Effect of Salinity on Growth and Genetic Diversity of Broad Bean (*Vicia faba* L.) Cultivars

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ABSTRACT

A study was conducted at Etay Elbaroud Research Station, Agriculture Research Center, MALR, Egypt, to investigate the effect of salinity on growth and genetic diversity of broad bean. The experiment was a randomized complete block design in a split-plot array with three replications. The main treatments were salt levels (0, 25, 50 and 100 mM of NaCl) and the sub treatments were broad bean cultivars (Etay1, Giza3, Giza843, Nubaria1, and Lozodo). Seeds were sowed in pots containing 1 Kg pre-washed quartz sand and irrigated three times per week by adding 100 mL of solution consisting of base nutrient solution and the salt level, to each pot. After four weeks from sowing the whole plants were collected. The results indicated that increasing salt concentration decreased the fresh and dry weight of shoots and roots, shoot height, and leaf area of all cultivars. However, shoot/root ratio on fresh and dry weight basis and moisture content of shoots and roots were increased with increasing salt concentration. Chlorophyll *a* and *b* and carotenoids content tended to decrease with increasing salt concentration. The genetic diversity analyses allowed classifying the broad bean cultivars into three main clusters; Cluster A includes Giza3 and Giza843, cluster B includes Lozodo and Itay1, and cluster C includes Nubaria1. The different salt levels caused the synthesis and increased the intensity of the original protein bands and caused the appearance of additional new bands of broad bean total protein. The broad bean cultivars were grouped into tolerant (Lozodo and Itay1), moderately tolerant (Giza3 and Giza843), and sensitive (Nubaria1).

Keywords: Salt stress, faba bean, RAPD markers, salt tolerance.

INTRODUCTION

Plant responses to salinity is a complex and vary throughout the season, depending on factors such as soil properties, environmental conditions, cultural practices, and water management. Broad bean (*Vicia faba* L.) or faba bean, a common food in the Mediterranean region, is an important winter legume crop worldwide. Around 4.46 million tonnes of broad bean were produced in 2012 worldwide; almost 35 percent from China, the major producer, and slightly more than 3 percent from Egypt (FAOSTAT, 2014). The crop is beneficial to soil health because of its fixing nitrogen capabilities, improving fertility, especially in sandy soils. Mature seeds of broad bean are good source of protein, which ranges from 270 to 320 g kg^{-1} of dry seeds (Crépon et al., 2010). Broad bean is considered moderately sensitive to salt (Maas and Hoffman, 1977; Katerji et al., 2003). It is more sensitive to salinity during early vegetative stages (Al-Tahir and Al-Abdulsalam, 1997), and reduction in growth can be as much as 50 percent at 6.7 dS m^{-1} salinity (Maas and Hoffman, 1977). In Egypt, broad bean is usually produced in saline soils (Abdelhamid et al., 2010). The prospect of limited fresh water in the country also limits the potential increase of broad bean production.

Nile River is the main source of fresh water in Egypt and is limited to 55.5 billion cubic meters annually, evidencing the need for alternative sources of water if irrigation needs are expanded. Agricultural drainage water or treated wastewater are nonconventional water sources for irrigation but restrictions due to salinity-related issues limit their reuse for irrigation. Worldwide, the problem of salinization is steadily increasing and is more common in arid and semi-arid regions (Evangelou and McDonald, 1999). By the end of the 20th century, about a third of the irrigated land was already affected by salinity (Jacoby, 1999), because of the use of poor quality water for irrigation and because of poor drainage. Globally, salinity has reached 19.5 percent and 2.1 percent in irrigated and dryland areas, respectively (FAO, 2000).

Salt is one of the major types of abiotic stressors that adversely affect growth and development of legumes in arid and semi-arid regions, particularly because these plants depend on symbiotic N_2 fixation for their nitrogen requirements. Salinity damages soil structure and decreases the productivity of most crops as plant growth is affected in several aspects of its metabolism. These include photosynthesis (Nieman and Clark, 1976), osmotic adjustment (Bernstein, 1963), nutritional imbalance and specific ion toxicity (Cordovilla et al., 1994; Gunes and Alpaslan, 1996; Jacoby, 1999), ion uptake (Greenway et al., 1966), enzyme activities (Weimberg, 1970), protein and nucleic acid synthesis (Nieman, 1965), photosynthesis (Downton, 1977), and hormonal balance (Itai et al.,

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Received August15, 2016, Accepted September 21, 2016

1968). Salinity also reduces growth rate by reducing the uptake of water by plants (Munns, 1993; Munns, 2002). Several studies have revealed that growth retardation under salinity is mostly related to osmotic and specific-ion effects (Cramer et al., 1990; Khan et al., 2000). One of the first consequences of plant exposure to high saline concentrations is the formation of reactive oxygen species (ROS). Salt stress leads to excess ROS production that induces stress and plants showed reduction of photosynthetic efficiency and loss of chlorophyll and protein contents (Astolfi and Zuchi, 2013). A study on the physiological responses of three bean cultivars to stress from two sources of salt found that the bean cultivars showed different reaction to salinity and salt type (Kaymakanova and Stoeva, 2008).

While differences on salt tolerance between crop species is well documented (Maas and Hoffman, 1977; Katerji et al, 2003), differences on salt tolerance within a crop, the subject of this study, will continue to be needed. This is particularly important in semi-arid and arid regions as it affects the sustainability of irrigated crop production.

The use of molecular tools has made possible the identification of differences on tolerance to salt within a crop. For instance, Afiah et al. (2007a) used Random Amplified Polymorphic DNA (RAPD) for genotypic identification of broad bean tolerance to drought. Esmat et al. (2012) used RAPD markers to detect the genetic variability and relationships among five faba bean lines. Khan et al. (2013) used RAPD technology to investigate the influence of genetics on salt tolerance in 10 soybean genotypes concluding that variations on tolerance to salt can be partially accounted by plant physiological measures. More recently, Agarwal et al. (2015) used RAPD to determine the genetic homogeneity of in vitro raised medicinal plants. As a result, the objectives of this study, therefor, were to: (i) evaluate the response of five broad bean cultivars to salinity, and (ii) determine the genetic diversity of broad bean cultivars to salt stress.

