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Effect of Drought and Salt Stress on Growth, Osmolytes Protein and Isozymes in *Vicia faba* L. Genotypes

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PLANTS regularly face adverse growth conditions, such as drought and salinity. These stresses can delay growth and development, reduce productivity, and, in extreme cases, cause plant death. Plant stress responses are dynamic and involve complex cross-talk between different regulatory levels, including adjustment of metabolism and gene expression for physiological and morphological adaptation. In this concern, two pot experiments in split plot design were conducted to investigate the response of five divergent faba bean genotypes namely (NBL- Mar.3(G1), NBL-5(G2), L3(G3), Nubariya-1(G4) and Misr-1(G5)) against drought or salt stress. Expose the faba bean genotypes (1, 2, 3, 4 and 5) to water and salt stress leads to significant decrease in fresh and dry weight, total protein and NPK%. Tolerant genotypes (G1 and G2) have high protein content than sensitive genotypes (G4 and G5). Drought and salt stress induced an accumulation of total phenolic compound and total free amino acids. Aliphatic unsubstituted amino acids and cyclic amino acids increased under drought and salt stress in all genotypes. This was accompanied by a marked increase in the proline content. Aliphatic substituted amino acids decreased in tolerant genotypes and increased in sensitive genotypes under drought and salt stress. The biochemical response diversity in susceptible and tolerant faba bean varieties is discussed.

Keywords: Amino acids, Phenolic compounds, SDS protein PAGE, Isozymes.

Faba bean (*Vicia faba* L.) is considered the first legume crop in Egypt, where it's the main nutritional source of plant proteins (Bakry *et al*., 2011). Egypt ranked the fifth order of ten biggest producers of the world after China, Ethiopia, Australia and France with 175000 ton /year (130952 fed), (FAO, 2011). The total production of faba bean is still insufficient to cover the local consumption so there is great need to increase our production by expansion through reclaimed areas which represent the hope of cultivated lands and to overcome the deficiency food requirements, as well as, increasing the vertical production through production of new varieties with high yield potential. Moreover, fresh water resources for agricultural use are becoming limited due to the competition

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with human and industrial use. Therefore, use of low saline water is a subject of increasing interest. The salinity problem has been aggravated by the requirement of irrigation for crop production in arid and semiarid environments. This imposes a threat to the global food production with respect to the increasing population and the limitation in soil and water resources.

Drought and salinity are among the major environmental constraints to crop productivity worldwide. Exposure of plants to a water - limiting or salt environments during various developmental stages appears to activate various physiological and developmental changes (Ashraf & Foolad, 2007). Salt stress as a major adverse factor can lower leaf water potential, leading to reduced turgor and some other responses, and ultimately lower crop productivity in arid and semi arid zones (Mehr *et al*., 2012), inhibits the growth of plants at early seedling and developed seedling stages (Silva *et al*.,2014). Salinity causes a range of deleterious effects such as inhibition of photosynthesis, damage to plasma membrane permeability and other metabolic disturbances (Karimi *et al*., 2005).

Some plants exhibit a number of physiological adaptations that allow them to tolerate water stress conditions. Although genotypic differences in the response of faba bean to drought have been documented (Abdelmula *et al*., 1999). The physiological processes associated with drought tolerance are less understood than for other crop species. Tolerant genotypes respond to abiotic stress with complex changes in their physiological and molecular status. Differential responses of different genotypes of a common species to abiotic stresses imply that mechanisms conveying tolerance differ between genotypes and stresses (Morsy *et al*., 2007). Tolerance to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo *et al*., 2008).

Our study aims to evaluate faba bean varieties for some growth and physiological parameters under salinity and drought conditions. Some attention is given to difference in salt and drought tolerance between varieties of the same crop.

Materials and methods

Plant materials

Five faba bean (*Vicia faba* L.) genotypes which have different response to salt and drought stress and are adapted to cultivation in Egyptian agricultural conditions were used. The used genetic materials were NBL (Mar.3) (G.1), NBL-5 (G.2) and L3 (G.3) chosen through faba bean breeding program of Plant Genetic Resources Department, Desert Research Center, Cairo, Egypt, while, Nubariya-1 (G.4) and Misr-1(G.5) from Agricultural Research Center. Names, pedigree/or selection history and origin are illustrated in Table 1. According to yield components, (G.2) was the most variety tolerant to the drought stress while

(G.5) was the most one susceptible to the drought stress. Genotype (G.1) was the most variety tolerant to the salt stress while the (G.4) was the most one susceptible to the salt stress (Afiah *et al.* , 2015).

TABLE 1. Names, pedigree/or selection history and origin of the five faba bean (*Vicia faba* L.) genotypes tested.

Genotype	Name	Pedigree/or selection history	Origin
G.1	NBL(Mar.3)*	ILB1179//(L 3457/3460 W.H.)/(L 3495/3198)#	Egypt
G.2	NBL-5*	G. 716 // A2 / ILB 1179	Egypt
G.3	L3	A2 / ILB 1179	ICARDA
G.4	Nubariya-1	An individual plant selection from the Spain variety Reina Blanka	Egypt
G.5	Misr-1	(G.3x123A/45/76)x(62/1570/66xG.2)x(Romi x Habashi)	Egypt

*: F_8 Newly bred lines produced through legume breeding program, Desert Research Center
#: S. Giant (Spain)/ ERESEN-87 (Turkey)
ICARDA; International Center of Agricultural Research in the Dry Areas

Greenhouse experiments

Two experiments were carried out in the greenhouse in the experimental garden of Botany Department, Faculty of Women, Ain Shams University, Heliopolis, Cairo, during winter growing seasons (2011-2012) to investigate the response of the five faba bean genotypes to salt and drought stress treatments. The experiments were carried out in split-plot design for both drought and salinity stresses in 50 cm diameter plastic pots, filled with 10 kg soil. Soil physical characteristics were: clay in texture, sand 19.05%, silt 16.4%, clay 64.5% and chemical characteristics listed in Table 2. Five plants were grown in each pot and three pots for each treatment.

TABLE 2. Chemical analysis of soil.

OM (%)	$CaCO_3$ (%)	pH	EC (dSm^{-1})	Anions (meq/L.)			Cations (meq/L.)			
				Cl^-	HCO_3^-	SO_4^{--}	Na^+	K^+	Ca^{+2}	Mg^{+2}
1.7	1.6	7.5	0.8	9.8	1.15	7.1	8.7	0.35	5.7	3.2

The first experiment

Irrigation every one week, soil moisture content depleted from 100% to 70 % of field capacity (Control.). Irrigation every two weeks, soil moisture content depleted from 100% to 50% of field capacity (First level). Irrigation every three weeks, soil moisture content depleted from 100% to 30% of field capacity (Second level). (soil FC was determinated on dry weight basis of irrigated pots after keeping saturated soil for 24hr under free drainage).

The second experiment

Three concentrations of salt [tap water (Cont.), 30mM (1753 ppm) (First level) and 60 mM (3506 ppm) (Second level)] in the form of NaCl were used as irrigation water salinity starting from two weeks after sowing.

Growth criteria

Randamized replicates were taken from each treatment at the flowering stage for the determination of fresh and dry weight/plant.

Physiological and biochemical analysis

Samples were taken at 45 days old from sowing for all physiological and biochemical analysis.

