Effects of Salt Stress on Gene Expression and Activities of POD and PPO in of Broad Bean (*Vicia faba* L.) Cultivars

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ABSTRACT

Soil salinity is one of the most significant abiotic stresses for crop plants, including legumes. The effect of different concentrations of NaCl (0, 25, 50 and 100 mM) on dry weight of shoots and roots, shoot height. The results indicated that increasing of salt concentration was a significant decrease fresh and dry weight of shoots and roots, The effect of different concentrations of NaCl on peroxidase (POD) and Polyphenol oxidase (PPO) enzymes activities in faba bean (*Vicia faba*) cultivars were investigated. POD and PPO activities increased significantly with increasing NaCl concentrations, and the maximal levels increased at 50 mM of NaCl concentration while the activity decreased at high NaCl concentration in all broad bean cultivars except Nubaria1 cultivar. The optimum NaCl concentration for POD and PPO activities were at 25 mM and 0 mM in all cultivars accept Nubaria1 the high activity was at 50mM throw addition of NaCl concentration in reaction of enzyme. The level expression of POD gene in lozoda and Nubaria1 were high gene expression at 50 mM, while giza843 and giza3 were high gene expression at 25 mM. Itay1 was high gene expression at 25 mM. The level expression of PPO gene in lozoda and giza3 were high gene expression at 25 mM, ,while giza843 and Nubaria1 were high gene expression at 50 mM. . Itay1 was high gene expression at 100 mM. lozoda and Itay1 were high gene expression, while giza843 and giza3 were moderately gene expression. Nobaria1 was low POD gene expression.

Key words: salinity, faba bean, significantly, gene expression.

INTRODUCTION

Salinity in soil or water is one of the most severe abiotic stress factors and, especially in arid and semiarid regions, can severely limit crop production (Shannon, 1998). Today over 800 million hectares of land are salt affected, an area equivalent to 6% of the world's total land (FAO, 2005). Two million feddans in Egypt suffer from salinization problems (FAO, 2007). Traditional breeding has few solutions for this problems but biotechnology has been very successful moving the genes that allow a mangrove to live in seawater, into crop plants. These salt tolerant plants will help keep the 750 million acres of salty soil in production (Wager, 2004) In order to improve the performance of crops growing under salt stress, it is important to understand how plants cope under such conditions. Salt tolerance of plants is a complex phenomenon that involves physiological, biochemical, and molecular processes as well as morphological. Furthermore, salinity tolerance is unlikely to be determined by a single gene or gene product (Huang *et al.,* 2005), but probably results from the expression of a number of polygenes, the importance of which is dependent upon their interaction with other salt tolerance genes and the external salt concentration. Salinity tolerance is more likely to be controlled by the complex interaction of several genes than by a single gene. The expression of these genes is influenced by multifarious environmental factors (Foolad, 2004). Changes in their expression can be detected by studying the protein and isozyme pattern of expression (El-Tayeb and Hassanein, 2000). Plants growing in saline environments exhibit various strategies at both the whole plant and cell level that allow them to overcome salinity stress. The problems posed to higher plants by a saline environment results from osmotic stress as a result of the difficulty of absorbing water from soil of unusually high osmotic pressure, and ionic stress resulting from concentrations of potentially toxic salt ions higher than the limit to which most plants are adapted for optimum growth. Both of these components of salt stress affect a growing plant by causing changes in cell membrane chemistry، cell and plant water status, enzyme activities, protein synthesis and gene expression (Alamgir *et al.,* 2008; Turkan and Demiral, 2009).

Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have been considered as a defensive team, whose combined purpose is to protect cells from oxidative damage (Mittler, 2002). Increased SOD, POD and CAT activities are closely related to salt tolerance of many plants as reported in various researches (Rahnama and Ebrahimzadeh 2005,

