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Physiological studies on tolerance of different genotypes of faba bean plant to NaCl stress.

El-Kafafi S.H and Essam A.M.
Dept. of Agri. Bot., Faculty of Agri., Al-Azhar univ., Cairo, Egypt
Corresponding author: esambaset@yahoo.com

Abstract

Salinity stress is one of the major abiotic stresses that are a threat to crop production worldwide. Therefore, the aim of the present experiment was to select the tolerant genotype/s based on morphological, physiological and biochemical characteristics of five *Vicia faba* genotypes (Giza 716 "Egypt", Gza "Yemen", ALB "Algeria", Triple white TW "Sudan" and NA112"Bakestane") under salt stress. We studied the effect of different levels of NaCl stress (0-100 mM-150 mM and 200 mM) on growth parameters (length, fresh weight and dry weight) of shoot and root, also some physiological and biochemical traits such as membrane stability index (MSI), leaf relative water content (RWC), chlorophyll (a, b and carotenoids) content, proline biomarker and activities of catalase (CAT, EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7) polyphenol oxidase (PPO) enzymes in the five faba bean genotypes. The results indicated that, salt stress reduced all growth parameters, MSI, RWC and photosynthetic pigments content with all genotypes. However, the deteriorating effect of salt stress on the growth performance of genotype ALB were relatively low due to its better RWC, higher membrane stability, more stabile for photosynthetic pigments, relatively high accumulation of proline and the high expression of some antioxidant enzymes inside plant cells. In the present study, genotype ALB and GZA were found to be relatively tolerant to salt stress while TW and NA112 genotypes were sensitive to salt stress.

Keywords: *Vicia faba*, salinity stress, growth parameters, proline, antioxidant enzymes, nutrient accumulation.

Introduction

Faba bean (*Vicia faba* L.) is one of the most important crops due to the richness of protein content in seeds. The developed range of faba bean diminished in the last 3th years in Egypt from 26,700 to 34,871 ha **(FAO, 2013).** Therefore, the total production of this crop is yet inadequate to cover the local consumption **(Khafaga *et al.*, 2009)**. Soil salinity is one of the most important environmental problems in the world, which limits plant growth and development and thus diminishes the yield **(Krasensky and Jonak, 2012).** Salinity problem in Egypt has a special importance for both old cultivated area and the recently reclaimed soils **(Hellal *et al.*, 2012). Ahmed (2003)** revealed that 60% of the cultivated lands of northern delta area are salt-influence, 20% of the southern delta and middle Egyptian area and 25% of the upper Egypt region are salt-affected soils.

Salinity stress is an intricate stress which incorporates osmotic stress, specific ion effect and nutrient deficiency, and so on, thereby affecting different physiological and biochemical mechanisms related with plant growth and development **(Kukreja *et al.*, 2005)**. Salinity stress leads to a clear stunting of plants **(Cherian *et al.*, 1999).** Many reviews have demonstrated that the fresh and dry weights of the shoot system are influenced, either negatively or positively, by changes in salinity concentration, kind of salt present and type of plant species (**Memon *et al.*, 2010).** Fresh and dry weight of mung bean significantly reduced under three levels of saline water irrigation compared to control plants **(Sajid *et al.*, 2016).**

Changes in water relations of plants that are stressed by salinity can be found in certain reviews that confirm that many plants undergo osmotic regulation when they are exposed to salt stress by increasing the negativity of the osmotic potential of the leaf sap **(Kaymakanova and Stoeva, 2008)**. Water potential and osmotic potential of plants turn out to be more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity **(Meloni *et al.*, 2001)**. Chlorophyll a, chlorophyll b and carotenoid are main photosynthetic pigments and they play important role in photosynthesis. Changes of pigment system contents under salt stress are used as parameter for determination of tolerant and sensitivety in plants **(Eryilmaz, 2007)**. The salt stress can induce oxidative stress because of generation of reactive oxygen species (ROS), including singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical (**Attia *et al.*, 2011**). ROS attack the cellular macromolecules, for example proteins, nucleic acids and membrane lipids causing their damage. In addition, membrane permeability has been accounted for as an effective selection criterion for salt tolerance in different crops (**Ashraf and Ali, 2008).** The plants have developed different protective mechanisms to wipe out or reduce ROS, which are effective at various levels of stress-induced deterioration **(Beak and Skinner, 2003)**. The enzymatic antioxidant system including (Catalase, peroxidase and polyphenol oxidase) is one of the protective mechanisms, which can be found in

different cell compartments and it catalyzes the imbalance of two $O_2^{\cdot-}$ radicals to H_2O_2 and O_2 **(Scandalias, 1993)**. The present study aimed to: Evaluate the salinity-induced modulation and comprehensive understanding of how plants respond to salinity stress and an integrated approach of combining mechanisms based on some key morphological, physiological and biochemical screening in five genotypes of faba bean.

Materials and Methods

This study was carried out at the laboratory of Plant Physiology, department of Agriculture Botany, Fac. of Agric., Al-Azhar Univ., Cairo, Egypt during 2013 - 2014. Five genotypes of faba bean (*V. faba*.L) were screened for their tolerance to salinity: Giza 716 (Egypt), GZA (Yemen), ALB (Algeria), Triple white (Sudan) and NA112 (Pakistan). They differed in geographical origins, growth habitats, seed sizes and earlinesses.

Table 1. Origin and description of five faba bean genotypes used of this study.

Genotypes	Origin	Seed		Hilum colo
		Size	Color	
Giza 716	Egypt	Large	Beige	Black
G.Z.A	Yemen	Medium	Beige	Black
A.L.B	Algeria	Medium	Beige	White
T.W	Sudan	Medium	White	White
NA112	Pakistan	Small	Black	Black

Seeds were acquired from the Field Crops Research Institute (F.C.R.I), Genetic Resources Department, Bahteem (G.R.D) Kaliobia, Egypt (Table1).