Physiological Analysis

Total protein was estimated using the method of Bradford (1976). Total phenolic compounds were estimated using the method of Sadasivam & Manickam (2008). Total nitrogen (%) was determined by Kjeldahl method according to Hesse (1971). Phosphorus (%) was estimated colorimetrically at wave length 650 nm using spectrophotometer as described by Jackson (1967). Potassium (%) was determined using a Gallen Kamp Flame Photometer as described by Jackson (1967). Chloride was estimated according to the method of Higinbotham *et al.* (1967). Sodium was determined using Flame Photometer apparatus according to Brown & Lilleland (1946). Calcium was determined using Atomic Absorption Spectrophotometer, Pye unican SP1900, According to Brandifeld & Spincer (1965). Total free amino acids were determined using ninhydrin reagent according to Lee & Takahashi (1966). Protein amino acids content were determined according to Pellet & Young (1980) by amino acid analyzer.

Biochemical studies

SDS-PAGE analysis of protein: Separation of proteins was performed using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), according to the method of Laemmli (1970).

Isozymes extraction and assay: Isozymes variations were identified by using native-polyacrylamide gel electrophoresis (Native–PAGE). Four isozymes: α-esterase (α-EST), β -Esterase (β -EST) (EC 3.1.1.1). peroxidase (POD)(EC 1.11.1.7) and Polyphenol oxidase (PPO) (EC 1.10.3.1), were extracted from the plant samples. These isozymes were separated on polyacrylamide gel according to Stegmann *et al.* (1985). After electrophoresis, the isozyme of interest was identified by incubating the gel in an appropriate substrate solution such that a coloured product was produced at the site of the enzyme (Wilson & Walker, 2000). The reactions mixture for α -Esterase (α-EST) and β -Esterase (β -EST) were prepared according to Jonathan & Wendel (1990). The reaction mixture for Peroxidase (POD) was prepared according to Graham *et al.* (1964). The reaction mixture for Polyphenoloxidase (PPO) was prepared according to Yu *et al.* (1992).

Statistical analysis

The experimental design was split plot where, the genotypes arranged in main plots and levels of treatments (water deficit or salinity) were arranged in sub-plots. Each of the two experiments were replicated three times. Data were analyzed as outlined by Snedecor & Cochran (1989). Means were compared by LSD at 5 %using SPSS program version16.

Results and Discussion

Shoot fresh weight and shoot dry weight

Tables 3-a, and 3-b shows the effect of drought and salinity stress levels on fresh and dry weight of the shoots/plant. It can be observed that shoot fresh weight decreased with increasing water deficit and salt stress levels in all genotypes under study. Genotype (1) gave the highest value of shoot fresh weight in control treatment where genotype (3) recorded the lowest shoot fresh weight. Genotype (1) gave the highest shoot fresh weight under drought stress levels, but genotype (2) recorded the highest shoot fresh weight under salt stress levels.

TABLE 3-a. Shoot fresh weight and shoot dry weight (g/plant) of the five faba bean genotypes tested under three levels of water deficit .

Genotype	Shoot fresh weight				Shoot dry weight			
	Drought level			Mean of D.	Drought level			Mean of D.
	Cont.	First	Second		Cont.	First	Second	
G.1	15.73	13.6	11.39	13.57	1.35	1.30	1.09	1.28
G.2	15.26	13.81	10.08	13.05	1.34	1.28	0.96	1.21
G.3	12.47	9.87	8.14	10.16	1.16	0.93	0.80	1.04
G.4	15.49	9.69	5.73	10.30	1.42	0.91	0.47	0.85
G.5	14.49	10.3	5.15	9.98	1.43	1.00	0.49	0.92
Mean of G.	14.68	11.45	8.10	11.41	1.33	1.12	0.76	1.06
LSD 0.05	D.:1.63	G.: 1.95	D x G. :- NS		D.: 0.11	G.: 0.18	D x G. :- NS	

TABLE 3-b. Shoot fresh weight and shoot dry weight (g/plant) of the five faba bean genotypes tested under three levels of salt stress.

Genotype	Shoot fresh weight				Shoot dry weight			
	salinity level			Mean of S.	salinity level			Mean of S.
	Cont.	First	Second		Cont.	First	Second	
G.1	15.73	12.49	8.96	12.39	1.35	1.14	0.77	1.07
G.2	15.26	13.37	8.96	12.53	1.43	1.29	0.96	1.22
G.3	12.47	10.40	6.10	9.66	1.16	0.87	0.51	0.85
G.4	15.49	12.43	7.79	11.90	1.42	1.15	0.68	1.08
G.5	14.49	13.69	9.94	12.71	1.34	1.13	0.84	1.10
Mean of G.	14.68	12.48	8.35	11.84	1.33	1.11	0.75	1.07
LSD 0.05	S.:2.18	G.: 1.80	S x G. :- NS		S.: 0.15	G.: 0.16	S x G. :- NS	

Also, shoot dry weight decreased with increasing the drought and salinity stress levels. Genotype (1) under drought stress and genotype (2) under salt stress were recorded the highest values of shoot dry weight, where genotype (3) recorded the least shoot dry weight under the salinity stress conditions. The results of the present study demonstrated that both drought and salinity stresses reduced the plant fresh and dry weight. The reduction in growth parameters of faba bean plants under salinity and drought stresses might be attributed to the reduction in cell division, cell elongation and meristematic activity due to higher concentration of Na+ (Table 6, b) which causes membrane disorganization and also due to reduction in water absorption which reduced metabolic activities. These results are similar to Sharma *et al.* (2013) and Abdelgawad *et al.* (2014) who found that salinity stress was significantly decreased fresh weight or dry weight in soybean and rice plants, respectively. Decreased total DW may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in *Abelmoschus esculentum* (Bhatt & Srinivasarao, 2005). Imposition of water deficit condition decreased the shoot fresh and dry weights (Jabeen *et al*., 2008).

Our study revealed that tolerant cultivars produce greater FW and DW than sensitive cultivars. Similar data were recorded by Sumithra *et al.* (2006) and Kumar *et al*. (2009). They stated that the salt tolerant cultivars produce greater biomass than salt sensitive mung bean and rice cultivars, respectively, when irrigated with NaCl dominated waters. Our data is in disagreement with Kov´acs *et al*. (2012) who stated that the fresh and dry weight data did not differ significantly between the treated and control plants at the end of the osmotic stress.

Physiological analysis

Metabolic products

It represents the summation of the effect of environmental factors and heredity of the species. Total free amino acids, total protein and total phenolic compounds of the five faba bean genotypes were estimated to study their response to water deficit and salinity levels.

Total Protein, free amino acids and total phenolic compounds: The values of total protein in the five faba bean genotypes tested under water and salt stress are represented in Tables 4-a and 4-b. The result showed that total protein decreased with increasing water and salt stress level. Genotype (G.2) and genotype (G.1) recorded the highest value of total protein at the second stress levels under drought and salt stress, respectively, but genotype (G.5) and genotype (G.4) recorded the lowest value under water deficit and salinity, respectively. Water stressed plants had lower mean values of total protein (12.05mg/g F. wt.) than salt stressed plants (12.84 mg/g F. wt.).Control plants (irrigated every week with fresh water) had the higher amounts of total protein than plants subjected to the first and the second level of water deficit. Our results cleared that tolerant genotypes have higher protein content than sensitive genotypes. These results are

in agreement with Zengin & Munzuroglu (2005) who said that proteins are important constituents of the cell that easily damage under environmental stress conditions and the protein degradation to amino acids is in fact an adaptation of the cells to the carbohydrate deficiency. Moreover, the reduced amount of total protein content in sensitive genotypes most probably is a result of the reduced biosynthesis or the accelerated protease activity or catabolic processes.