MATERIALS AND METHODS

The experiment

A pot experiment was conducted under field conditions during the winter season of 2013 at Etay Elbaroud Research Station, El Beheira Governorate, Ministry of Agriculture and Land Reclamation (MALR), Agriculture Research Center, Giza, Egypt. A randomized complete block design in a split-plot array with three replicates was used. The main plot was salt levels (0, 25, 50 and 100 mM NaCl) and the sub plot was broad bean cultivars (Etay1, Giza3, Giza843, Nubaria1, and Lozodo). Ten seeds of every broad bean cultivar were sown in plastic pot of 15 cm diameter and 12 cm depth containing 1 Kg pre-washed quartz sand of size fraction between 0.25 and 1 mm. The number of plants per pot was kept to four, manually thinned 11 days after sowing. Each pot was irrigated three times per week with 100 mL of irrigation solution. The irrigation solution was prepared as follow:

The tenth strength modified Hoagland and Arnon nutrient solution was used as the base solution (Hewitt, 1966). The concentrations of macronutrients in the base solution were 16.87, 8.47, 11.92, 29.99, 12.00, 4.78, and 6.38 mg L^{-1} for $N\text{-}NO_3$, $N\text{-}NH_4$, P, K, Ca, Mg, and S, respectively. The concentrations of micronutrients in the base solution were 0.50, 0.11, 0.05, 0.01, 0.01 and 0.005 mg L^{-1} for Fe, Mn, B, Zn, Cu and Mo, respectively. The irrigation solution consisted of both the base nutrient solution and the salt level: 0, 25, 50 or 100 mM of NaCl.

Growth analysis

Four weeks after sowing the whole plants were collected. The fresh seedlings were subjected to washing by tap water then by distilled water. The seedlings were then separated into shoots and roots. The fresh weight of shoots and roots, shoot height, and leaf area were measured. Half gram of the fresh leaves was cut to small pieces and extracted with 10 mL N, N-Dimethylformamide for the determination of chlorophyll *a* and *b* and carotenes according to Moran and Porath (1980). The plant samples were then dried at 70°C for 48 hours and the dry weight of shoots and roots were measured. The shoot/root ratio on fresh and dry weight basis was calculated.

Crop diversity to salt tolerance

Leaf samples from each cultivar and salinity treatment were submitted to RAPD analysis by PCR amplification (Promega, Germany). The PCR program consisted on an initial denaturation cycle at 95°C for 5 min, 40 cycles at 95°C for 1 min, annealing at 30°C for 1 min and extension at 72°C for 1 min and finally an extra final extension step at 72°C for 10 min (Istock et al., 2001). Two µL of loading dye were added prior to loading of 10 µL sample per gel slot. Electrophoresis was performed at 100 volt with 0.5 x TBE as running buffer in 1.5 percent agarose. Gel was stained in 0.5 $\mu g/cm^3$ (w/v) ethidium bromide solution and destained in deionized water. Finally, the gel was visualized and photographed using gel documentation system. Random primers were used to differentiate and fingerprint the broad bean cultivars under study (Table 1).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE), a proteins separation procedure, was performed using 12 percent separating gel with a 5 percent stacking gel (Laemmli, 1970). We used three replicates,

each consisting one leaf per cultivars and salinity treatment. The protein bands were visualized by staining with 0.1 percent Coomassie Brilliant Blue R-250. Afterwards, the gels were de-stained in a methanol-acetic acid-water mixture (3:1:6) until protein bands became clearly visible.

Table 1. Primer names and their nucleotide sequences employed in the RAPD-PCR analysis

Primer Names	Nucleotide Sequence (5′ to 3′)
C10	TGT CTG GGT G
C11	AAA GCT GCG G
C14	TGC GTG CTT G
C16	CAC ACT CCA G
C18	TGA GTG GGT G

Statistical analysis

The obtained results were submitted to analysis of variance using the CoStat statistical analysis software (CoStat 6.400). Differences among means were identified using Fisher's Least Significant Difference (LSD) test at the 0.05 probability level. Data obtained by RAPD-PCR DNA band patterns were scored for cluster analysis. A dendogram was constructed based on the presence and absence of the amplified bands for each primer. A band in a broad bean cultivar was designated as present (1) and absent (0) after excluding common bands. Pair-wise comparison of broad bean cultivars, based on the presence or absence of unique and shared polymorphic bands, was used to generate similarity coefficients according to Jaccard (1980). The similarity coefficients were used to construct a dendrogram; the Unweighted Pair-Group Method with Arithmetical Averages (UPGMA) was the clustering method.

RESULTS AND DISCUSSIONS

Early growth response of broad bean cultivars to salt stress

The increase of salt concentration in the irrigation solution significantly ($p < 0.05$) decreased the total plant fresh weight of all broad bean cultivars (Table 2). The relative decrease were in the order of 1.02, 21.97, and 54.71 % for 25, 50, and 100 mM NaCl levels, respectively as compared to the control (0. T mM Nacl). There were no significant differences in whole plant fresh weight under salt stress treatments among broad bean cultivars. No differences were also found between the interaction of salinity and broad bean cultivars on whole plant fresh weight. The increase of salt concentration significantly ($p < 0.05$) effected the whole plant dry weight of all five cultivars (Table 2). The average relative decrease in whole plant dry weight, under all salinity levels, was 28.11, 30.12, 37.94, 39.13, and 14.18 % for Itay1, Giza3, Giza843, Nubaria1, and Lozodo cultivars, respectively. The order of these cultivars with respect to tolerance to salinity was Lozodo > Itay1 > Giza3 > Giza843 > Nubaria1.