A sand-culture technique was utilized to give controlled concentrations of the nutritive elements as well as of the salinizing agent (NaCl). Seeds of five genotypes of faba bean specified above were grown in pots with a mesh bottom and filled with sand washed with tap water over night. Each of these pots was submersed to 4 cm on container which contained 7 Liters of quarter strength Hoagland's nutrient solution **(Hoagland and Arnon, 1950).** Four seeds were planted in each pot and after germination diminished to tow. After the seedlings had grown for about 20 days, they were transferred to another container of the same solution yet with varying concentrations of sodium chloride (0, 100,150 and 200 mM) and after that left to grow for another 25 days. To keep the concentrations of supplement components and the levels of salinization as close to their initial values as possible the culture solutions were adjusted on pH 6, recharged weekly and aerated continuously. The experiment was arranged in a complete randomized design and did in four replicates.

Data recorded at vegetative stage for the following studied traits:

- ***Growth traits*:** recorded on five seedlings from each treatment for the following traits; root and shoot length (cm), fresh and dry weight (g) of root and shoot.

Salt tolerance index: Salt tolerance index (STI) is the ratio of total dry weight at control treatment and dry weight at salt concentration. Salt tolerance index was calculated from the formula of **Ali *et al.*, (2007).**

- ***Physiological and Biochemical traits:***

-***Relative water content (RWC):*** The estimation of leaf relative water content conducted by incubating leaf samples (0.1 g) in 20 ml distilled water for 4 h **(Weatherley, 1950).** The turgid weight of leaf samples was recorded. The leaf samples were oven dried at 65 ºC for 48 h. Dry weights of the samples were taken after confirming that the samples were completely dried out. RWC = (fresh weight- dry weight)/ (turgid weight - dry weight).

-***Membrane stability index (MSI):*** was determined according to **Sairam *et al.*, (2002).** Leaf samples (0.1 g) were placed in 10 ml of double-distilled water. Samples were kept at 40 °C for 30 min and its conductivity recorded (C1) utilizing a conductivity meter. Subsequently the same samples were kept in a boiling water bath (100 °C) for 15 min and its conductivity was additionally recorded (C2). The membrane stability index (MSI) was calculated using following formula: MSI = [1 – C1/ C2]/100.

-***Chlorophyll content:*** Fresh leaves samples (0.1g) were ground in liquid N2 and homogenized in 6 ml 90% aqueous methanol solution and leaved for 3 hours. The analytical determination was performed with spectrophotometer at the following wavelengths: 666, 653 and 470 nm for chlorophyll a, b and carotenoids respectively and the amount of these pigments was calculated according to the formulas of (**Lichtentaler and Wellburn, 1985).**

Chl a=15.65 A666 - 7.340 A653 ,
Chl b=27.05 A653 - 11.21 A666

Cx+c = 1000 A470 - 2.860 Chl a - 129.2 Chl b/245

-***Antioxidant enzymes:*** Tissue preparation for enzymatic antioxidants. Fresh leaves samples (0.2 g) were ground in liquid N2 and homogenized in an ice-bath in 4 mL homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v polyvinylpyrrolidone (pH 7.8). The homogenate was centrifuged at 14000 rpm at 4°C for 10 min and the resulting supernatant was utilized for enzyme assays.

Assay of catalase: Catalase activity was precise according to **Aebi (1984).**

Assay of peroxidase: was measured spectrophotometrically at 420 nm. and determined in fresh leaves as activity per the method of **Chance and Maehly (1955).**

Assay of polyphenol oxidase (PPO): was determined according to **Duckworth and Coleman (1970).**

- ***Estimation of proline content:*** Free proline was extracted and determined in fresh leaves as per the method of **(Bates *et al.*, 1973).**

- ***Determination of nutrient elements in leaves:*** Sodium (Na^+) and potassium (K^+) were determined by flame photometry as described by **Page *et al.*, (1982).** Calcium (Ca^{2+}) was assayed by atomic absorption spectrometry as described by **Chapman and Pratt (1982).** the accumulation (content) ppm/ g = conc. Of element × DWt of leaves.

- ***Statistical analysis:*** Data were subjected to analysis of variance (ANOVA) and significant differences among means were calculated by Duncan's multiple range test ($p \leq 0.05$). All calculations were performed in CoStat program version 6.3

Results and Discussion

1- *Effect of salt stress (NaCl) on growth traits:*

The data revealed that growth performance of the five faba bean genotypes were affected significantly, depending on the level of salt stress (Table 2). The values of salt tolerance index (STI) gradually decrease with increasing salinity levels from 100 to 200 mM NaCl for all faba bean genotypes. Under severe stress (200 mM NaCl), ALB genotype gave the highest value of fresh and dry weight for shoot (75.07% and 92.07%) respectively, when compared with the other genotypes under same concentration. While minimum value recorded by T.W (32.9%) for fresh weight and Giza 716 (60.7%) for dry weight. Also the genotype ALB gave maximum value of fresh and dry weight of root (84.3% and 113.3%) respectively, but the minimum value recorded by NA112 (44.1%) and (64.04%) for fresh and dry weight of root respectively. The maximum value for shoot length was recorded by genotype ALB (83.47%) and (78.24%) for root length. While the minimum value observed by T.W (54.33%) and NA112 (60.55%) for shoot and root respectively.

Table 2. Salt tolerance indices (STI) for the growth parameters of 5 faba bean genotypes at different concentrations of NaCl.

