proline content in leaves of three *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks)



LSD at 5% → 2.87

Fig. (12). Total free amino acids in leaves of three *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks)

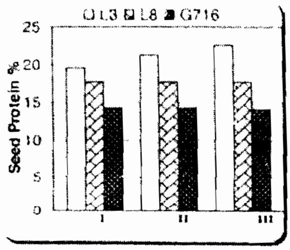
Seed Chemical Analysis

Protein

The highest seed protein content was seen in L3 irrigated every three weeks. There were no significant differences in seed protein content in L8 and G716 genotypes under the three water regimes (Fig. 13).

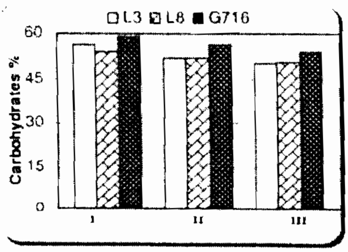
Carbohydrates

Figure (14) shows that total carbohydrate content in seeds was decreased in all genotypes with decreasing water supply. The percentage of carbohydrate in the G716 genotype was higher than that in L3 and L8.



LSD at 5% → 1.47

Fig. (13). Seed protein of three *Vicia faba* genotypes as affected by different irrigation regimes
(I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).



LSD at 5% → 1.28

Fig. (14). Seed carbohydrates of three *Vicia faba* genotypes as affected by different irrigation regimes (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).

Water Use Efficiency (WUE)

Figure (15) shows that the drought-tolerant L3 genotype had the highest WUE (g/L) in plants irrigated every two weeks. These results suggest that L3 genotype (irrigated every two weeks) may be an economic use of water in faba bean cultivation. However, reduction of irrigation water by one-half caused a one-third reduction in yield. The increased WUE of some genotypes under water stress has also been noted by Ismail and Hall (1992). Passioura (1994) demonstrated that WUE is one trait that has been studied because it can give an idea of the variation among genotypes in stress-tolerance.

Fig. (15). Water use efficiency (WUE) of three *Vicia faba* genotypes as affected by different irrigation regime (I- irrigation every week, II- Irrigation every 2 weeks and III- Irrigation every 3 weeks).

SDS-PAGE Analysis

A maximum of 20 bands were detected with molecular weights ranging from 13.5 to 116.4 KDa. All bands were detected as monomorphic bands except bands No. 14 and No. 15 which were recorded as unique bands for the L8 genotype when irrigated every three weeks as shown in Fig. (16). These results are in agreement with Sakova *et al.* (1997) who didn't find substantial differences between polypeptide profiles in drought-stressed and well-watered *Vicia sativa* plants.

Isozymes Analysis

Acid phosphatase

Figure (17) and table (2) show that acid phosphatase patterns of all samples examined are highly polymorphic. Differences in the intensity of bands were also noticed among and within samples. Five bands were identified. While band No. 2 was monomorphic, all the other bands were polymorphic. Band No. 5 was unique to drought-tolerant L3, under irrigation every three weeks. Band No. 4 was specific to L8 (sensitive genotype) and was not affected by water stress. Band No. 1 was observed in all plants except L3 genotype under weekly irrigation. Band No. 3 was unique for G716 under weekly irrigation every week, but it disappeared under water stress.

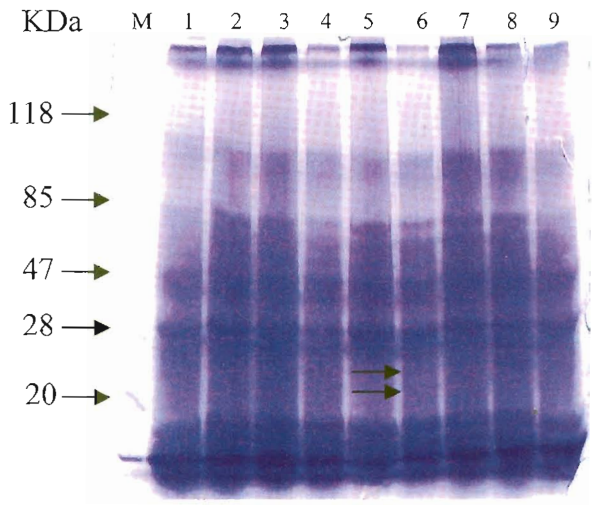


Fig. (16). SDS-PAGE protein banding pattern in seeds of three *Vicia faba* genotypes under different irrigation regimes.

Esterases

The electrophoretic patterns of the α-esterase isozyme are shown in figure (18) and table (3). Five bands were observed; three of them, No. 1, 4 and 5, were monomorphic bands. Band No. 2 and No. 3 were specific for the L3 genotype; these two bands appeared under weekly and biweekly irrigation, but they disappeared under severe stress. The α-esterase isozyme patterns of G7 16 and L8 genotypes were not affected by different irrigation intervals.