TABLE 4-a. Total protein, free amino acid and total phenolic compounds of the five faba bean genotypes tested under water deficit.

Genotype	Total protein (mg/g F. Wt.)				Free amino acid (mg/g F. Wt.)				Total Phenolic Compound (mg/g D. Wt.)			
	Drought level			Mean of D.	Drought level			Mean of D.	Drought level			Mean of D.
	Cont.	First	Second		Cont.	First	Second		Cont.	First	Second	
G.1	14.75	13.13	9.81	12.56	2.74	3.93	4.28	3.65	0.204	2.745	3.686	2.181
G.2	13.75	13.13	11.00	12.63	1.65	2.61	4.12	2.79	0.136	0.398	3.007	1.180
G.3	13.13	12.25	11.38	12.25	1.86	3.48	3.87	3.04	2.84	3.478	4.438	3.585
G.4	13.25	13.13	10.13	12.17	2.13	6.43	7.05	5.20	0.432	1.644	2.571	1.814
G.5	14.19	13.13	7.63	11.65	2.51	3.95	6.23	4.23	0.335	0.684	4.143	1.721
Mean of G.	13.81	12.954	10.00	12.05	2.18	4.08	5.11	3.78	0.789	1.789	3.569	2.096
$LSD_{0.05}$	D.: 1.86			G.: 1.25	D.: 0.11			G.: 0.32	D.:0.62			G.: 1.04
	G x D.: ns				D x G.: 0.55				G x D.: 1.45			

TABLE 4-b. Total protein, free amino acid and total phenolic compounds of the five faba bean genotypes tested under salt stress.

Genotypes	Total protein (mg/g F. Wt.)				Free amino acid (mg/g F. Wt.)				Total Phenolic Compound (mg/g D. Wt.)			
	Salinity level			Mean of S.	Salinity level			Mean of S.	Salinity level			Mean of S.
	Cont.	First	Second		Cont.	First	Second		Cont.	First	Second	
G.1	14.75	13.88	12.19	13.60	2.74	2.89	3.23	3.00	0.204	0.272	2.745	1.073
G.2	13.75	12.63	11.81	12.73	1.65	2.18	3.11	2.31	0.136	2.503	3.832	2.157
G.3	13.13	12.63	12.06	12.60	2.10	2.23	2.66	2.33	2.84	3.255	3.478	2.277
G.4	13.25	12.19	11.38	12.27	2.13	2.77	2.79	2.57	0.432	0.461	1.64	0.802
G.5	14.19	13.50	11.44	13.04	2.51	3.88	4.10	3.50	0.335	3.667	3.798	2.600
Mean of G.	13.81	12.96	11.78	12.84	2.23	2.79	3.18	2.74	0.789	2.031	3.099	1.782
LSD 0.05	S.: ns			G.: 1.01	S.: 0.12			G.: 0.21	S.: 0.56			G.: 0.72
	G x S.: ns				S x G.: 0.37				G x S.: 1.46			

The changes in soluble protein showed a reverse trend to that of free amino acids implying that the increase in protein content may be at the expense of amino acids and that the salinity influenced the inter conversion of these compounds **(**Abdelgawad, 2014). Protein content in the tissues of many plants declined under drought or salinity stress, because of proteolysis and decreased protein synthesis (Joshi & Misra, 2000).

*Total free amino acids:*Total Free amino acids which showed in Tables 4-a and 4-b were significantly increased in the studied faba bean genotypes as water deficit and salinity levels increased. The highest value of total free amino acid

(7.05 mg/ g F. Wt.) was recorded in genotype (G.4) under second drought stress where the lowest value (2.66 mg/g F. Wt.) was recorded in genotype (G.3) under the second level of salinity stress. Salt stressed plants had lower values of total free amino acids than water stressed plants.

In the recent study the increasing in total free amino acids in plant growing under drought or salt stress may be referd to hydrolysis of protein. These results are in agreement with Ashraf & Iram (2005) who said that the accumulation of amino acids may be due to the hydrolysis of protein and also may occur in response to the changes in osmotic adjustment of their cellular contents. Drought stress caused an increase in the free amino acid content, (Sankar *et al*., 2007). Free amino acid accumulation is more important to account for most of the changes in osmotic potential. The accumulation of free amino acids under stress at all the growth stages indicates the possibility of their involvement in osmotic adjustment (Yadav *et al*., 2005). EL-Beltagi *et al.* (2013) stated that increasing salinity in the soil (25, 50, 100 and 200 mM NaCl) increased total free amino acids.

Total phenolic compounds: Total phenolics (mg/g) increased with increasing water and salt stress levels as shown in Tables 4-a and 4-b. Genotype (G.3) recorded the highest value under second level of water stress (4.438 mg/g D.wt.) where genotype (G.4) recorded the lowest value under the same level of water stress (2.571 mg/g D.wt.) and salt stress conditions (1.64 mg/g D.wt.). Total phenolic compound was higher in mean value under water stress (2.096 mg/g D.wt.) than salt stress (1.78 mg/g D.wt.). Phenol accumulation could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress. These data are in good agreement with those obtained by Mohamed & Aly (2008) on onion plant and El Hariri *et al.* (2010) on flax plant. It is well known that, phenolic compounds play a key role as protective components of plant cells. The potential activity of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet O_2 (Zhang & Wang, 2001). Phenolic compounds have important physiological role in the protection and development of plants and these compounds are affected by a biotic stresses (Peer & Murphy, 2007).

Phenol accumulation in salt tolerant plants could be a defense mechanism for scavenging the free radicals of oxygen and preventing cell membrane damage during stress (Singh, 2004). Abdelgawad *et al*. (2014) showed that as salinity increases, accumulation of phenols increases in cultivars and the magnitude of increase was at the salt tolerance cultivar. These results concur with the findings of Sonar *et al.* (2011) who reported increased contents of phenols in chickpea and *Colubrina asiatica* under salinity stress. Mehr *et al*. (2012) results support the idea that the accumulation of total phenol is associated with salt tolerance as oxidative responses.

Mineral contents

Water and salinity stress caused decrease in nitrogen, phosphorous and potassium contents of the five faba bean genotype as shown in Tables 5-a and 5-b relative to control. NPK % was lower mean value under water stress condition than salt stress condition.

TABLE 5-a. Minerals content (NPK %) in dry leaves of the five faba bean genotypes tested under water deficit.

Genotype	Nitrogen (%)				Phosphorus (%)				Potassium (%)			
	Drought levels				Drought levels				Drought levels			
	Cont.	First	Second	Mean of D.	Cont.	First	Second	Mean of D.	Cont.	First	Second	Mean of D.
G.1	2.36	2.1	1.89	2.12	0.68	0.49	0.28	0.48	2.41	2.21	1.99	2.20
G.2	2.04	1.12	2.1	1.75	0.93	0.4	0.19	0.52	2.54	2.36	2.08	2.33
G.3	2.1	1.96	1.82	1.96	0.78	0.38	0.20	0.46	2.59	2.47	2.13	2.40
G.4	2.12	2.1	2.1	2.11	0.73	0.19	0.14	0.35	2.21	2.18	1.76	2.05
G.5	2.27	2.1	1.54	1.97	0.90	0.24	0.08	0.41	2.42	2.16	1.81	2.13
Mean of G.	2.13	2.04	1.87	1.982	0.80	0.35	0.178	0.444	2.44	2.33	2.10	2.22
LSD 0.05	D.: NS G.: NS G x D: NS				D.: 0.127 G.: 0.18 G x D: 0.27				D.: NS G.: 0.29 G x D: NS			

TABLE 5-b. Minerals content (NPK %) in dry leaves of the five faba bean genotypes tested under salt stress.