Genotypes	NaCl mM	Shoot			Root			TSTI
		F.W	D.W	Length	F.W	D.W	Length	
Giza 716	100	68.89 ^{b}	82.50 ^{cd}	74.71 ^{cde}	94.34 ^{a}	80.63 ^{abcde}	95.85 ^{a}	82.8 ^{bc}
	150	59.44 ^{c}	71.36 ^{de}	70.11 ^{def}	88.91 ^{a}	89.93 ^{abcd}	81.46 ^{abc}	76.9 ^{cd}
	200	38.73 ^{ef}	60.75 ^{e}	69.13 ^{defg}	80.45 ^{abc}	96.95 ^{abcd}	67.07 ^{abc}	68.8 ^{de}
	Mean	55.68 ^{b}	71.54 ^{bc}	71.31 ^{b}	87.9 ^{a}	89.17 ^{a}	81.46 ^{a}	76.2 ^{bc}
GZA	100	71.78 ^{b}	96.25 ^{ab}	81.30 ^{bcd}	99.54 ^{a}	100.14 ^{abc}	97.09 ^{a}	91.0 ^{ab}
	150	61.58 ^{c}	106.25 ^{a}	70.29 ^{def}	87.54 ^{a}	112.30 ^{a}	74.79 ^{abc}	85.5 ^{bc}
	200	38.85 ^{ef}	82.53 ^{cd}	64.97 ^{efgh}	65.20 ^{bcd}	76.04 ^{bcde}	74.80 ^{abc}	67.1 ^{e}
	Mean	57.40 ^{b}	95.01 ^{a}	72.19 ^{b}	84.09 ^{a}	96.16 ^{a}	82.23 ^{a}	81.18 ^{b}
ALB	100	90.23 ^{a}	95.66 ^{abc}	103.84 ^{a}	93.03 ^{a}	104.33 ^{ab}	91.27 ^{abc}	96.4 ^{a}
	150	83.91 ^{a}	92.19 ^{bc}	84.42 ^{bc}	87.84 ^{a}	103.62 ^{ab}	87.20 ^{abc}	89.9 ^{ab}
	200	75.07 ^{b}	92.07 ^{bc}	83.47 ^{bc}	84.29 ^{ab}	113.33 ^{a}	78.24 ^{abc}	87.7 ^{ab}
	Mean	83.07 ^{a}	93.31 ^{a}	90.57 ^{a}	88.39 ^{a}	107.09 ^{a}	85.57 ^{a}	91.3 ^{a}
T. W	100	47.79 ^{d}	66.15 ^{e}	70.51 ^{def}	59.44 ^{cde}	68.01 ^{cde}	92.53 ^{ab}	67.4 ^{e}
	150	42.68 ^{de}	65.51 ^{e}	57.68 ^{gh}	57.82 ^{de}	67.43 ^{cde}	81.29 ^{abc}	62.1 ^{efg}
	200	32.92 ^{f}	64.63 ^{e}	54.33 ^{h}	57.02 ^{de}	65.96 ^{de}	61.61 ^{bc}	56.1 ^{fg}
	Mean	41.13 ^{c}	65.43 ^{c}	60.84 ^{c}	58.09 ^{b}	67.13 ^{b}	78.48 ^{a}	61.8 ^{d}
NA112	100	71.76 ^{b}	82.48 ^{cd}	92.33 ^{ab}	98.56 ^{a}	83.21 ^{abcde}	83.70 ^{abc}	85.3 ^{bc}
	150	57.65 ^{c}	84.12 ^{bcd}	77.01 ^{cde}	61.54 ^{cde}	52.14 ^{e}	57.95 c	65.1 ^{ef}
	200	41.94 ^{de}	63.31 ^{e}	58.65 ^{fgh}	44.13 ^{e}	64.04 ^{de}	60.55 ^{bc}	55.4 ^{g}
	Mean	57.11 ^{b}	76.63 ^{b}	75.99 ^{b}	68.07 ^{b}	66.46 ^{b}	67.40 ^{a}	68.6 ^{cd}

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's Multiple-Range Test.

According to the mean of total salt tolerance index (TSTI) for F.W, D.W and length of shoot and root a range value decreased with increased NaCl concentration for all genotypes. The genotype ALB gave high mean value (91.3%) when compared with other genotypes followed by GZA (81.2%). While the genotype T.W showed low mean value (61.8%) when compared with other genotypes.

The effect of salinity levels on faba bean was studied by different researchers who reported that, salt stress of (NaCl) caused decreases in growth parameters. **Hamada and El-Enany (1994)** found

that increasing salinity of soil up to 160 mM NaCl reduced growth and transpiration in pot grown *Vicia faba*. **Ahmed *et al.*, (2008)** indicated that growth parameters (shoot length, fresh and dry weights) for faba bean decreased in stressed plants at 90 days age compared with non-stressed plants in the same age. **Dawood and El-Awadi (2014)** reported that in faba bean plant all the measured growth parameters (plant height, leaves number/plant, fresh and dry weights) decreased as a result of application of two salinity levels (3.85 dS/m and 7.69 dS/m) relative to control plants. **Al-Ashkar and El-Kafafi (2014)** observed that salinity stress resulted in significantly reduction in growth parameters (fresh, dry weight and length) of shoot and root in wheat plant. **Abdelgawad *et al.*, (2014)** found that salinity stress was significantly decreased in fresh and dry weight in rice plants.

Salt stress is first perceived by the root system and impedes plant growth both in the short term, by inducing osmotic stress caused by reduced water availability, and in the long term, by salt-induced ion toxicity because of nutrient imbalance in the cytosol **(Munns, 2005)**. The two main threats forced by salinity are promoted by osmotic stress and ionic toxicity related with excessive Cl^- and Na^+ uptake, promoting Ca^{2+} and K^+ deficiency and to other nutrient imbalances **(Marschner, 1995)**.