The electrophoretic patterns of β-esterase isozyme are shown in figure (19) and table (4). The β-esterase isozyme patterns revealed a total of five bands. Band No. 1 was monomorphic, while the other four bands were polymorphic. Band No. 5 was unique in the L3 genotype, observed only under weekly irrigation. El-Saied and Afiah (2004) also detected a band associated with tolerant lentil genotypes.

TABLE (2). Presence (+) and absence (-) of acid phosphatase isozymes bands in *Vicia faba* genotypes tesed.

Band no	1	2	3	4	5	6	7	8	9
1	-	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	-	-	-	-	-	-	+	-	-
4	-	-	-	+	+	+	-	-	-
5	-	-	+	-	-	-	-	-	-

TABLE (3). Presence (+) and absence (-) of α- esterase isozymes bands in *Vicia faba* genotypes tesed.

Band no.	1	2	3	4	5	6	7	8	9
1	+	+	+	+	+	+	+	+	+
2	+	+	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+

TABLE (4). Presence (+) and absence (-) of β-esterase isozymes bands in *Vicia faba* genotypes tesed.

Band no.	1	2	3	4	5	6	7	8	9
1	+	+	+	+	+	+	+	+	+
2	-	-	+	-	+	-	+	+	-
3	-	-	-	-	+	-	-	-	-
4	-	+	+	+	+	+	+	+	+
5	+	-	-	-	-	-	-	-	-

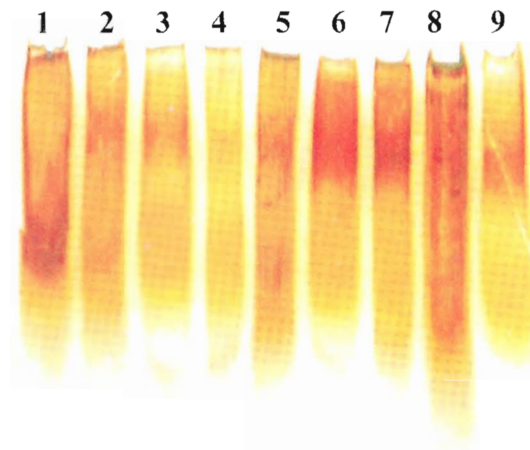


Fig. (17). Isozyme patterns of acid-phosphatase in faba bean genotypes under different irrigation regime.

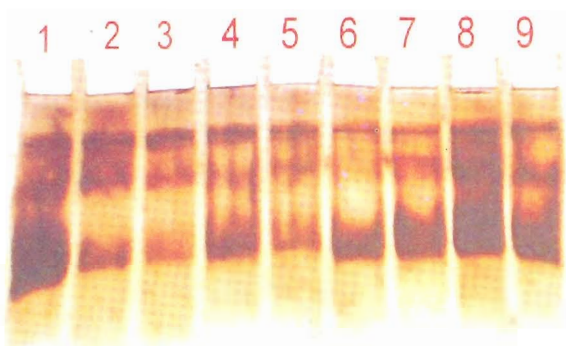
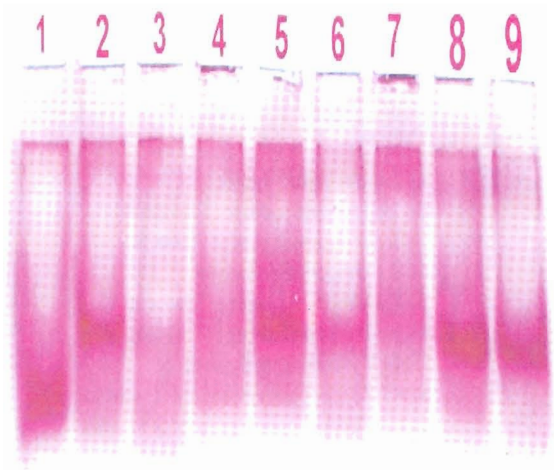


Fig. (18). Isozyme patterns of α- esterase in faba bean genotypes.



1- L_3 irrigated weekly
2- L_3 irrigated every 2 weeks
3- L_3 irrigated every 3 weeks
4- L_8 irrigated weekly
5- L_8 irrigated every 2 weeks
6- L_8 irrigated every 3 weeks
7- G 716 irrigated weekly
8- G 716 irrigated every 2 weeks
9- G 716 irrigated every 3 weeks

Fig. (19). Zymogram of β-esterase in faba bean genotypes under different irrigation regimes.

CONCLUSION

Irrigation every 7 days increased growth and yield for all genotypes. L3 genotype recorded the highest growth, yield, total soluble carbohydrates, protein content and proline content. L3 genotype was economic in water use under irrigation every two weeks. However, reduction of about half the amount of irrigation water caused a reduction of about one third of the yield. Therefore, L3 genotype is recommended for cultivation in new reclaimed area where limited water supply.