Genotype	Nitrogen (%)				Phosphorus (%)				Potassium (%)			
	Salinity levels				Salinity levels				Salinity levels			
	Cont.	First	Second	Mean of S.	Cont.	First	Second	Mean of S.	Cont.	First	Second	Mean of S.
G.1	2.36	2.22	1.95	2.18	0.68	0.67	0.60	0.65	2.41	2.28	1.86	2.18
G.2	2.04	2.02	1.89	1.98	0.93	0.62	0.42	0.66	2.54	2.32	2.28	2.38
G.3	2.10	2.02	1.93	2.02	0.78	0.76	0.69	0.74	2.59	2.44	2.19	2.41
G.4	2.12	1.95	1.82	1.96	0.73	0.65	0.53	0.63	2.21	2.14	1.94	2.10
G.5	2.27	2.16	1.83	2.09	0.90	0.64	0.64	0.73	2.42	2.4	1.98	2.27
Mean of G.	2.13	2.07	1.87	2.05	0.80	0.67	0.57	0.68	2.44	2.33	2.10	2.27
LSD 0.05	S.: NS G.: 0.18 G x S: NS				S.: NS G.: 0.18 G x S: 0.22				S.: NS G.: NS G x S: NS			

Tables 6-a and 6-b showed that the mean performance of minerals content (Ca %) and (Na^+, Cl^- (mg/100g)) of the five faba bean genotypes tested under water deficit and salinity stress. Calcium content increased under the first level of stress and then decreased under the second level of stress in both water and salinity stress but the differences were non significant under water stress. Sodium was significantly increased under salt stress relative to control but recorded a non-significant increase under water stress. Chloride recorded significantly increase under both water and salt stress, where water stress plants were lower mean of chloride value than salt stressed plants.

TABLE 6-a. Minerals content (Ca %) and (Na^+, Cl^- (mg/100g)) in dry leaves of the five faba bean genotypes tested under water deficit.

Genotype	Calcium (%)				Sodium (mg/100g)				Chloride (mg/100g)			
	Drought levels				Drought levels				Drought levels			
	Cont.	First	Second	Mean of D.	Cont.	First	Second	Mean of D.	Cont.	First	Second	Mean of D.
G.1	0.66	0.63	0.89	0.73	0.78	0.79	0.93	0.83	8.78	14.63	14.63	12.68
G.2	0.60	0.75	0.72	0.69	0.87	0.90	0.94	0.90	7.31	14.63	21.94	14.63
G.3	0.52	0.72	0.60	0.61	1.03	1.11	1.16	1.10	11.7	10.97	12.43	11.70
G.4	0.69	0.78	0.55	0.67	0.94	0.97	0.98	0.96	5.85	7.31	12.43	8.53
G.5	0.63	0.66	0.66	0.65	0.96	1.02	1.05	1.01	8.48	13.89	14.63	12.33
Mean of G.	0.61	0.70	0.60	0.67	0.95	1.00	1.03	0.96	8.304	11.70	15.36	11.97
LSD 0.05	D.: NS G.: NS G x D: NS				D.: NS G.: NS G x D: NS				D.: 1.14 G.: 2.72 G x D: 3.46			

TABLE 6-b. Minerals content (Ca %) and (Na^+, Cl^- (mg/100g)) in dry leaves of the five faba bean genotypes tested under salt stress.

Genotype	Calcium (%)				Sodium (mg/100g)				Chloride (mg/100g)			
	Salinity levels				Salinity levels				Salinity levels			
	Cont.	First	Second	Mean of S.	Cont.	First	Second	Mean of S.	Cont.	First	Second	Mean of S.
G.1	0.66	0.69	0.58	0.64	0.78	1.55	1.58	1.30	8.78	13.16	14.63	12.19
G.2	0.6	0.81	0.72	0.71	0.87	1.87	2.1	1.58	7.31	12.29	16.82	12.14
G.3	0.52	0.92	0.78	0.74	1.03	1.58	2.36	1.66	11.7	11.7	19.01	14.14
G.4	0.69	0.75	0.81	0.75	0.94	1.44	1.87	1.43	5.85	7.31	18.43	10.53
G.5	0.63	0.78	0.66	0.69	0.96	1.41	2.21	1.53	8.48	10.24	16.09	11.66
Mean of G.	0.61	0.82	0.74	0.71	0.95	1.58	2.10	1.5	8.34	10.39	17.59	12.12
LSD 0.05	D.: NS G.: 0.11 G x D: 0.34				D.: 0.12 G.: 0.44 G x D: 0.58				D.: 1.68 G.: 2.06 G x D: 3.14			

The N, P, and K^+ are primary nutrient elements which are very essential for plant growth and development (Mishra *et al.*, 2014). Also most plants use K^+ and Ca^{2+} rather than Na^+ as an important component of osmotic adjustment, K^+ and Ca^{2+} are essential macronutrients for all plants (Kaya *et al.*, 2007 and Zhang *et al.*, 2010). Consequently, crops growing in saline soils may suffer dual injury, Na^+ toxicity and K^+ or Ca^{2+} deficiency (Kaya *et al.*, 2007). Abdelgawad *at al* (2014) indicated that accumulation of Na^+ in the leaves of both rice cultivars increased significantly under saline conditions. But accumulation of K^+, N, and P was significantly reduced due to salt stress. Imposition of water deficit condition decreased the shoot N and K in all cultivars (Jabeen *et al.*, 2008).

Amino acids content.

Amino acids and type are very important to evaluate protein. In this respect, Tables 7-a and 7-b indicated the 16 amino acids that detected including acyclic amino acids (ACAA) and cyclic amino acids (CAA). ACAA contain: aliphatic unsubstituted amino acids (AUAA) such as (Glycine, Alanine, Valine, Leucine, and Isolucine) and aliphatic substituted (ASAA): hydroxy (Serine, Threonine), thio (Methionine), carboxy (Aspartic, Glutamic), Diamino (Lysine), Guanidino (Arginine). Cyclic amino acids include: aromatic (Phenylalanine, Tyrosine), heterocyclic (Histidine), imino acid (Proline).

TABLE 7-a. Amino acids fractions (mg/g D.Wt) in dry leaves of the five faba bean genotypes tested under water deficit.