The inhibitory effect of salinity stress on growth parameters under sodium chloride stress conditions, could be due to the negative effect of this salt on the rate of photosynthesis, changes in enzyme activity (that therefore influences protein synthesis) and furthermore the diminishing in the level of carbohydrates and growth hormones, both of which can prompt inhibition of the growth **(Mazher *et al.*, 2007). Lin and Kao (2000)** reviewed that the inhibitory effect of salinity stress on root growth may be attributed to the increasing concentrations of NaCl. Also, salinity stress increased the hydrogen peroxide content and diminished the root growth. Furthermore, hormonal control of cell division and elongation is evident in roots. Salinity has differential effects on root elongation rates and lateral root initiation **(Rubinigg *et al.*, 2004).**

2- *Effect of salt stress (NaCl) on physiological and biochemical traits:*

- ***Relative water content (RWC):*** Relative water content (RWC) is a good indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues. One of the early symptoms of salinity stress in plant tissue is the decrease of relative water content (RWC). Results in (Table 3) clearly showed that significantly decrease in RWC of treated plants with different concentrations of NaCl compared with non-stressed plants. Under severe stress (200 mM NaCl) showed that ALB genotype gave the highest value of STI for RWC (84.62%) when compared with other genotypes, while minimum value recorded by GZA (77.5%) at the same concentration of NaCl.

Our results are in agree with some previous studies in various crops. **Dawood and El-Awadi (2014)** observed that relative water content (RWC) diminished in stressed faba bean plants subsequently in application of two salinity levels (3.85 dS/m and 7.69 dS/m) relative to control plants. **Ehsan *et al.*, (2010)** reported that water content of faba bean leaves decreased when plants treated with NaCl. **Al-Ashkar and El-Kafafi (2014**) confirmed that RWC of wheat plant leaves diminished with increase of NaCl levels. **Chaudhuri and Choudhuri (1997)** indicated that relative water content, leaf water potential and water uptake decreased under short-term of NaCl stress in jute plant.

It is outstanding that the accumulation of salts in the root zone causes a decrease in osmotic potential and, consequently, a decrease in the water potential, diminishing the water available to the root **(Franco *et al.*, 2011).** High salt concentration in soil and water make high osmotic potential, which reduces the availability of water to plants. Diminish in water potential cause's osmotic stress **(Allakhverdiev *et al.*, 2000).** Moreover, **Neumann (1995)** demonstrated that salinity can quickly inhibit root growth and subsequently limit of water uptake and essential mineral nutrition from soil. Evaluation of relations of plants grown under stress conditions including saline stress is important to ascertain that up to what extent cellular water content is maintained, because almost all metabolic activities within the cell are reliant on availability of sufficient amount of water therein **(Ashraf *et al.*, 2011).** This observation could be expressed that the ALB genotype was able to keep up relatively high RWC than the other four genotypes under salt stress. This is one of a favorable feature with regard to salt stress tolerance of this genotype. Genotypes which were believed to be more salt tolerant usually keep up higher leaf RWC under salinity stress.

- ***Membrane stability index (MSI):*** Membrane stability index was significantly affected by salt stress as appeared in (Table 3). Results revealed that salt stress had decreased the MSI values with increasing of NaCl concentration except genotype (ALB). The highest value recorded by genotype ALB (100.5%) at 200 mM concentration. While the lowest value recorded by genotype NA112 (57.3%) at the same concentration of NaCl. These results are substantially in accordance with those of **Hassanein *et al.*, (2012)** they reported that (MSI) in faba bean decreased with increasing of NaCl stress levels from (0 to 200 mM). **Shahid *et al.*, (2012)** indicated that MSI decreased under salt stress in pea genotypes at all NaCl treatments. **Sairam *et al.*, (2005)** who reported a lower decrease in membrane stability index in tolerant genotypes plant than in salt-sensitive ones under salt stress. Under environmental stresses plant membranes are subject to changes often associated with the increase in permeability and loss of integrity **(Blokhina *et al.*, 2003)**. The cell

membrane stability an indicator of the structural integrity is influenced by salinity stress **(Thomes and Dias 1997).** Salinity stress induce enhanced production of the reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) in plants **(Kholová *et al.,* 2010)**. Different works have examined the generation of ROS and lipid peroxidation in plants because of salinity stress and have demonstrated that there is an increase in lipid peroxidation and a decrease of MSI by increasing senescence and salinity **(Wang and Jian-guo, 2009)**. **Jamil and Eui (2013)** recommended that decrease in membrane stability reflects the degree of lipid peroxidation caused by reactive oxygen species. Salt stress may also displace calcium from plasma membrane binding sites, accordingly causing membrane leakiness as a primary cellular response to salt stress **(Cramer *et al.,* 1988).**

- ***Photosynthetic pigments***: The effects of salt stress on the Chl a, b and carotenoids of 5 faba bean genotypes were studied in (Table 3). Statistical analysis of the data revealed that different salinity levels and the interaction between salinity and genotypes had significantly affected chlorophyll a, b and carotenoids content. The results indicated that increasing salinity levels from 0 to 200 mM, leads to decrease in these three photosynthetic pigments value and the maximum reduction was observed when plants were exposed to high salinity level in all genotypes. At sever concentration (200 mM) the genotype ALB recorded high STI values of Chl a, Chl b and carotenoid (81.36, 78.52 and 83.49%) respectively. While genotype GZA recorded minimum values (48.75 and 43.59%) for Ch a and Ch b respectively. But, genotype T.W recorded lower value (54.64%) for carotenoids. According to TSTI for all pigments, genotype GZA gave the maximum value (92.5%) at 150 mM NaCl concentration, while under severe concentration (200 mM NaCl) the genotype ALB recorded the highest value (81.1%) and lowest value (52.2%) recorded by GZA genotype.