Azevedo Neto *et al.,* 2006, Koca *et al.,* 2000). An enzyme needs to be in certain atmosphere to be more affective. One of the factors that can effect the enzyme reaction is salt concentration (Cummings, 2005). Numerous studies report increased activity of antioxidant enzymes in plants subjected to salt stress (Meneguzzo and Navari-Izzo, 1999; Hernandez *et al.,* 2000). Salt concentration has to be in its intermediate state for an enzyme to work properly. For instance, if the salt concentration is too high, then the enzyme site will be blocked by the salt ions (Huystee, 1987). Therefore, it will lower the reaction activity rate. The main intention of this experiment was to figure out the salt concentration and its effect on enzymes. To perform this experiment, use the turnip peroxidases. Peroxidases are an enzymes found in plant and animal cells (Gjesing, 1985). Because salt concentration denatures the enzyme we did an experiment to see how the salt concentration would effect the reaction. It is believed that the increase in salt concentration will lower the absorbance rate of turnip peroxidases. The antioxidant enzymes were analyzed activities and transcript levels of genes encoding antioxidant enzymes in Limonium sinense seedlings under salt stress. Catalase showed the largest increase in activity, peaking on day 4 of the 7-day NaCl treatment. Peroxidase and superoxide dismutase activities also increased, peaking on days 2 and 3 of the NaCl treatment, respectively (Zhang *et al.,* 2014). The aim of the present study were to: (i) study the effects of increasing NaCl concentrations on Peroxidase and polyphenol oxidase enzyme activities in broad bean cultivars (ii) determine the optimum NaCl concentration for POD and PPO activities. (iii) Quantification of the level of Peroxidase and polyphenol oxidase genes expression using real-time PCR.

MATERIALS AND METHODS

1. Plant material and growth condition

A pot experiment was conducted under field conditions during the winter season of 2013 at Etay Elbaroud Research Station, Agriculture Research Center, Giza, Egypt to investigate the effect of salinity stress on various cultivars of broad bean (Vicia faba L.) plants grown in sand culture. A randomized complete block design in a split-plot array with three replicates was used. The main plot treatments were four salt levels (0, 25, 50 and 100 mM NaCl) and the subplot treatments were five 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. Each pot was irrigated three times in a week with 100 mL of irrigation solution. After 11 days of sowing the plants were thinned to four seedlings per pot. Tenth strength modified Hoagland and Arnon nutrient solution was used as the base solution according to Hewitt (Hewitt, 1966). The concentrations of macro-nutrients in this base solution were: 16.87, 8.47, 11.92, 29.99, 12.00, 4.78, and 6.38 mg L-1 for N-NO3, N-NH4, P, K, Ca, Mg, and S, respectively, and those of micro-nutrients 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 used water for irrigation consisted of both the base nutrient solution containing 0, 25, 50 or 100 mM of NaCl. After four weeks from sowing the whole plants were collected. The seedlings were then separated into shoots and roots. The fresh and dry weight of shoots and roots were obtained. The plant organs were then dried at 70 □C for 48 hrs, and the dry weight of shoots and roots were measured obtained. The data obtained were analyzed for the least significant difference using the CoStat statistical analysis software (CoStat 6.400)

2. Estimation of peroxidase (POD)

The peroxidase activity was assayed as described by Hammerschmidt and Kuc (1982). Extraction was carried out by homogenizing 1 g of the leave sample in 2 mL of 0.1M sodium phosphate buffer (pH 6.5) using pre chilled pestle and mortar (4 oC). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 oC. The supernatant served as enzyme source and the reaction mixture consisted of 1.5 mL of 0.05 M Pyrogallol, 0.5 mL of enzyme extract, and 0.5 mL of 1% H2O2. The reaction mixture was incubated at 28±2 oC. At the start of enzyme reaction, the absorbance of the mixture was set to zero at 420 nm in the spectrophotometer and the change in the absorbance was recorded at 20 s interval for 3 min. Boiled enzyme preparation served as control. The peroxidase activity was expressed as change in the absorbance of the reaction mixture min-1 g-1 of fresh tissue.

3. Estimation of polyphenol oxidase (PPO) activity

One gram of the sample was homogenized in 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 ^{o}C and the supernatant served as enzyme source. Polyphenol oxidase activity was determined as the procedure given by Mayer *et al.,* (1965). The reaction mixture consisted of 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µL of the enzyme extract. To start the reaction, 200 µL of 0.01 M catechol was added. The reaction mixture was incubated at room temperature and the absorbance was set to zero at 495 nm. The changes in absorbance were recorded at 30 s interval for 2 min and the activity was expressed as change in absorbance min-1 g-1 of fresh tissue.