Geno-type	Drought levels	Amino acids (mg/g dry wt.)																						
		Acyclic amino acids																Cyclic amino acids						
		Aliphatic unsubstituted							Aliphatic substituted									Aromatic			Heterocyclic	Imino		
		Glycine	Alanine	Valine	Leucine	Isoleucine	Total	Hydroxy		Thio	Carboxy		Diamino		Guanidino	Hydroxy	Total	Phenyl Alanine	Tyrosine		Histidine	Proline		Total
								Serine	Threonine	Methionine	Aspartic	Glutamic	Lysine		Arginine									
G.1	Cont.	11.203	12.187	12.128	19.333	10.898	65.75	14.823	15.098	0.906	44.53	31.151	15.821		16.754	139.81		12.186	8.143		7.374	13.698		41.40
	D1	14.856	17.545	12.873	20.549	10.171	75.99	12.97	10.989	1.073	36.6	26.281	13.276		12.616	113.805		12.645	8.688		8.349	13.748		43.43
	D2	16.171	19.112	15.352	23.611	11.242	85.49	9.693	11.356	3.264	27.22	22.223	13.286		10.73	97.772		14.266	7.316		9.886	15.671		47.139
G.2	Cont.	12.256	15.995	12.905	19.708	8.819	70 .68	14.575	15.535	1.007	49.17	31.064	17.551		16.088	144.99		13.429	9.76		9.77	15.038		47.997
	D1	14.366	17.593	13.574	21.902	10.971	78.41	13.745	13.964	1.325	44.38	31.182	15.765		18.534	138.895		14.402	9.106		9.875	22.691		56.074
	D2	16.585	19.636	15.886	25.146	12.687	89.94	11.194	10.87	0.971	34.47	25.097	15.467		12.097	110.166		15.184	10.523		9.602	23.813		59.122
G.3	Cont.	15.366	17.203	13.236	21.227	10.053	77.09	14.265	13.484	1.502	39.917	30.906	16.788		17.243	134.105		12.717	8.457		9.642	14.105		44.921
	D1	16.744	19.238	15.827	24.487	10.312	86.61	12.959	11.982	1.194	38.315	27.436	14.309		15.498	124.693		14.384	9.136		8.437	18.981		50.938
	D2	16.859	19.425	15.74	24.596	12.595	89.22	14.129	13.99	1.407	34.888	27.553	13.414		13.882	119.263		14.981	10.744		9.226	22.911		57.862
G.4	Cont.	12.773	14.450	12.841	21.535	11.229	72.83	6.562	6.898	1.196	39.471	15.502	14.861		12.133	83.16		11.481	8.665		7.867	9.238		37.251
	D1	16.144	20.267	15.584	24.431	12.381	88.81	13.937	13.845	1.204	40.722	30.41	16.716		13.518	130.352		14.867	9.57		9.601	22.819		56.857
	D2	16.966	19.375	15.851	24.407	12.59	89.19	14.235	13.699	1.341	35.519	35.767	16.755		18.032	135.338		14.968	10.62		10.63	23.639		59.857
G.5	Cont.	11.144	12.331	9.669	15.299	7.697	56.14	9.444	8.856	1.159	29.523	20.094	10.652		11.972	91.7		9.468	6.45		7.294	10.676		33.889
	D1	13.372	13.693	11.643	81.499	10.917	70.12	12.7	12.645	1.063	37.416	27.01	13.798		15.12	119.75		13.076	8.243		8.556	12.223		42.098
	D2	13.381	15.419	12.466	20.495	10.171	71.93	10.88	14.55	1.318	35.791	29.474	16.674		19.176	127.863		12.043	8.579		8.196	14.967		43.785

TABLE 7-b. Amino acid fractions (mg/g D.Wt) in dry leaves of the five faba bean genotypes tested under salt stress.

Geno-type	Salinity levels	Amino acids (mg/g dry wt.)																		
		Acyclic amino acids														Cyclic amino acids				
		Aliphatic unsubstituted						Aliphatic substituted								Aromatic		Heterocyclic	Imino	
								Hydroxy		Thio	Carboxy		Diamino	Guanidino	Hydroxy					
		Glycine	Alanine	Valine	Leucine	Isoleucine	Total	Serine	Threonine	Methionine	Aspartic	Glumatic	Lysine	Arginine	Total	Phenyl Alanine	Tyrosine	Histidine	Proline	Total
G.1	Cont.	11.203	12.187	12.128	19.333	10.898	65.75	14.823	15.098	0.906	44.53	31.151	15.821	16.754	139.083	12.186	8.143	7.374	13.698	41.40
	S1	11.111	12.895	13.388	22.491	11.097	70.98	10.912	8.777	1.296	24.780	15.058	14.545	12.282	87.65	13.010	8.598	8.864	15.824	46.296
	S2	14.455	15.229	13.606	23.895	11.691	78.88	6.308	8.875	1.616	14.952	16.483	13.867	13.800	75.901	14.598	9.004	9.297	17.699	50.598
G.2	Cont.	12.256	15.995	12.905	19.708	8.819	70 .68	14.575	15.535	1.007	49.17	31.064	17.551	16.088	144.99	13.429	9.76	9.77	15.038	47.997
	S1	14.840	18.397	14.112	22.236	11.221	80.81	13.497	12.20	0.892	39.244	27.089	14.914	15.817	123.653	13.486	9.092	9.995	17.423	49.996
	S2	15.316	18.745	14.817	23.174	11.816	83.87	13.287	12.95	0.920	36.191	28.571	16.228	16.280	124.427	14.127	9.459	9.784	19.208	52.578
G.3	Cont.	15.366	17.203	13.236	21.227	10.053	77.09	14.265	13.484	1.502	39.917	30.906	16.788	17.243	134.105	12.717	8.457	9.642	14.105	44.921
	S1	14.174	17.238	13.130	20.253	10.319	75.11	13.27	11.51	1.625	33.296	26.653	13.366	14.793	114.513	12.446	9.972	8.463	16.968	47.849
	S2	13.730	18.718	14.864	20.387	12.245	79.94	12.944	11.71	0.821	31.279	26.352	13.635	14.738	111.479	13.131	9.996	7.679	16.313	47.119
G.4	Cont.	12.773	14.450	12.841	21.535	11.229	72.83	6.562	6.898	1.196	39.471	15.502	14.861	12.133	83.16	11.481	8.665	7.867	9.238	37.251
	S1	13.880	16.964	13.370	20.792	10.587	75.59	13.86	11.87	0.960	45.787	27.000	14.621	15.175	129.273	12.825	8.898	8.694	12.049	42.466
	S2	15.142	18.596	14.762	22.843	11.513	82.86	14.174	12.71	1.121	41.065	29.163	15.977	15.510	129.72	13.894	9.359	8.742	16.348	48.343
G.5	Cont.	11.144	12.331	9.669	15.299	7.697	56.14	9.444	8.856	1.159	29.523	20.094	10.652	11.972	91.7	9.468	6.45	7.294	10.676	33.889
	S1	15.873	14.439	12.075	20.954	13.954	77.30	13.964	12.79	1.343	38.662	29.792	15.635	16.677	128.863	11.533	9.419	9.309	16.090	46.351
	S2	15.460	18.658	14.332	23.250	23.250	94.95	13.950	13.10	1.179	36.662	29.517	15.384	15.971	125.763	13.997	9.231	9.814	18.855	51.897

In general, stress causes an oxidative attack on proteins results in; site specific amino acid modifications, fragmentation of peptide chain, aggregation of cross-linked reaction products, alteration of electrical charge and increasing susceptibility to proteolysis. The amino acids in a peptide differ in their susceptibility to attack, and various forms of activated oxygen differ in their potential reactivity. Primary, secondary and tertiary protein structures alter the relative susceptibility of certain amino acids (Farr & Kogama, 1991).

Acyclic amino acids (ACAA)

Table 7-a and 7-b showed the amino acids fractions in dry leaves (mg/g D.Wt.) of the five faba bean genotypes tested under water deficit and salt stress, the application of drought stress and salt stress increased the AUAA (glycine, alanine, valine, leucine and isoleucine). These results of drought are in agreement with Bowne *et al*. (2012) who stated that the levels of the branched chain amino acids leucine, isoleucine, and valine were increased under drought stress in all cultivars, and also in agreement with Mansour, (2000) who stated that free amino acids and other soluble N-containing compounds accumulated in plants grown under salt stress. These amino acids include alanine, glycine, serine, leucine and valine could play a substaintial role in mitigation of the effect of salt stress leading to plant adaptation to salinity (Cuin & Shabala, 2007).