Table 3. Salt tolerant index (STI) for photosynthetic pigments (Chl a, Chl b and carotenoids), Relative water content (RWC) and membrane stability index (MSI) for five faba bean genotypes at different concentrations of NaCl.

NaCl mM	Genotypes					Mean
	Giza 716	GZA	ALB	T. W	NA112	
			chl *a*			
100	98.83 ^{a}	95.09 ^{ab}	86.29 ^{bcde}	88.0 ^{bcd}	78.35 ^{defg}	89.31 ^{a}
150	85.45 ^{bcde}	92.26 ^{abc}	84.04 ^{cde}	85.0 ^{cde}	72.14 ^{fg}	83.77 ^{a}
200	50.26 ^{h}	48.75 ^{h}	81.36 ^{def}	77.09 ^{efg}	70.83 ^{g}	65.66 ^{b}
mean	78.18 ^{bc}	78.69 ^{abc}	83.89 ^{a}	83.37 ^{ab}	73.77 ^{c}	
			chl *b*			
100	95.25 ^{ab}	103.82 ^{a}	81.05 ^{abc}	80.23 ^{abcd}	79.04 ^{abcd}	87.9 ^{a}
150	77.57 ^{bcd}	91.66 ^{ab}	87.72 ^{ab}	72.36 ^{bcd}	75.42 ^{bcd}	80.94 ^{a}
200	55.69 ^{de}	43.59 ^{e}	78.52 ^{bc}	60.18 ^{cde}	76.14 ^{bcd}	62.82 ^{b}
mean	76.17 ^{a}	79.69 ^{a}	82.43 ^{a}	70.92 ^{a}	76.86 ^{a}	
			cx+c			
100	103.27 ^{a}	92.41 ^{ab}	84.39 ^{abc}	77.45 ^{abcd}	76.84 ^{abcd}	86.87 ^{a}
150	97.84 ^{a}	93.68 ^{ab}	84.64 ^{abc}	67.38 ^{bcd}	80.30 ^{abcd}	84.77 ^{a}
200	58.46 ^{cd}	64.38 ^{cd}	83.49 ^{abc}	54.64 ^{d}	78.72 ^{abcd}	67.94 ^{a}
mean	86.52 ^{a}	83.49 ^{a}	84.18 ^{a}	66.49 ^{b}	78.62 ^{ab}	
100	99.1 ^{a}	97.1 ^{a}	83.9 ^{b}	81.9 ^{b}	78.1 ^{b}	
TSTI 150	86.9 ^{ab}	92.5 ^{a}	86.2 ^{b}	75.9 ^{b}	78.1 ^{b}	
200	54.8 ^{c}	52.2 ^{c}	81.1 ^{a}	63.9 ^{bc}	75.2 ^{ab}	
			RWC			
100	89.82 ^{bc}	96.63 ^{a}	96.76 ^{a}	92.57 ^{ab}	96.39 ^{a}	94.43 ^{a}
150	89.07 ^{bc}	87.57 ^{bcd}	91.39 ^{ab}	83.34 ^{de}	90.73 ^{b}	88.42 ^{b}
200	82.59 ^{def}	77.45 ^{f}	84.62 ^{cde}	81.75 ^{ef}	84.41 ^{cde}	82.17 ^{c}
Mean	87.16 ^{b}	87.21 ^{b}	90.92 ^{a}	85.88 ^{b}	90.51 ^{a}	
			MSI			
100	96.46 ^{ab}	87.15 ^{abc}	100.29 ^{a}	85.12 ^{bcd}	77.64 ^{cde}	89.33 ^{a}
150	94.99 ^{ab}	82.54 ^{bcd}	101.22 ^{a}	78.69 ^{cde}	59.27 ^{f}	83.34 ^{a}
200	64.36 ^{ef}	70.95 ^{def}	100.54 ^{a}	59.79 ^{f}	57.34 ^{f}	70.59 ^{b}
Mean	85.27 ^{b}	80.20 ^{bc}	100.68 ^{a}	74.53 ^{c}	64.74 ^{d}	

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's Multiple-Range Test.

The effect of salinity levels on photosynthetic pigments was studied by different researchers. **Hassanein *et al.*, (2012)** recorded that the contents of photosynthetic pigments (chl. a, chl. b, chl. a/b ratio, carotenoids and total pigments) in faba bean significantly decreased with increasing salinity level in treated plants compared with those of untreated plants. **Ahmed *et al.*, (2008)** demonstrated that chl a and chl b content of leaves decreased in all faba bean stressed plants at 90 days age. **Khaled *et al.*, (2016)** indicated that chl a, chl b and carotenoids adversely influenced with increase in NaCl levels in bean plant. **Santos (2004)** recommended that decrease in chlorophyll content might be due to an increase of chlorophyll degradation or to a reduction of chlorophyll biosynthesis. The reduction in chlorophyll and other pigments content because of salinity may decrease carbon fixation that eventually supply energy and substrates for metabolic pathways. This at last may cause reduction in plant growth and development **(Yadav *et al.*, 2011). Taffouo *et al.*, (2010)** showed that reduction in leaf chlorophyll content has been related to salt-induced increasing chloropyllase activity, adverse effects on membrane stability and weakening of protein-pigment-lipid complex. Also variation in specific enzymes under saline conditions **(Keutgen and Pawelzik, 2007). Hanafy *et al.*, (2002)** revealed that salinity could increase chlorophyllase activities, which may be due to the salinity adverse effects on some ions absorption, such as Mg and Fe, which were included in the chloroplast formation.