Table 1: Sequence of primers used in the real-time PCR.

Primers	Primer sequence 5→3	Annealing (°C)
peroxidase (F)	CTCGACCTACAAGGAC	60
peroxidase (R)	ATGTAGGCGCTGGTGA	
polyphenol oxidase (F)	GCTTTGTCAGGGGTTGTGAT	60
polyphenol oxidase (R)	TGCATCTCTAGCAACCAACG	

4. Determine the optimum NaCl concentration for POD and PPO activities throw the Addition of NaCl concentration in reaction of enzyme

To determine the optimal NaCl concentrations for activity of POD and PPO, standard assay was done at different NaCl concentrations were four salt levels (0, 25, 50 and 100 mM NaCl). The concentrate of reaction mixture of enzyme was 0 or 25 or 50 or 100 mM NaCl. The relative activities as percentages were expressed as the ratio of the POD or PPO activity at a certain concentration to the maximum activity at the given concentrations.

5. Quantification of the Peroxidase and polyphenol oxidase Genes Expression Using Real-time PCR

5.1. RNA isolation protocol

Total RNA was extracted from plant tissue using GStract™ RNA Isolation kit II (Guanidium Thiocynate) according to the manfacture procedures.

5.2. Reverse transcription-polymerase chain reaction (RT-PCR) of mRNA

Reverse transcription(RT) or first strand reaction was performed for converting the mRNA to complementary DNA (cDNA) in the presence of deoxynucleotide triphosphates (dNTPS) and reverse transcriptase. The components are combined with a DNA primer in a reverse transcriptase buffer for an hour at 42°C. The exponential amplification via reverse transcription polymerase chain reaction provides a highly sensitive technique, where a very low copy number of RNA molecules can be detected.

Reverse transcription reaction was performed using oligo(dT) primer (5'-TTTTTTTTTTTTTTT-3'). Each 25 µl reaction mixture contained 2.5 µl (5x) buffer with $MgCl_2$, 2.5 µl (2.5 mM) dNTPs, 1 µl (10 pmol) primer, 2.5 µl RNA (2mg/ml) and 0.5 unit reverse transcriptase enzyme. PCR amplification was performed in a thermal cycler programmed at 42 °C for 1 hr, 72 °C for 10 min (enzyme killing) and the product was stored at 4 °C until use.

5.3. Estimation of Quantitative of Peroxidase and polyphenol oxidase genes expression using RT-qPCR

Samples were analyzed using the Fermentase kit: Each reaction contained 12.5 µl of 2x Quantitech SYBR® Green RT Mix, 1µl of 25 pm/µl forward primer, 1 µl of 25 pm/µl reverse primer,1 µl of the cDNA (50ng), 9.25 µl of RNase free water for a total of 25 µl. Samples were spun before loading in the Rotor's wells.

The real time PCR program was as follows : initial denaturation at 95 °C for 10 min.; 40 cycles of at °C for 15 sec.; annealing at 60°C for 30 sec and extension at 72 °C for 30 sec. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene- 6000-system (Qiagen, USA).

5.4. Data analysis

Comparative quantification analysis was done using Rotor-Gene-6000 Series Software based on the following equation (Rasmussen, 2001)

Ratio target gene expression (Experimental/control) =

$$\frac{\text{Fold change in target gene expression (expt/control)}}{\text{Fold change in reference gene expression (expt/control)}}$$

Fold change in target gene expression (expt/control) =

$$\frac{\text{copy number experimental}}{\text{copy number control}}$$

Fold change in reference gene expression (expt/control) =

$$\frac{\text{copy number reference}}{\text{copy number control}}$$

The equation shows a mathematical model of relative expression ratio in real-time PCR. The ratio of a target gene is expressed in a sample versus a control in comparison to a reference gene.

The sample and control dataset of real-time PCR data were analyzed with appropriate Bioinformatics and Statistical program for the estimation of the relative expression of genes using real-time PCR and the result normalized to ITS housekeeping gene (Reference gene). The data were statistically evaluated, interpreted and analyzed using Rotor-Gene-6000 version 1.7.