The mean relative decreases in whole plant dry weight of all broad bean cultivars with increasing salinity levels were 9.23, 26.86, and 54.77 percent at 25, 50, and 100 mM NaCl, respectively compared to the control. The broad bean cultivars varied significantly with respect to whole plant dry weight under salt stress, where the cultivar Lozodo showed the highest whole plant dry weight and the cultivar Giza3 the lowest whole plant dry weight. Significant differences of the interaction between salinity levels and broad bean cultivars, on whole plant dry weight, were also observed (Figure 1).

Table 2. The effect of salinity levels and cultivar on the growth characters of broad bean

Treatment	Fresh weight (g plant^{-1})			Dry weight (g plant^{-1})			Shoot/Root ratio	
	Whole	Shoot	Root	Whole	Shoot	Root	Fresh Weight	Dry Weight
Salinity levels (mM NaCl)								
0	11.79 a*	5.24 a	6.55 a	0.95 a	0.51 a	0.45 a	0.82 b	1.18 a
25	11.67 a	5.07 a	6.60 a	0.87 b	0.46 b	0.41 a	0.78 b	1.17 a
50	9.20 b	4.35 b	4.85 b	0.70 c	0.39 c	0.31 b	0.92 ab	1.31 a
100	5.34 c	2.61 c	2.73 c	0.43 d	0.23 d	0.20 c	1.00 a	1.18 a
Cultivar								
Itay 1	9.41 a	4.44 ab	4.97 a	0.69 bc	0.38 ab	0.31 b	0.92 a	1.259 a
GiZa 3	8.75 a	3.88 c	4.87 a	0.67 c	0.35 b	0.32 b	0.84 a	1.166 a
Giza 843	9.26 a	4.12 bc	5.14 a	0.72 bc	0.37 b	0.35 ab	0.82 a	1.107 a
Nubaria 1	9.50 a	4.42 ab	5.08 a	0.76 ab	0.43 a	0.33 b	0.91 a	1.348 a
Lozodo	10.58 a	4.73 a	5.85 a	0.83 a	0.44 a	0.39 a	0.90 a	1.167 a

*Within column, means with the same letter are not significantly different according to LSD comparison at the $P \leq 0.05$ probability level.

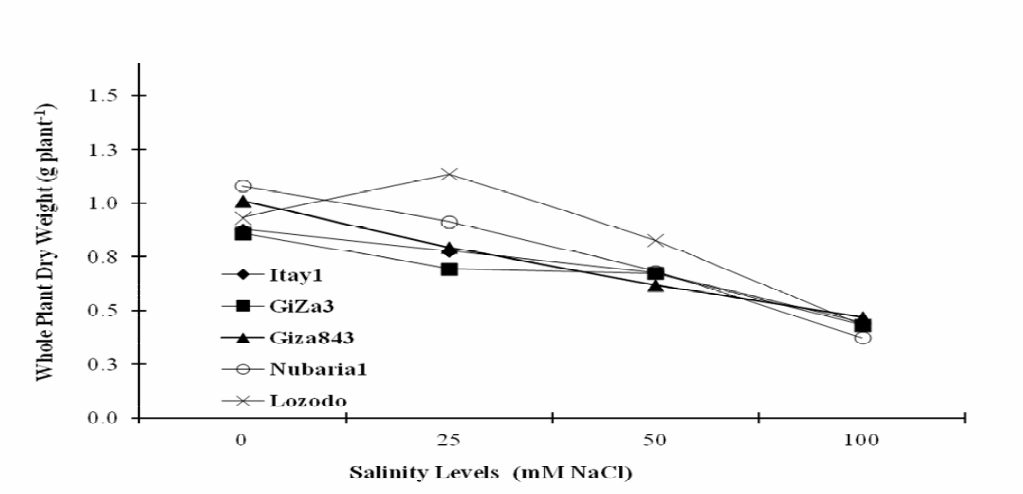

Figure 1. The relation between salinity and the dry weight of broad bean cultivars

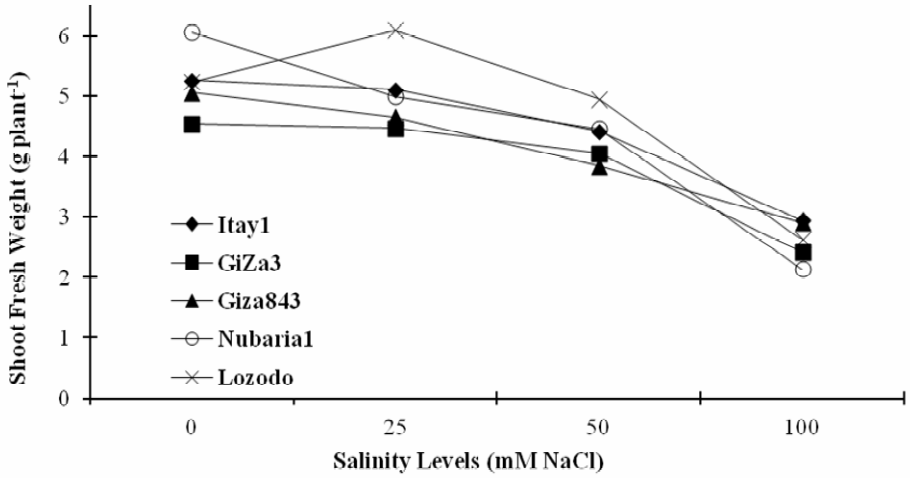

Figure 2. The relation between salinity and shoot fresh weight of broad bean cultivars