- ***Antioxidant enzymes activities:*** The results presented in (Table 4) show the effect of different concentrations of NaCl on the activities of the antioxidant enzymes CAT, POD and PPO in the five V. faba genotypes plants at the vegetative stage. The activities of POD and PPO showed progressively increased with increasing salinity level, whereas CAT activity significantly decreased with increasing NaCl concentration in all genotypes, compared with those of the non-salt stressed plants. Catalase activity was increased in ALB and TW at 150 mM NaCl (227.2% - 208.2%) respectively, but at 200 mM NaCl recorded (100.5% - 140.3%) respectively in compare with the control. According to data genotype T.W recorded the maximum value of CAT and PPO activities under 200mM concentration (140.3% and 323%) respectively. While ALB genotype recorded high value of POD activity at 200 mM NaCl concentration. The minimum value recorded by Giza 716 (10.8%) for CAT and NA112 (100.2 and 69.5%) for PPO and POD respectively.

Our results agreed with some previous studies. **(Bekheta *et al.*, 2009)** they Observed that the activities of POD showed progressively increased with increasing salinity level, whereas CAT activity significantly decreased with increasing NaCl concentration, compared with those of the control faba bean plants. In other words, the reduction in catalase activity resulted in accumulation of toxic amounts of hydrogen peroxide (H_2O_2) which might restrict shoot growth, elevated (H_2O_2) concentrations that could release peroxidase from the cell membrane structure **(Nguyen *et al.*, 2005). Magdi *et al.*, (2013)** indicated that the activities of POD and PPO in the shoots of faba bean grown under NaCl stress, showed progressively increased with increasing salinity levels.

Tolerance of salinity stress in higher plants correlates with the levels of antioxidant systems and substrates, these changes in the levels of antioxidant molecules are signals of plant tolerance/adaptation to stress conditions **(Koca *et al.*, 2007).** An increase in enzyme activity in salt stress condition as compared to control was different among cultivars. It can be indicator of their ability to detoxify ROS and oxidative stress tolerance. Early studies indicated that improvement in the salt tolerance in different plant species is possible through the genetically engineered overexpression of specific enzymes for scavenging reactive oxygen species **(Ashraf, 2009).** However, under salinity stress, the highest CAT, POD and PPO enzymes activities were noted in genotypes ALB. Thus, it could be possible and reasonable to suggest that this genotypes was more tolerant than the other genotypes, because the maximum values for these enzymes activity were recorded.

- ***Proline accumulation:*** The effect of different NaCl levels on proline accumulation in five faba bean genotypes was studied. Results in (Table 4) clearly showed that Proline content significantly increase in treated plants in comparison to control. The maximum value of proline accumulation (1121.5%) showed at 150mM NaCl concentration, with ALB genotype. While Giza 716 gave the maximum value (1360.2%) under 200 mM. but, genotype TW recorded the minimum value under 150 and 200 mM (508.5- 692.5%) respectively. According to total salt trait index (TSTI) for biochemical triats (antioxidant enzymes and proline), recorded the highest value at 200 mM Nacl concentration (423.8%) with ALB genotype, and followed by genotype GZA (400.8%). While genotype NA112 showed the lowest value (22.8%) in compare with other genotype under the same concentration.

Our results agreed with some previous studies. **Magdi *et al.*, (2013)** and **Bekheta *et al.*, (2009)** they Indicated that saline solutions resulted in significant increases in proline content of faba bean leaves and that this increment corresponded to increasing salinity concentration. **Suriyan and Chalermpol, (2009)** reported that in saline condition proline content in stressed maize plants increased in comparison of control plants.

Table 4. Salt tolerance index (STI) for antioxidant enzymes (Catalase, Peroxidase and Poly phenol oxidase) and proline content of five faba bean genotypes at different concentrations of NaCl.

NaCl (mM)	Genotypes					mean
	Giza 716	GZA	ALB	T. W	NA112	
			Catalase (CAT)			
100	67.5 ^{abc}	60.1 ^{abc}	192.8 ^{abc}	167.6 ^{abc}	52.2 ^{abc}	108.1 ^{a}
150	30.8 ^{bc}	32.6 ^{bc}	227.2 ^{a}	208.2 ^{ab}	45.0 ^{abc}	108.8 ^{a}
200	10.8 ^{c}	35.2 ^{bc}	100.5 ^{abc}	140.3 ^{abc}	27.8 ^{bc}	62.9 ^{a}
Mean	36.4 ^{b}	42.6 ^{b}	173.5 ^{a}	172.0 ^{a}	41.7 ^{b}	
			Polyphenol oxidase (PPO)			
100	146.7 ^{cdef}	99.1 ^{f}	171.9 ^{bcd}	103.3 ^{ef}	168.5 ^{cde}	137.9 ^{b}
150	158.9 ^{cdef}	131.7 ^{cdef}	183.5 ^{bc}	99.1 ^{f}	191.9 ^{bc}	153.0 ^{ab}
200	110.6 ^{def}	236.5 ^{b}	184.8 ^{bc}	353.0 ^{a}	100.2 ^{f}	197.0 ^{a}
Mean	138.7 ^{b}	155.8 ^{ab}	180.1 a	185.2 ^{a}	153.6 ^{ab}	
			Peroxidase (POD)			
100	69.3 ^{g}	97.42 ^{efg}	144.9 ^{cde}	60.2 ^{g}	103.6 ^{efg}	95.1 ^{b}
150	125.4 ^{def}	84.9 ^{fg}	336.2 ^{a}	127.3 ^{def}	159.1 ^{cd}	166.6 ^{a}
200	109.2 ^{defg}	181.2 ^{bc}	215.5 ^{b}	194.9 ^{bc}	69.5 ^{g}	154.1 ^{a}
Mean	101.3 ^{b}	121.2 ^{b}	232.2 ^{a}	127.4 ^{b}	110.7 ^{b}	
			Proline accumulation			
100	232.7 ^{fg}	419.5 ^{ef}	506.1 ^{de}	222.6 ^{fg}	180.8 ^{g}	312.2 ^{a}
150	884.6 ^{c}	889.0 ^{c}	1121.5 ^{b}	508.5 ^{de}	589.6 ^{de}	798.6 ^{b}
200	1360.2 ^{a}	1150.5 ^{ab}	1194.2 ^{ab}	692.5 ^{cd}	713.9 ^{cd}	1022.3 ^{a}
Mean	825.8 ^{a}	819.7 ^{a}	940.6 ^{a}	474.5 ^{b}	494.7 ^{b}	
100	129.1 ^{b}	169.1 ^{ab}	253.9 ^{a}	138.4 ^{b}	126.2 ^{b}	
TSTI 150	299.9 ^{ab}	284.6 ^{ab}	467.1 ^{a}	235.8 ^{b}	246.4 ^{b}	
200	397.7 ^{a}	400.8 ^{a}	423.8 ^{a}	345.2 ^{a}	227.8 ^{a}	