ASAA were decrease in the tolerant genotypes and increased in the sensitive genotypes under both water stress and salt stress levels. Glutamic and aspartic acid recorded the highest values in all genotypes compared to other amino acids, where methionine recorded the lowest values. These results are in agreement with Aranjuelo *et al*. (2010) who stated that drought decreased amino acid content such as aspartic and glutamic acid, and Marur *et al.* (1994) who stated that an increase in methionine.

Concentration can be an indication of water stress in plant tissues. In another work, Caputo & Barneix (1997) found that, the amino acids composition of phloem sap is different in different species, in barley, glutamic acid accounts for approximately 50% of the total amino acids, while aspartic acid accounts for roughly 20% and in wheat, glutamic amounted to 30% of the total amino acids, and aspartic acid to 20%, with these proportions changing with plant age.

Cyclic amino acids (CAA)

Data in Tables 7-a and 7-b showed that water deficit increased aromatic and heterocyclic acid content. Under salinity stress levels. Proline increased in all genotypes under stress. Proline content recorded the highest values in genotype (G.2) and genotype (G.1) under water and salinity stress conditions, respectively. Luecine content recorded the highest values in both genotypes (G.5 and G.4) under drought and salinity stress, respectively. This gradual increase in amino acid at high salinity level could be due to increased degradation of protein. The result was in agreement with Mafakheri *et al.* (2010) who reported that the proline content of the leaf increased at growth stages in all varieties of chickpea in response to drought and also with Rajaravindran &Natarajan (2012) who reported that amino acid accumulation occurs not only under salinity but also under water stress in higher plants.

Osmotic adjustment is an important mechanism of salinity tolerance and occurs through compatible solutes accumulation in stressed plants (Talaat & Shawky, 2014). Many functions have been postulated for proline accumulation in plant tissues, proline and free amino acids could be involved in the osmotic adjustment of plants (Delavari *et al.*, 2010) and could also be a protective agent of enzymes and membranes (Abdalla & Selem, 2014). Proline may not only act as an osmoregulator but also play a role in the protection of enzymes and the structure of macromolecules, and as a reservoir of energy and nitrogen for utilization upon exposure to salinity (Tounektia *et al.*, 2011).

On the other hand, higher level of proline content in stem and leaf may be due to expression of genes encoding enzymes of proline synthesis such as pyrroline-5-carboxylate or decrease in enzymes of proline oxidative such as proline dehydrogenase which is controlled by osmotic and salinity stress (Amini & Ehsanpour, 2005).

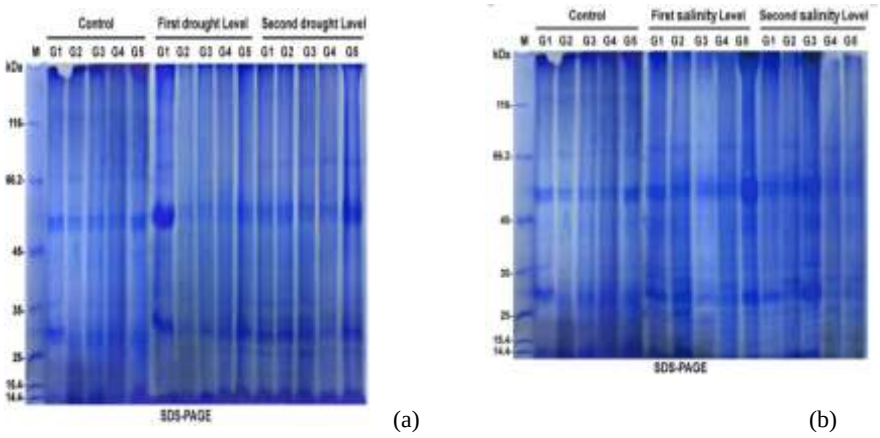
SDS-PAGE of protein

Sodium dodecyl sulphate polyacylamide gel electrophoresis (SDS- PAGE) is employed to detect differences in polymorphic proteins in different cultivars (Atoyebi *et al.* 2014), to evaluate the genetic variation among the accessions of the wild species (Elham *et al.*, 2010 and Vishwanath *et al.*, 2011), to determine of genetic diversity between and within different plant species (Arsalan & Ertugrul 2010 and Rdawan *et al.*, 2013) and to identify genetic relationship between cultivated species and their wild relatives (Folorunso *et al.*, 2006).

According to yield components, the variety ‘genotype (G.2) was the most variety tolerant to the drought stress while the variety ‘genotype (G.5) was the most one susceptible to the drought stress. Genotype (G.1) was the most variety tolerant to the salt stress while the variety ‘genotype (G.4) was the most one susceptible to the salt stress (Afiah *et al*., 2015).

In order to find out biochemical markers associated with the above findings, SDS-PAGE for the total leaves protein of all varieties (control and drought stress treated) had been performed.

Using one-dimensional SDS-PAGE analysis, A maximum of (15) bands were detected with molecular weight ranging from 126 kD to 14 kDa (Fig. 1-a and b) and Tables 8-a and 8-b. Under drought stress, all bands were detected as polymorphic bands except bands No.3 and No. 15 which were recorded as monomorphic bands. On the other hand, in genotype (G.2) bands No. 4 and 14 were absent in control treatment. Genotype (G.5) had an absent band No. 12.



(a) (b)

Fig. 1-a, b. SDS-Protein banding pattern in leaves of the five *Vicia faba* L. genotypes tested under water deficit (a) and salt stress (b).

TABLE 8-a. SDS-protein banding pattern in leaves of five *Vicia faba* L. genotypes tested under the three levels of water deficit.

Band No.	M.W KDa	NBL-(Mar.3) (G1)			NBL-5 (G2)			L3 (G3)			Nubaria-1 (G4)			Misr-1 (G5)		
		C	D1	D2	C	D1	D2	C	D1	D2	C	D1	D2	C	D1	D2
1	126	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1
2	108	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
3	78	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	58	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
5	56	1	1	1	0	1	0	0	0	1	0	1	1	0	1	1
6	47	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0
7	43	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
8	36	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
9	30	1	0	1	0	1	0	0	0	1	1	1	1	0	1	1
10	28	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
11	26	0	1	1	0	0	0	0	0	1	1	1	1	0	1	1
12	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
13	22	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
14	21	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
15	14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total		12	14	15	7	14	15	12	12	12	11	13	15	12	15	11

(0) : Absence of band (1) : Presence of band

TABLE 8-b. SDS-protein banding pattern in leaves of the five *Vicia faba* L. genotypes tested under the three levels of salt stress.

Band No.	M.W KDa	NBL-(Mar.3) (G1)			NBL-5 (G2)			L3 (G3)			Nubaria-1 (G4)			Misr-1 (G5)		
		C	S1	S2	C	S1	S2	C	S1	S2	C	S1	S2	C	S1	S2
1	126	1	1	0	0	1	1	1	0	0	1	1	1	1	0	1
2	108	1	1	1	0	1	1	0	0	0	1	1	0	1	0	0
3	78	1	1	0	1	1	0	1	1	1	1	0	0	1	1	0
4	58	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
5	56	1	1	1	0	1	1	0	1	1	0	1	1	0	0	1
6	47	1	1	0	1	1	0	0	1	1	0	0	1	1	1	0
7	43	1	1	0	1	1	0	0	1	1	0	1	1	1	1	1
8	36	1	1	1	0	1	1	0	1	1	1	1	0	1	1	0
9	30	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1
10	28	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1
11	26	0	1	1	0	1	1	0	1	1	1	1	1	0	1	1
12	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	22	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	21	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
15	14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total		12	15	10	7	15	12	9	13	12	12	13	12	12	12	10

(0) : Absence of band (1) : Presence of band

Under salinity stress, all bands were detected as polymorphic bands except bands No .12 and No. 15 which were recorded as monomorphic bands. The total number of bands increased in tolerant genotypes under the second level of stress while decreased in susceptible genotypes. These results are in accordance with EL-Fadly *et al*. (2007) who detected that there was an increase in number and intensity of some bands in some parents in wheat which might be attributed to water stress treatment**.**

results also revealed that both drought and salinity tolerant and susceptible varieties of faba bean differed from each other in their protein patterns but there was no clear cut marker in protein banding pattern between the studied tolerant and susceptible faba bean genotypes.