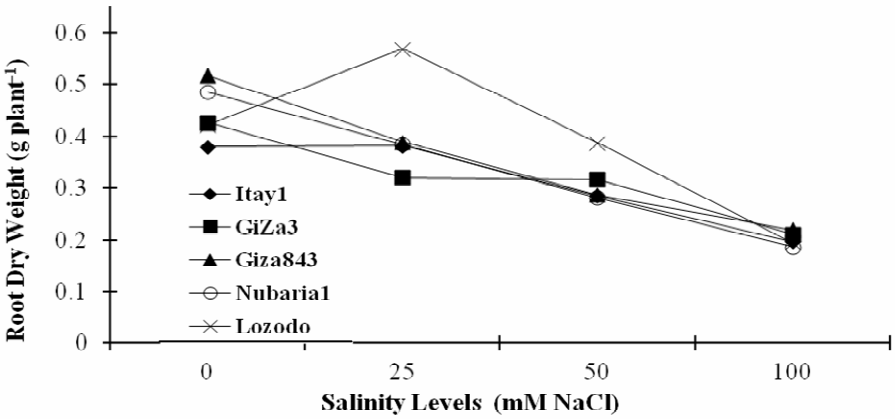

Figure 3. The relation between salinity and root dry weight of broad bean cultivars

Increasing salt concentration decreased the fresh weight of shoot of all broad bean cultivars except Lozodo cultivar, which was increased at 25 mM NaCl (Table 2, Figure 2). In addition, the decrease in shoot fresh weight was not significant at 25 mM NaCl but was significantly different ($p < 0.01$) at 50 and 100 mM NaCl. The relative decrease in shoot fresh weight of all broad bean cultivars with increasing salinity levels were 3.24, 16.98, and 50.19 % with 25, 50, and 100 mM NaCl, respectively compared to the control. There were significant differences ($p < 0.01$) between broad bean cultivars with respect to shoot fresh weight under salt stress. Moreover, there were significant differences ($p < 0.05$) with respect to shoot fresh weight between the interaction of salinity and broad bean cultivars. The mean relative decreases in shoot fresh weight under all salinity levels were 21.00, 19.71, 24.77, 36.14, and 12.98 % compared to the control of Itay1, Giza3, Giza843, Nubaria1, and Lozodo cultivars, respectively. Therefore, the shoot fresh weight of Nubaria1 was the most sensitive to salinity while Lozodo was the least sensitive. Shoot dry weight of broad bean cultivars decreased with the increase of salt concentration in the irrigation water, since the relative decrease in shoot dry weight of all broad bean cultivars with increasing salinity levels were 10.24, 24.02, and 54.92 % at 25, 50, and 100 mM NaCl, respectively compared to the control (Table 2). Also, there were significant differences ($p < 0.05$) between broad bean cultivars with respect to shoot dry weight under salt stress, while no significant differences between the interaction of salinity and broad bean cultivars, on shoot dry weight, were observed.

Salinity levels 50 and 100 mM NaCl significantly ($p < 0.01$) decreased root fresh weights of broad bean cultivars by relative mean value of 25.95 and 58.32 %, respectively compared to the control, while 25 mM NaCl salinity level had no significant effect on root fresh weight of broad bean cultivars. No differences on root fresh weight were observed between broad bean cultivars. Moreover, no differences on root fresh weight were observed for the interaction between salinity and broad bean cultivars (Table 2).

The root dry weight of broad bean cultivars decreased with increasing salt concentration in the irrigation water (Table 2, Figure 3). The decrease in root dry weight was not significant at 25 mM NaCl but was significantly different ($p < 0.01$) at 50 and 100 mM NaCl. The mean relative decreases in root dry weight of all broad bean cultivars with increasing salinity were 8.52, 30.27, and 54.71 % at 25, 50, and 100 mM NaCl, respectively compared to the control. There were significant differences ($p < 0.05$) between broad bean cultivars with respect to root dry weight under salt stress. Moreover, there were significant differences ($p < 0.05$) between the interaction of salinity and broad bean cultivars on root dry weight. The mean relative decreases in root dry weight under all salinity levels were 24.10, 33.65, 42.30, 41.44, and 8.95 % compared to the control of Itay1, Giza3, Giza843, Nubaria1, and Lozodo cultivars, respectively. Therefore, the root dry weight of Giza843 was the highest sensitive to salinity while that of Lozodo was the lowest.

Salt levels of 50 and 100 mM NaCl increased shoot/root ratio on fresh and dry weight basis of broad bean cultivars (Table 2), while 25 mM NaCl salt level had decreased but not significantly shoot/root ratio on fresh and dry weight basis of broad bean cultivars. Significant differences ($p < 0.05$) on fresh weight shoot/root ratio of broad bean cultivars between salt concentration treatments were found. However, no differences on dry weight shoot/root ratio between salt concentration treatments were observed. There were no differences in shoot/root ratio on fresh and dry weight between broad bean cultivars subjected to salt stress. Also, there were no differences on shoot/root ratio, on fresh and dry weight basis, between the interaction of salinity and broad bean. It could be concluded that the increased values of shoot/root ratio with the higher concentrations of salinity indicated that the magnitude of reduction in root growth was greater than that in shoot. This indicates that roots are more sensitive to high salt concentration than shoot. It is also clear that the values of shoot/root ratio were higher on dry weight basis than on fresh weight basis. This is due to higher moisture content in roots than in shoots.

Increased salt concentration significantly decreased the shoot height of broad bean cultivars (Table 3). The mean relative decrease in shoot height of broad bean cultivars with increasing salinity were 16.17, 24.19, and 46.69 % at 25, 50, and 100 mM NaCl, respectively compared to the control. There were significant differences between broad bean cultivars with respect to shoot height under salt stress. On the other hand, there were no differences on shoot height between the interaction of salinity and broad bean cultivars.

High salt levels decreased leaf area of broad bean cultivars (Table 3), and the mean values of the relative decreases were 13.79 and 45.64 % at 50 and 100 mM NaCl, respectively compared to the control, while, 25 mM NaCl salt level increased leaf area. The mean relative increase at 25 mM NaCl was 6.79 % compared to the control.