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's Multiple-Range Test.

Proline accumulation is one of the most frequently reported modifications induced by salt stress in plants and is often considered to be involved in stress resistance mechanisms. It is possible roles have been attributed to stabilizing the structure of macromolecules and organelles through stabilizing proteins and membranes against the denaturating effect of high concentrations of salts and other harmful solutes **(Munns, 2002)**. It is believed that proline protect plant tissue against stress by acting as a nitrogen-storage compound, osmo-solute and hydrophobic protectant for enzymes and cellular structures **(Greenway and Munus, 1980)**. The accumulation of proline concomitant with increasing salinity in faba bean plants was in agreement with the results obtained by **Kavi *et al.*, (2005)** they reported that proline accumulation in response to several types of environmental stress, such as exposure to salinity, protected the cell by balancing the osmotic strength of the cytosol with that of the vacuole and external environment. Proline is also considered to be the only osmolyte able to scavenge free radicals, thereby ensuring membrane stabilization and preventing protein denaturation during severe osmotic stress **(Szabados and Savouré, 2010)**. In addition, proline accumulation was reported to serve as a nitrogen storage compound and protect cellular structure **(Hare and Gress, 1997)**.

- Effect of salt stress (NaCl) on nutrients accumulation: The results presented in (Table 5) showed the effect of different concentrations of NaCl on accumulation of Na^+, K^+ and Ca^{2+} elements in leaves of five faba bean genotypes. It was observed that increase of NaCl concentration lead to increase in Na^+ accumulation in leaves with all genotypes. The Na112 genotype gave the maximum of accumulation (45.74 ppm) Under 200 mM NaCl, while T.W genotype gave the minimum of Na^+ accumulation (21.54 ppm) when compared with other genotypes in same concentration. But, for K^+ accumulation was decreased with increase of concentration of NaCl. The highest decreased for K^+ accumulation was recorded with NA112 genotype gave reduction in K^+ (2.69 ppm) at 200 mM the followed by Giza 716 (4.39 ppm). Ca^{2+} accumulation increased in all genotypes leaves except GZA it was decreased.

Our results agreed with some previous studies **(Lana *et al.*, 2014)** on faba bean, **(Amirjani, 2010)** on soybean and **(Anna *et al.*, 2014)** on green bean. The Na^+ accumulation in plants causes many deleterious effects such as necrosis of leaves and reduced shoot and root growth **(Munns, 2002).**

The accumulation of Na^+ interferes with the K^+ selective ion channels in the root plasma membrane due to similar structure of sodium and potassium. So,

there is a competition between both of them for plant uptake under salinity stress **(Tester and Davenport, 2003)**. According to **Ioneva, (1988)**, increase in Na^+ contents, decrease in K^+ contents and K^+/ Na^+ ratios in plant leaves can be attributed to the effect of competition between Na^+ and K^+ ions on the absorptive sites of the plant roots. The reduced intake of K^+ ions hinders protein synthesis as it plays a major role in binding tRNA to ribosomes **(Blaha *et al.*, 2000)**. Na^+/K^+ ratio is considered as indicator of salinity tolerance in plants **(Munns, 2005)** because it has been found that low Na^+/K^+ ratio in cytosol is essential for normal cell metabolism **(Chinnusamy *et al.*, 2005)**. It's reported that Na^+ transport from root to shoot is unidirectional and the resultant build up of Na^+ in leaves causes osmotic damage **(Flowers *et al.*, 1991).**

Table 5. Effect of different concentrations of NaCl on Na^+, K^+ and Ca^{2+} accumulation in leaves of five faba bean**.**