The appearance and disappearance of some protein bands means that drought stress resulted in an increase of some proteins and a decrease of others (Amini *et al*., 2007).The appearance of new protein bands under rainfed stress conditions suggests that these proteins may be the cause of drought tolerance in different barley genotypes (Zoro *et al*., 2006). One possible explanation for disappearance of some protein bands under stress is that the genes responsible for proteins synthesis had been completely suppressed as result of stress.

Mohammed *et al.* (2012) suggested that salt stress lead to difference in gene expressions where alterations in protein could be due to alteration in regulation of transcription, mRNA processing or due to altered rates of protein degradation.

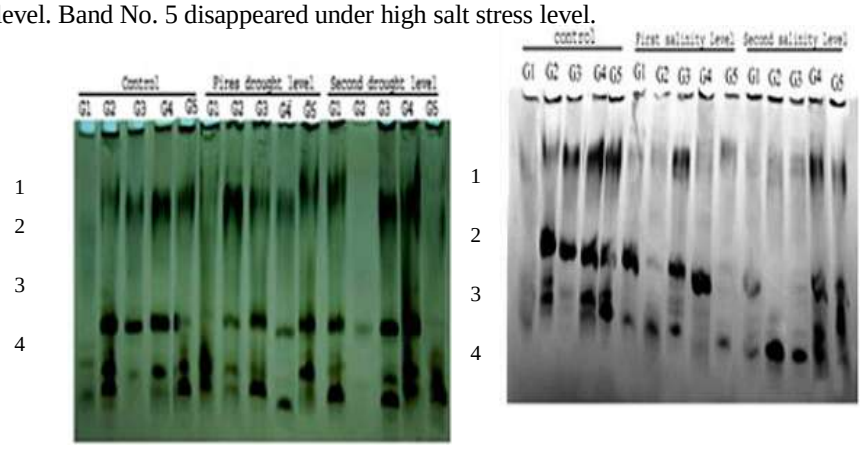
These proteins may be synthesized in response to salt stress or the increase of presently consecutive expression proteins when plants are exposed to salt stress

(Zhang *et al*., 2013). There are common pathways of tolerance to drought and/or salinity (Al-Ansary *et al*., 2007).

Isozymes

Isozyme patterns of α- esterase: The electrophoretic patterns of α – esterase isozymes for the five faba been genotypes under water deficit levels are presented in Fig.2, a. The resulted α–esterase patterns of all studied samples revealed a high polymorphism differences in band's intensity were also noticed among and within studied samples. Five bands were identified, bands were polymorphic bands. Band No. 2 appeared in all genotypes under all treatments except the control treatment for genotype (G.1). Bands (No. 1, 3 and 5) disappeared in genotype (G.2) in the second drought level. Band No.3 disappeared in genotype (G.3) in all treatments. But appeared in genotype (G.4) in the second drought level. On the other hand, all bands appeared in all treatments in genotype (G.5).

The resulted α–esterase patterns of all studied samples under salt stress levels (Fig.2, b) revealed a high polymorphism. Differences in band's intensity were also noticed among and within studied samples. Five bands were identified, all bands were polymorphic bands except band No. 1. Band No. 2 appeared in all genotypes under all treatments except the control treatment for genotype (G.1) and genotype (G.2) under the highest salt stress level. Bands (No. 3 and 4) disappeared in genotype (G.1) under the two salt stress level. Band No.3 disappeared in genotype (G.3) in all treatments, but appeared in genotype (G.4) in the second salt stress level. Band No. 5 disappeared under high salt stress level.

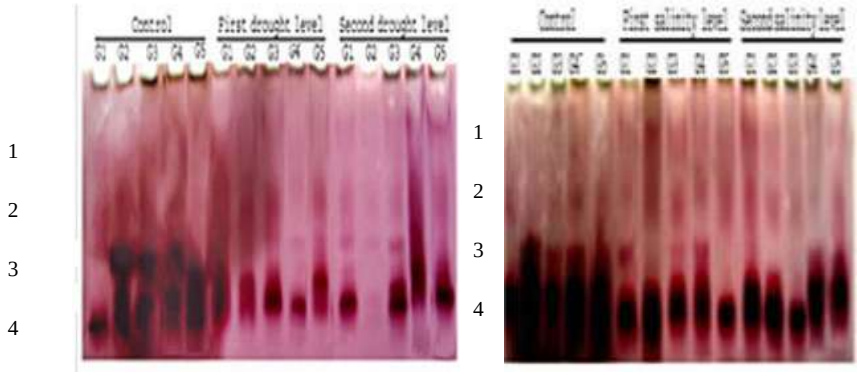


(a) (b)

Fig. 2-a, b. Isozyme patterns of α- esterase in the five faba bean genotypes tested under water deficit (a) and salt stress (b).

Isozyme patterns of β- esterase: The electrophoretic protein of β -esterase isozyme in the five faba been genotypes under different drought and salt stress levels are shown in Fig. 3-a,b. Under different drought stress levels, β -esterase

isozyme exhibited four bands, all four bands were polymorphic. Bands (No. 1, 3 and 4) absent in second drought level for genotype (G.2), genotype (G.1) had two absent band in control treatment and genotype (G.3) had either two bands but in control treatment. Genotype (G.4 and G.5), each one had absent band in control treatment, also the later had another one in second drought level.



(a) (b)

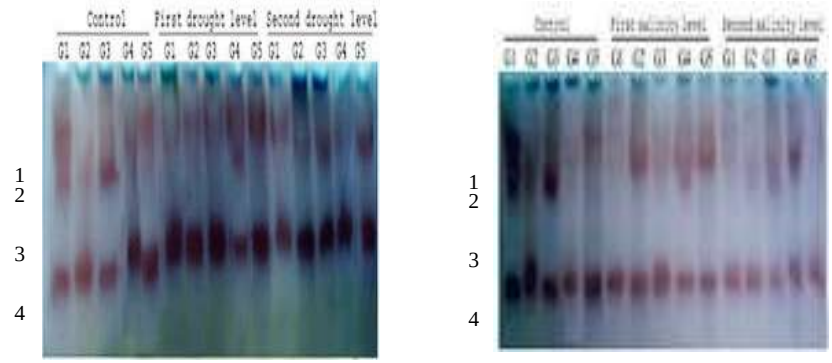
Fig. 3-a, b. Isozyme patterns of β- esterase in the five faba bean genotypes tested under water deficit (a) and salt stress (b).

The electrophoretic protein of β -esterase isozyme in the five faba been genotypes under different salt stress levels exhibited four bands, three bands were polymorphic, and one band (No. 4) was monomorphic. Band No. 3 was absent in all levels in genotype (G.1). Bands (No. 1 and 2) were absent in control treatment for genotype (G.2 and G.3), where band No.3 disappeared under stress in all genotypes except genotype (1). Band No. 1 appeared under stress in genotype (4 and 5).