Table 3. The effect of salinity levels and cultivar on shoot height, leaf area, moisture and pigments contents of broad bean

Treatment	Shoot height (cm)	Leaf area (cm^2)	Moisture content (%)			Photosynthetic pigments (mg/100g Fresh Weight)		
			Whole	Shoot	Root	Chl. *a*	Chl. *b*	Carotenoid
Salinity levels (mM NaCl)								
0	16.94 a*	66.56 a	91.85 a	90.29 a	93.11 ab	71.61 a	38.92 a	32.24 a
25	14.20 b	71.08 a	92.63 a	91.04 a	93.89 a	65.33 a	35.35 a	30.49 a
50	12.85 b	57.38 a	92.43 a	91.15 a	93.59 a	64.59 a	33.60 a	30.39 a
100	9.03 c	36.18 b	91.77 a	91.14 a	92.29 b	62.20 a	33.37 a	29.95 a
Cultivar								
Itay 1	14.15 a	58.47 ab	92.52 a	91.39 a	93.56 a	68.03 a	36.93 a	30.20 bc
Giza 3	12.69 b	54.71 b	92.21 a	91.00 a	93.17 a	59.93 a	32.89 a	28.72 c
Giza 843	13.05 b	53.98 b	92.20 a	91.03 a	93.18 a	62.99 a	34.50 a	29.84 bc
Nubaria 1	12.32 b	54.57 b	92.00 a	90.43 a	93.34 a	68.50 a	36.09 a	31.70 ab
Lozodo	14.07 a	67.27 a	91.91 a	90.67 a	92.86 a	70.20 a	36.13 a	33.38 a

*Within column, means with the same letter are not significantly different according to LSD comparison at the $P \leq 0.05$ probability level.

There were significant differences ($p < 0.05$) in leaf area between broad bean cultivars under salt stress but the interaction of salinity and broad bean cultivars did not affect leaf area.

Very close results have been reported by El-Sayed (2011), Gauch and Wadleigh (1951) and Younis *et al.* (2003). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, water content, cause ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan and Panda, 2008). Kaymakanova and Stoeva (2008) found that the reduction of the biomass of beans grown under saline condition was indicative of several growth limitations, so, the salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio

The influence of two salinity levels (50 and 100 mM NaCl) on the growth and nutrients composition of relatively salt-tolerant or moderately salt-sensitive broad bean seedlings grown in a greenhouse in pots containing perlite was investigated by Bulut and Akinci (2010). Their results indicated reductions in plant height, number of internodes and leaf dry weights of both cultivars as a result of exposure to the two salinity levels. Similar studies reported growth inhibition in soybean (Elsheikh and Wood, 2000), chickpea (Elsheikh and Wood, 1990), pea, and faba bean (Delgado et al., 1994), reduced shoot and root weights in broad bean (Yousef and Sprent, 1983; Zahran and Sprent, 1986) and soybean (Grattan and Maas, 1988), and reduction in biomass and relative growth rate of bean (Gama et al., 2007). A study on beans conducted by Wignarajah (1990) showed reduced shoot growth at NaCl concentrations of 0.05 mol L^{-1} and 0.1 mol L^{-1}; the biomass reduction were based on reduction in root-nodule number and mass, percentage of nitrogen, and dry tissue mass. Mohsen et al., (2014) found that the fresh and dry weights of faba bean (Vicia faba cv. Misr 2) were decreased with salt treatment. The shoot length was hardly, if at all, affected by salinity stress either in the plants treated with ascorbic acid or not.

Effect of salt stress on moisture content of broad bean cultivars

Increased salt concentration had no significant effect on whole plant moisture content of broad bean cultivars (Table 3). Also, there were no differences on whole plant moisture content between broad bean cultivars and between the interaction of salinity and broad bean cultivars. It is clear also that the increase of salt concentration did not affect the moisture content in shoots of broad bean cultivar. In addition, there were no differences for shoot moisture content between broad bean cultivars and also the interaction of salinity and broad bean cultivars.

Salt concentration of 100 mM NaCl significantly ($p < 0.05$) decreased root moisture content of broad bean cultivars (Table 3). On the other hand, salt levels of 25 and 50 mM NaCl increased, but not significantly, the root moisture content of broad bean cultivars. There were no differences in root moisture content between broad bean cultivars under salt stress. Also, there were no differences for root moisture content between the interaction of salinity and broad bean cultivars. As shown in Table 3 the moisture content in roots was

higher than in shoots. Generally, salt stress increased markedly and not significantly moisture content of whole plant, shoot and root of broad bean cultivars. It is clear that the broad bean plants tolerated the adverse effects of salt stress by succulence, which mean the plant increased the shoot and root volume by increasing the moisture content more than the biomass content. These results are in agreement with those reported by Elsokkary *et al*. (2010, 2011).

Effect of salt stress on photosynthetic pigments content of broad bean cultivars

Increased salt concentration in the irrigation solution of broad bean cultivars did not affect chlorophyll *a* and *b* content in plant leaves (Table 3 and Figure 4). Also, there were no significant differences between broad bean cultivars with respect to chlorophyll *a* and *b* content under salt stress. There was no salt stress effect on chlorophyll *b* content and for the interaction of salinity and broad bean cultivars.

However, there was significant ($p < 0.05$) effect of salt stress on chlorophyll *a* content and for the interaction of salinity levels with broad bean cultivars which was clearly observed. As shown in Figure 4, the highest content of chlorophyll *a* was 81.85 mg/100g fresh weight of Nubaria1 at 0 mM NaCl and the lowest value was 45.15 mg/100g fresh weight of the same cultivar (Nubaria1) at 100 mM NaCl. Table 3 showed that the increase of salt concentration did not affect carotenoids content in the leaves of broad bean cultivars. However, there were significant differences ($p < 0.05$) in carotenoids content between broad bean cultivars under salt stress