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دراسات فسيولوجية بيئية ووراثية بيوكيميائية على بعض التراكيب الوراثية من الفول

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أجريت تجربة أصص خلال موسم نمو٢٠٠٣/٢٠٠٤ لدراسة استجابة ثلاثة تراكيب وراثية من نبات الفول البلدي: جيزة ٧١٦ ، L3 ، L8 لمعاملات الإجهاد المائي ودراسة العلامات الحيوية الكيميائية المرافقة لتحمل الإجهاد المائي.

وقد أجريت هذه التجربة في ظروف البيوت المحمية في الحديقة التجريبية لكلية البنات جامعة عين شمس. واستخدم تصميم القطع المنشقة فى ستة مكررات. وطبقت ثلاثة نظم للري: رية كل ٧ أو ١٤ أو ٢١ يوما بدأ تنفيذ هذه المعاملات بعد ٢١ يوما من الزراعة، ويمكن تلخيص النتائج كما يلي :

١- ازدادت قياسات النمو للثلاثة تراكيب الوراثية تحت ظروف الري كل ٧ أيام. وقد تأثر جيزة ٧١٦ ، L8 بدرجة أكبر بالنقص المائي عن L3 . وقد أظهر التركيب الوراثي L3 أعلى قيم لإرتفاع الساق والوزن الرطب والجاف والمساحة الكلية للأوراق والوزن الرطب والجاف للكتلة الحيوية تحت ظروف الري المختلفة.
٢- ظهرت فروق معنوية في المحصول ومكوناته بين التركيب الوراثي L3 والأخرين مسجلا أعلى عدد من القرون / نبات وعدد البذور / نبات. وسجل أعلى قيمة لدليل البذرة (١٠٣,٦ جم) تحت ظروف الري أسبوعياً.
٣- ازدادت صبغات البناء الضوئي في كل التراكيب الوراثية تحت ظرف الري كل أسبوعين وقلت تحت ظرف الري كل ثلاثة أسابيع وسجل L3 أعلى قيمة للأصباغ .
٤- قلت الكربوهيدرات الكلية لأصناف الفول البلدي الثلاثة معنوياً مع زيادة الإجهاد المائي، وكان ترتيب محتوى الكربوهيدرات الكلية كالاتي: جيزة ٧١٦ > L8 >L3 .
٥- زادت السكريات الذائبة الكلية مع زيادة فترات ما بين الري وسجل L3 أعلى قيمة للسكريات الذائبة الكلية.
٦- انخفض محتوى البروتين للثلاثة تراكيب الوراثية مع زيادة فترات ما بين الري من ٧-٢١ يوما، وكان محتوى L3 من البروتين أعلى من الصنفين الآخرين.
٧- ازداد محتوى البرولين في الثلاثة تراكيب الوراثية معنوياً نتيجة لزيادة فترات ما بين الري وأظهر L3 أعلى محتوى من البرولين تحت ظرف أعلى إجهاد مائي.
٨- إزداد محتوى الأحماض الأمينية الحرة الكلية زيادة معنوية في الثلاثة تراكيب الوراثية كلما زادت فترات ما بين الري.
٩- أظهر التحليل الكيميائي للبذور أن محتوى البروتين في صنف L3 زاد مع زيادة فترات ما بين الري ونقص محتوى البذرة من الكربوهيدرات في كل التراكيب الوراثية مع نقص الامداد المائي.
١٠- أظهرت L3 كفاءة في استخدام الماء فإنه تحت ظروف الري كل أسبوعين (نقص كمية ماء الري إلى النصف) ونقص المحصول حوالي الثلث فقط.
١١- أظهر التفريد الكهربي للبروتين ١٨ حزمة متشابهه وحزمتين متباينتين رقمي ١٤، ١٥ حيث ظهرا في L8 تحت ظروف الري كل ٣ أسابيع.
١٢- أظهر أنماط التفريد الكهربي لمشابهات أنزيم الفوسفاتيز تشكلا عديداً عاليا Polymorphism وكانت الحزمة رقم ٥ سجلت كحزمة وحيدة ميزت L3 تحت ظروف

الري كل ثلاثة أسابيع عن التراكيب الوراثية الأخرى. وكانت الحزمة رقم ٤ مميزة لـــــ L8 (حساس) والمتغير بزيادة الإجهاد المائي. وسجلت الحزمة رقم ٣ كحزمة منفردة فـــي جيـــزة ٧١٦ تحت ظرف الري كل أسبوع، ولم تظهر تحت ظروف الإجهاد المائي الاعلى.

وأظهرت أنماط تفريد شبه الإنزيم α-esterase خمسة حزم، والحزمتين رقما ٢، ٣ ميزتا السلالة L3 وظهرا تحت ظرفي الري كل أسبوع وكل أسبوعين ولم يظهرا تحت ظرف الإجهـــاد المائي القاسي. ولم يتأثر نمط انزيم α-esterase لصنفي جيزة ٧١٦ و L8 بتغير فترات الـــري. كما أظهرت أنماط مشابهات الإنزيم β-esterase خمسة حزم وظهرت الحزمة رقـــم ٥ كحزمـــة منفردة مميزة للسلالة L3 تحت ظروف الري كل أسبوع.