Genotypes	Nacl (mM)	p.p.m / g D.W		
		Na^+	K^+	Ca^{2+}
Giza 716	0	4.47 ^{o}	7.72 ^{a}	4.08 ^{j}
	100	4.43 ^{o}	4.92 ^{ef}	8.16 ^{h}
	150	17.61 ^{g}	5.19 ^{d}	8.21 ^{h}
	200	30.40 ^{b}	4.39 ^{g}	14.43 ^{e}
	Mean	14.22 ^{c}	5.56 ^{a}	8.72 ^{d}
GZA	0	6.31 ^{m}	4.36 ^{g}	8.33 ^{h}
	100	14.89 ^{i}	3.35 ^{kl}	2.14 ^{m}
	150	15.73 ^{h}	3.18 ^{lm}	2.85 ^{kl}
	200	23.85 ^{e}	3.01 ^{m}	2.78 ^{l}
	Mean	15.19 ^{b}	3.47 ^{d}	4.03 ^{e}
ALB	0	6.31 ^{m}	7.42 ^{b}	7.43 ^{i}
	100	6.44 ^{m}	6.41 ^{c}	8.25 ^{h}
	150	15.04 ^{i}	4.67 ^{f}	28.88 ^{b}
	200	24.76 ^{d}	3.52 ^{jk}	11.95 ^{f}
	Mean	13.14 ^{d}	5.51 ^{a}	14.13 ^{b}
T. W	0	5.79 ^{n}	6.16 ^{c}	8.37 ^{h}
	100	6.38 ^{m}	4.19 ^{gh}	19.59 ^{d}
	150	12.54 ^{j}	3.61 ^{j}	11.07 ^{g}
	200	21.54 ^{f}	3.91 ^{i}	11.61 ^{f}
	Mean	11.57 ^{e}	4.47 ^{b}	12.66 c
NA112	0	10.12 ^{l}	4.21 ^{gh}	3.17 ^{k}
	100	10.67 ^{k}	5.12 ^{de}	22.82 ^{c}
	150	26.73 ^{c}	3.99 ^{hi}	32.55 ^{a}
	200	45.74 ^{a}	2.67 ^{n}	14.35 ^{e}
	Mean	23.32 ^{a}	3.99 ^{c}	18.22 ^{a}

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's Multiple-Range Test.

Essa (2002) reported that the main response of the plant to salt stress is a change in Ca^{2+} homeostasis and attributed that the salt tolerance of plants is their ability to avoid Na^+ toxicity and to maintain Ca^{2+} and K^+ concentrations. Also Na^+ is said to maintain turgor but it is unable to substitute for specific functions of Ca^{2+} and K^+.

Conclusion

The assessment of the effect of salinity on some growth parameters in five five faba (*Vicia faba* L) bean genotypes can be concluded that all of the considered parameters were affected by salinity with a varietal difference. Indeed, the ALB was more tolerant genotype for NaCl stress, it may be correlated with it's ability to maintain higher RWC, higher membrane stability, more stable for photosynthetic pigments, high accumulation of proline inside different cells and the high expression of antioxidant enzymes in cell plant, which can be used as germplasm to introduce genes or valuable traits providing salt tolerance in V. faba via breeding.

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دراسات فسيولوجية على تحمل سلالات مختلفة من الفول البلدى للإجهاد الملحى

السيد حسن الكفافى ، عصام الدين عبد الباسط
قسم النبات الزراعى ، كلية الزراعة، جامعة الأزهر، القاهرة ،مصر

يعتبر إجهاد الملوحة واحد من أكبر المخاطر التى تهدد إنتاج المحاصيل على مستوى العالم. ولذلك تهدف هذه الدراسة إلى إنتقاء أفضل السلالات من الفول البلدى تحملا للإجهاد الملحى طبقا لبعض قياسات النمو ،والقياسات الفسيولوجية والبيوكيميائية التى أجريت على تلك السلالات تحت ظروف الإجهاد الملحى. ولتحقيق هذا الهدف تم دراسة تأثير التركيزات المختلفة (0 ، 100 ، 150 ، 200) مللى مول من ملح كلوريد الصوديوم على خمسة سلالات من الفول البلدى (Giza 716 "مصرى" – GZA " يمنى" – ALB " جزائرى" – TW " سودانى" – 112NA "باكستانى") خلال مرحلة النمو الخضرى.

وقد تم قياس كل من معدلات النمو (الوزن الرطب ، الوزن الجاف ، الطول) ومعدل ثبات الغشاء والمحتوى المائى النسبى ومحتوى الصبغات (أ ، ب ، كاروتين) وتراكم البرولين ومعدل نشاط الإنزيمات المضادة للأكسدة (الكتاليز ، البيروكسيديز ، البولى فينول أوكسيديز) وتراكم العناصر (الصوديوم، البوتاسيوم، الكالسيوم) فى أوراق تلك السلالات.

وقد أوضحت النتائج أن زيادة مستويات الملوحة قد صاحبها تناقص معدلات النمو المختلفة لكل السلالات. وحدوث نقص فى المحتوى المائى وإنخفاض فى ثبات الغشاء ومحتوى الصبغات.

من جهة أخرى أظهرت الدراسة بأن المعاملة بالملوحة أحدثت زيادة تراكم الأوراق من البرولين وزيادة معدل نشاط إنزيمى البروكسيديز والبولى فينول أوكسيديز بينما قل معدل نشاط إنزيم الكتاليز فى كل السلالات مقارنة بالنباتات الغير معاملة.

كما أوضحت النتائج أن الزيادة فى مستوى الملوحة يصاحبها زيادة محتوى الأوراق من عنصر الصوديوم والكالسيوم، بينما حدث إنخفاض في المحتوى من البوتاسيوم.

وقد أظهرت النتائج ان السلالة ALB قد أعطت معدل نمو أعلى نسبيا تحت الظروف الملحية مقارنة بالسلالات الأخرى، وقد يرجع ذلك إلى أن المحتوى المائى أعلى، ثبات الغشاء والصبغات أعلى، تراكم البرولين عالى نسبيا، زيادة نشاط بعض الإنزيمات المضادة للأكسده داخل الخلايا. وقد بينت الدراسة أن السلالتين ALB، GZA متحملتين نسبيا للإجهاد الملحى بينما كانت السلالتين TW، 112NA غير متحملتين.