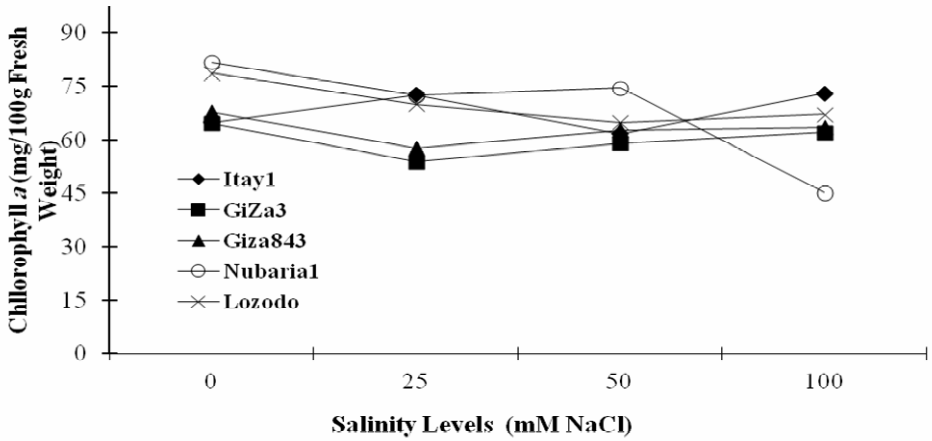

Figure 4. The relation between salinity and chlorophyll *a* contents in leaves of broad bean cultivars

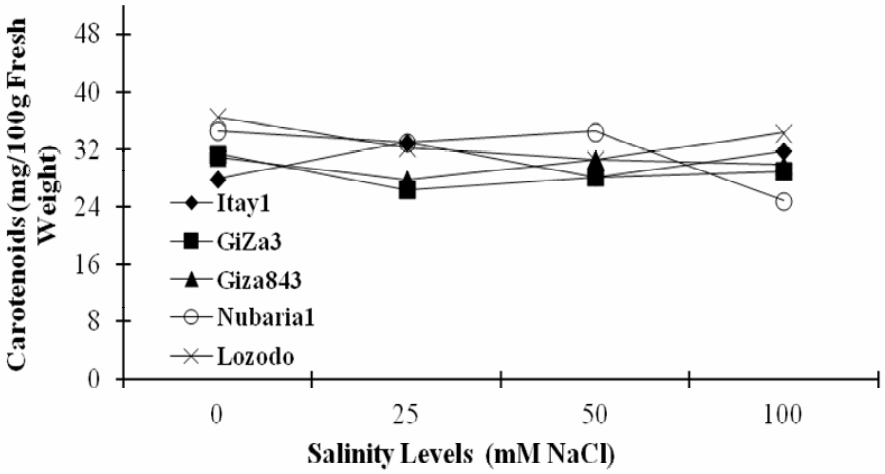

Figure 5. The relation between salinity and carotenoids contents in leaves of broad bean cultivars

There is a significant ($p < 0.05$) salt stress effect on carotenoids content of the interaction of salinity levels and broad bean cultivars, was observed. The highest content of carotenoids was 36.48 mg/100g fresh weight of Lozodo at 0 mM NaCl and the lowest value was 24.85 mg/100g fresh weight of Nubaria1 at 100 mM NaCl (Table 3 and Figure 5).Pigments contents of cultivar Nubaria1 were more affected by high salinity levels and consequently caused a decrease in whole plant dry weight. Similar results have been reported by El-Sayed (2011), who found that salinity tended to reduce chlorophyll content of broad bean cv. Giza2. It was observed by Nieman (1962) that plant species differed in their response to salinity with respect to chlorophyll content, in turnip and cabbage, chlorophyll content increased due to salinity and in wheat, salinity lowered total chlorophyll content. These results are in agreement with those reported by El-Shihaby et al. (2002) who found that salinity didn't significantly change carotenoids content of *Vigna sinesis* plant.

Mohsen et al. (2014) reported that biosynthesis of pigment was substantially affected by salinity levels. Astolfi and Zuchi (2013) showed that in salt-treated plants, an adequate sulfur supply allows adequate glutathione synthesis (high-thiol concentration) thus, avoiding the effects of relative oxygen species (ROS) on photosynthetic functions, whereas in S-deficient plants, salt stress leads to excess ROS production that induces stress and plants showed reduction of photosynthetic efficiency.

Effect of salt stress on genetic diversity of broad bean cultivars

a. Fingerprinting of broad bean cultivars

The use of primers in RAPD-PCR showed clear difference among the five broad bean cultivars based on amplified product band patterns observed with each primer. The amplification profiles with the primers showed in Fig. 6 revealed that all primers used successfully provided polymorphic patterns among cultivars. As compared to monomorphic bands, which are constant bands that cannot be used to study diversity, polymorphic bands reveal differences between genotypes so can be used to examine and establish systematic relationships among those genotypes (Hadrys et al., 1992). Our analysis found high similarity between the two cultivars; Lozodo and Itay1, in turn, both were found to be tolerant to salt stress.

Similar findings are reported by Abdel-Tawab et al. (1997) who differentiated salt tolerance and salt sensitive sorghum genotypes using RAPD markers. In previous studies (Hu and Wang 1997; Wang et al., 1997; and Liu et al., 2000), reported that several RAPD markers were found to be linked with salt tolerance characteristics of different plant species.