Our results revealed that there is no clear relationship between salt stress or drought stress and esterase isozymes as well as between esterases and genotypes. These results are in agreement with EL-Fadly *et al*. (2007) who stated that there is no clear relationship between esterase isozymes and water stress tolerance or susceptibility in wheat, but associated with water treatments in some bands.

Isozyme patterns of polyphenol oxidase: Studies on polyphenol oxidase patterns of the present genotypes reveled polymorphism. Differences in bands intensity were also noticed between and within the studies genotypes (Fig.4 –a, b). (Fig. 4-a) shows the polyphenol oxidase electrophoretic patterns of five Faba been genotypes under water deficit levels, A total of three bands cloud be identified for the studied genotypes which were present in some samples and absent in others. Two bands were scored as polymorphic bands and one was monomrphic (band No.3). Band No.2 was absent in genotype (G.1) under the

second drought level only. On the other hand, band No.1 was absent in the sensitive genotypes (G.4 and G.5) under the second drought level**.** Bands intensity increased under highly stress conditions, so this band act as a marker for sensitive genotypes under stress.



(a) (b)

Fig. 4-a, b. Isozyme patterns of polyphenol oxidase in the five faba bean genotypes tested under water deficit (a) and salt stress (b).

Figure 4-b shows the polyphenoloxidase electrophoretic patterns of five faba been genotypes under salinity levels. A total of three bands cloud be identified for the studied genotypes which were present in some samples and absent in others. One band was scored as polymorphic band and two bands were monomrphic.

Polyphenol oxidase (PPO) plays a defensive role in plants against pathogens and a biotic stresses (Radhakrishnan & Lee, 2014). PPO is also a functional mediator of salt stress adaptation in plants (Barbieri *et al.*, 2012). Kostopoulou *et al.* (2014) showed that the PPO activity was enhanced by NaCl treatment in citrus whereas chemical treatments further stimulated its activity.

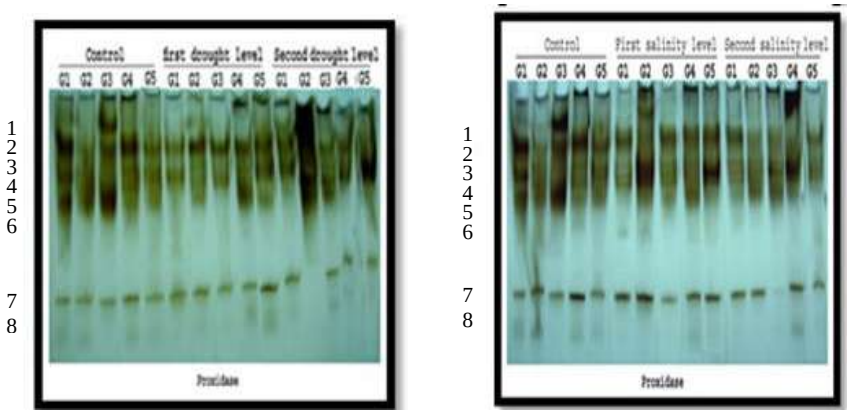
Barbieri *et al.* (2012) showed that enhanced PPO activity is linked with stress tolerance by reducing oxidative damage. Rajaravindran & Natarajan (2012) suggested that the high PPO activity under stress indicates its ability to oxidize and to degrade the toxic substances such as phenolic compounds which are generally reported to be accumulated during salt stress.

Isozyme patterns of peroxidase: Figure 5-a and b shows peroxidase electrophoretic patterns of the five faba been genotypes under different water stress level. The resulted peroxidase patterens of all studied samples reveled a high polymorphism. Differences in bands intensity were also noticed among and within studied samples. Eight bands were identified. All bands were polymorphic bands.

Genotype (G.1) had three bands (No. 1,6 and 8) disappeared under two water and salt stress levels but band No. 4 disappeared in control treatment and then appeared in the two other water stress levels . In genotype (G.2) band No.1 was absent in water deficit treatments but appeared under high salt stress. Band No.4 was absent only in control and then appeared in other water and salt stress treatments. Bands No. 7 and 8 disappeared in the water and salt stress levels. Generally Peroxidase patterns of genotype (G.2, tolerant one) have highly intensity under the second drought level than other genotypes, these results are in accordance with those obtained by Sairam *et al.* (2001), who found that peroxidase isozymes activities increased under water stress.

In genotype (G.3) band No. 1 disappeared under stress but bands No. 3 and 4 were absent in control and then appeared under water stress, bands No. 4 and 8 appeared under salt stress. On the other hand, in genotype (G.4); band No. 1 was present only under severe stress but band No. 4 was absent only under control treatment. Genotype (G.5) had three bands (No.3 .6 .8) which were absent under stress but band No. 1 was absent under all treatments.

Peroxidase activity that was expressed in terms of variation in band-intensity and/or presence or absence score proved to be not accurate criteria for characterization of faba bean genotypes at different water treatments, since the banding patterns differ extremely between water stress tolerance or susceptibility, these results are alined with those of EL-Fadly *et al.* (2007) on wheat genotypes. In shoots of susceptible varieties, the activity of the peroxidase isoenzymes decreased more than in tolerant varieties shoots with increasing in water stress (Mali &Mehta, 1977).



(a) (b)

Fig. 5-a,b. Isozyme patterns of peroxidase in the five faba bean genotypes tested under water deficit (a) and salt stress (b).

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تحمل اجهاد الملوحة و الجفاف فى بعض تراكيب الفول البلدى الوراثية وعلاقة ذلك بالكاشفات الجزيئية ودلائل تحمل الاجهاد

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يواجه النبات الظروف السلبية للنمو مثل الجفاف و الملوحة و التى تؤخر النمو و تحد من انتاجية النبات وفى الحالات القصوى تؤدى إلى موت النبات . استجابة النبات للاجهاد معقدة و تتطلب تنسيق بين المستويات التنظيمية المختلفة و تشمل تعديل التنظيم الغذائى و التعبير الجينى للتكيف الفسيولوجى و المورفولوجى. فى هذا الاطار اجري تجربتين اصص بنظام القطع المنشقة لدراسة استجابة خمس تراكيب وراثية متباينة من الفول البلدى (NBL- Mar.3(G1), NBL-5 (G2), L3 (G3), Nubariya-1(G4) and Misr-1 (G5)) لكل من ظروف إجهاد الجفاف و الملوحة. ادى تعرض التراكيب الوراثية من الفول البلدى لاجهاد الجفاف و اجهاد الملوحة إلى نقص معنوى فى الوزن الرطب و الوزن الجاف كذلك نقص المحتوى الكلى للنبات من البروتين و نسبة NPK كما احتوت الاصناف المتحملة (G1, G2) على نسبة اعلى من البروتين من الاصناف الحساسة (G4, G5) . سبب تعرض النبات لاجهاد الجفاف و الملوحة تراكم لمحتوى النبات من المواد الفينولية و الأحماض الأمينية كما زاد محتوى النبات من الأحماض الأمينية الأليفاتية الغير مستبدلة و كذلك الأحماض الأمينية الحلقية فى كل التراكيب الوراثية و قد رافق ذلك زيادة ملحوظة فى محتوى النبات من البرولين. انخفضت الأحماض الأمينية المستبدلة فى الأصناف المتحملة و زادت فى الأصناف الحساسة تحت تأثير الجفاف و الملوحة. تمت مناقشة التنوع فى الاستجابات البيوكيميائية فى التراكيب الوراثية المتحملة و الحساسة لنبات الفول البلدى.