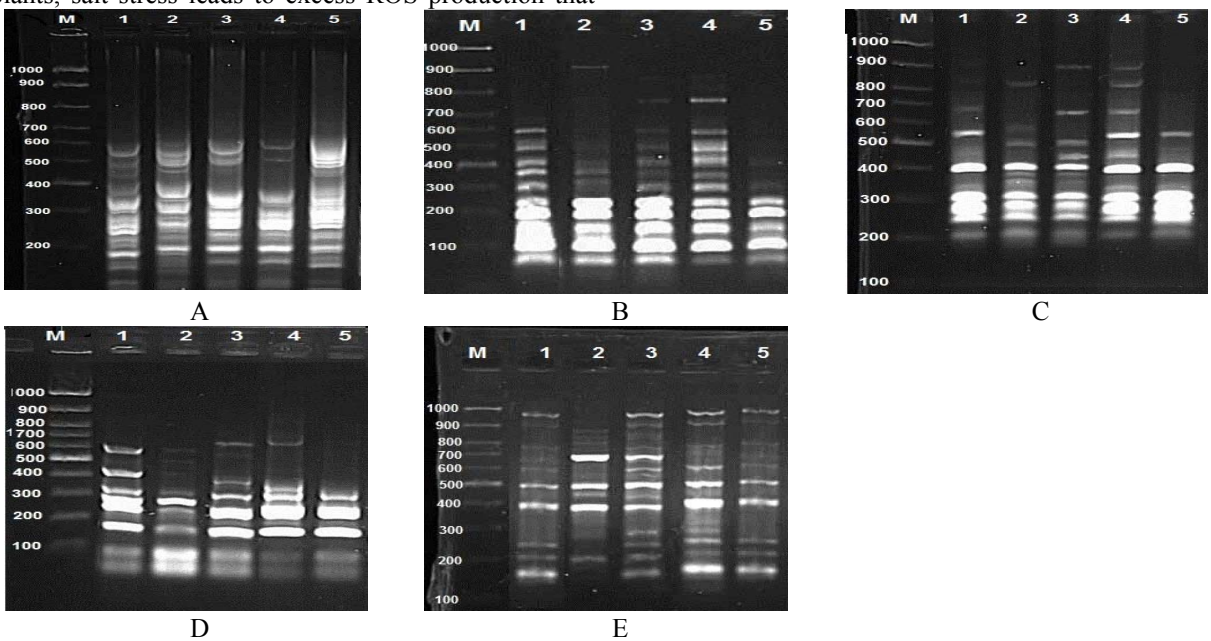

Fig. 6. RAPD-PCR using primer A (C10), B (C11), C (C14), D (C16), and E (C17); M corresponds to DNA marker; col 1-5 = Giza843, Giza3, Lozodo, Itay1, and Nubaria1 broad bean cultivars, respectively

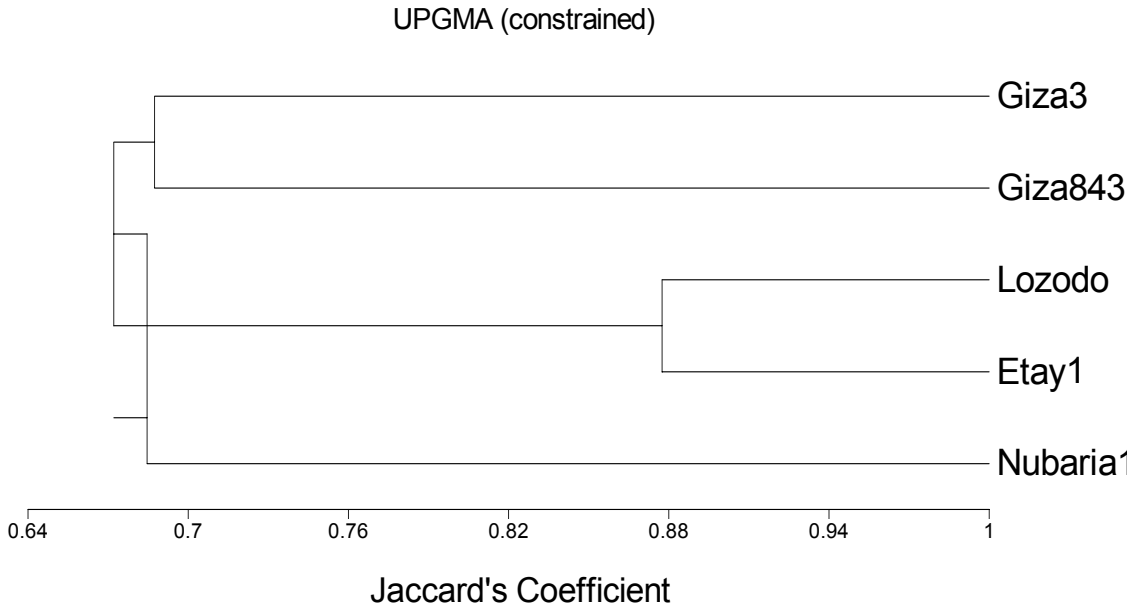

Figure 7. Dendrogram for the five broad bean cultivars obtained by clustering (UPGMA method) based on the band pattern from the RAPD-PCR analysis

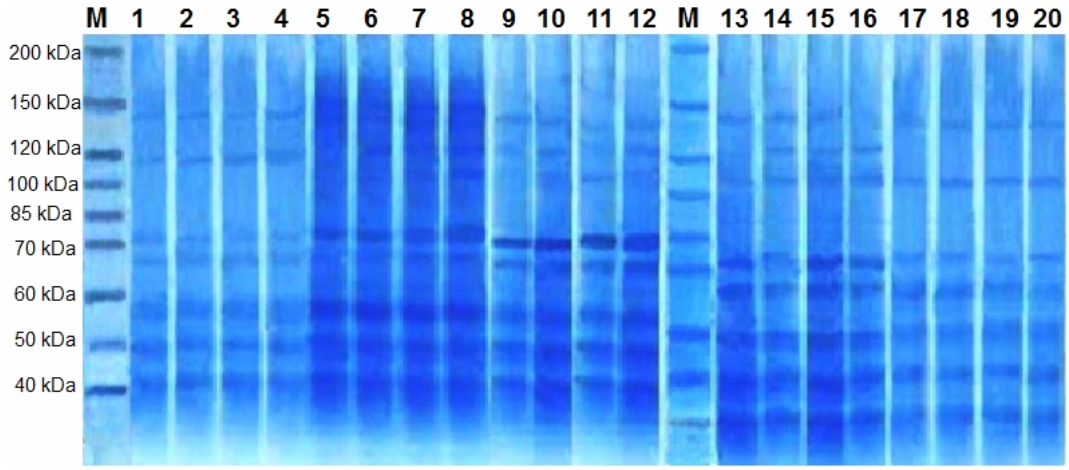

Fig. 8. SDS-PAGE of total protein profile of broad bean cultivars grown under different salt concentrations. Col M = markers; col 1, 5, 9, 13, and 17 = 0 NaCl; col 2, 6, 10, 14, and 18 = 25 mM NaCl; col 3, 7, 11, 15, and 19 = 50 mM NaCl; col 4, 8, 12, 16, and 20 = 100 mM NaCl; col 1-4 = Giza843; col 5-8 =Itay1; col 9-12 =Lozodo; col 13-16 = Giza3; and col 17-20 = Nubaria1

b. Cluster analysis of RAPD results

The RAPD band patterns were analyzed using the UPGMA approach as the clustering method to generate a dendrogram to indicate the relationship of salt tolerance between the five broad bean cultivars. The presence or absence of any particular DNA bands was the only factor considered in the computer analysis. The dendogram generated classified the broad bean cultivars into three main Clusters. As shown in Figure 7 Cluster A includes Giza3 and Giza843 (moderately tolerant), however cluster B includes Lozodo and Itay1 cultivars (tolerant). Cluster C includes Nubaria1 (sensitive). These results suggested that the RAPD approach showed considerable potential for identifying and

discriminating broad bean cultivars with respect to their tolerance to salt stress.

Abdel-Tawab et al. (2003) investigated the dendrogram derived from RAPD data of bread wheat genotypes under saline conditions and showed some divergence from the pedigree information available. Afiah et al. (2007a and 2007b) and Bahieldin and Ahmed (1994) reported that the RAPD technology provided a new alternative for genotypic identification in broad bean, canola and barley, respectively.

Total protein banding profiles

The different concentrations of salt stress on broad been cultivars caused the synthesis and increased of the intensity of the original protein bands. This in turn caused the appearance of additional new bands. It appeared that protein metabolism is greatly affected in plants growing under saline condition. Lozodo and Itay1 cultivars, high salt concentration caused an increase in the intensity band at 75 kDa and an appearance of new band at 107 kDa. Giza3 cultivar, high salt concentration caused appearance of new band at 126 kDa (Fig. 8).

In response to environmental stresses, increased gene expression have been noticed to provoke the synthesis of proteins, collectively called stress proteins, having putative role in stress protection. It has been reported that salinity adversely affects protein metabolism due to decrease in protein synthesis, accelerated proteolysis, decreased availability of amino acids and denaturation of enzymes associated with protein synthesis (Muthukumarasamy and Panneerselvam, 1997 and El-Mashad and Kamel, 2001).

CONCLUSION

Increasing salt stress decreased all growth parameter of all broad bean cultivars, while shoot root ratio and moisture content of shoots and roots of all broad bean cultivars generally increased with increasing salt concentration. Chlorophyll and carotenes content were affected with increasing salt concentration of all broad bean cultivars. Use of application of molecular markers namely, RAPD-PCR and SDS-PAGE would provide rapid and sensitive methods for detection of genetic variations among different broad bean cultivars. The broad bean cultivars Lozodo and Itay1 were tolerant; Giza3 and Giza843 were moderately tolerant, and Nubaria1 sensitive to salt stress.

Acknowledgment

Special thanks to Dr. Adel Elgarhy for providing the seeds of broad bean cultivars and the place to conduct the experiment at the Etay Elbaroud Research Station, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

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الملخص العربي

تأثير الملوحة على النمو والتنوع الجيني لأصناف الفول البلدي

السيد عبد الرءوف عبدالله، إبراهيم أحمد عدس، محمد زغلول دكروري

أجريت تلك الدراسة في محطة البحوث الزراعية بإيتاي البارود، مركز البحوث الزراعية، وزارة الزراعة واستصلاح الأراضي، مصر، لدراسة تأثير الملوحة على النمو والتنوع الجيني للفول البلدي. وكانت التجربة قطاعات منشقة في تصميم قطاعات عشوائية كاملة مع ثلاثة مكررات. وشملت المعاملات الرئيسية مستويات الملحوحة (٠، ٢٥، ٥٠ ، ١٠٠ مليمول من كلوريد الصوديوم) والمعاملات الفرعية أصناف الفول(Etay1، GiZa3، Giza843، Nubaria1، وLozodo). تم زراعة بذور الفول في اصص تحتوي على ١ كجم رمل مغسول كان يتم ريها ثلاث مرات إسبوعياً وذلك بإضافة ١٠٠ مل من محلول يحتوي على العناصر الغذائية ومستوى الملح لكل اصيص. تم حصد جميع النباتات بعد أربعة أسابيع من الزراعة. وأشارت النتائج إلى أن زيادة تركيز الملح أدي إلى انخفاض كل من الوزن الطازج والجاف للمجموع الخضري والجذري، أرتفاع المجموع الخضري، مساحة الورقة من جميع الاصناف. إلا أن نسبة المجموع الخضري / الجذر على أساس الوزن الطازج والجاف ومحتوى الرطوبة للمجموع الخضري والجذري قد إزداد مع زيادة تركيز الملح. أما محتوى الكلوروفيل أ و ب والكاروتينات فقد مالا إلى الانخفاض مع زيادة تركيز الملح.

وقد ساعدت تحليلات التنوع الجيني في تصنيف أصناف الفول إلى ثلاث مجموعات رئيسية. وتشمل المجموعة "A" كل من Giza3 وGiza843، وتشمل المجموعة "B" كل من Lozodo وItay1، وتشمل المجموعة "C" Nubaria1. وقد تسببت مستويات الملحوحة المختلفة في تخليق وزيادة كثافة حزم البروتين الأصلية وكذلك تسببت في ظهور حزم جديدة إضافية للبروتين الكلي في الفول. تم تقسيم أصناف الفول إلى مقاوم ويشمل أصناف(Lozodo وItay1)، متوسط المقاومة ويشمل أصناف(Giza3 وGiza843)، وحساسة وتشمل صنف(Nubaria1).