EXPERIMENTAL RESULTS

Soil salinity is a prevalent abiotic stress for plants. Growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance

as indicated by different studies (Parida and Das 2005). NaCl treatments caused a reduction in growth parameters (The fresh and dry weight of shoots and roots) as compared with control, particularly at high concentration (fig 1)

1. Effect of salt stress on growth parameters of broad bean cultivars

The increase of salt concentration in the growth media decreased the whole plant fresh weight of all broad bean cultivars with not significant decrease at 25 mM NaCl level by 1.02 % compared to control (0 mM NaCl) but there was a significant decrease at 50 and 100 mM NaCl levels by 21.97 and 54.71%, respectively compared to control (Table 2. There were no significant differences among broad bean cultivars in whole plant fresh weight under salt stress treatments; however Lozodo cultivar weighted 10.58 g/plant, which was the highest weight while the lowest weight was obtained on cultivar Giza3 with 8.75 g/plant as the average under all salt stress treatments.

The salt concentration increase had significant adverse effects on the whole plant dry weight of all broad bean cultivars (Table 2). The relative decrease in whole plant dry weight of all broad bean cultivars with increasing salinity levels were 9.23, 26.86, and 54.77 % at 25, 50, and 100 mM NaCl, respectively compared to the control. The broad bean cultivars varied significantly in 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 weight.

Table 2: The main effects of salinity levels and cultivars of broad bean on whole plant, shoot, and root fresh and dry weights and shoot/root ratio

Treatments	Fresh weight (g/plant)			Dry weight (g/plant)			Shoot/Root ratio	
	Whole	Shoot	Root	Whole	Shoot	Root	F. W.	D. W.
Salinity levels (mM NaCl)								
0	11.79	5.24	6.55	0.953	0.508	0.446	0.815	1.178
25	11.67	5.07	6.60	0.865	0.456	0.408	0.782	1.173
50	9.20	4.35	4.85	0.697	0.386	0.311	0.921	1.305
100	5.34	2.61	2.73	0.431	0.229	0.202	1.004	1.181
$LSD_{0.05}$	0.66	0.48	0.57	0.088	0.040	0.074	0.146	ns
Cultivars								
Itay 1	9.41	4.44	4.97	0.694	0.384	0.311	0.923	1.259
GiZa 3	8.75	3.88	4.87	0.667	0.348	0.318	0.840	1.166
Giza 843	9.26	4.12	5.14	0.724	0.371	0.353	0.824	1.107
Nubaria 1	9.50	4.42	5.08	0.764	0.430	0.334	0.913	1.348
Lozodo	10.58	4.73	5.85	0.834	0.441	0.393	0.904	1.167
$LSD_{0.05}$	ns	0.42	ns	0.089	0.059	0.053	ns	ns

Fig. 1: five broad bean cultivars throw treatment with NaCl concentrations.

2. Determine the POD and PPO activities

POD and PPO activities increased significantly with increasing NaCl concentrations compared to the control, and the maximal levels increased at 50 mM of NaCl concentration while the activity decreased at high NaCl concentration in all broad bean cultivars except Nubaria1 cultivar. The different degrees in susceptibility of brood bean cultivars to *salt stress*. Lozoda and Itay1 were the high POD and PPO activities. giza3 was the low POD activity (Table 3 and Fig 2), while Nubaria1 was the low PPO activity (Table 4 and Fig 3). Nubaria1 and giza843 cultivars proved to be moderately POD activity, while giza3 and giza843 cultivars proved to be moderately PPO activity to *salt stress*.

Table 3: Peroxidase (POD) activity in of broad bean cultivars after treatment with NaCl concentrations.

Cultivars.	POD activity (changes in Absorbance min^{-1}g^{-1} of fresh tissue)				
	NaCl (mM)				
	0	25	50	100	Mean
lozoda	0.93	1.41	1.9	1.62	1.465
Itay11	0.72	1.23	1.64	1.5	1.2725
giza843	0.77	1.01	1.54	1.33	1.1625
giza3	0.43	0.91	1.42	1.21	0.9925
Nubaria11	0.61	1.14	1.33	1.5	1.145
$LSD_{0.05}$ (Treat.) = 0.0014					

*, Values are means of three replications.

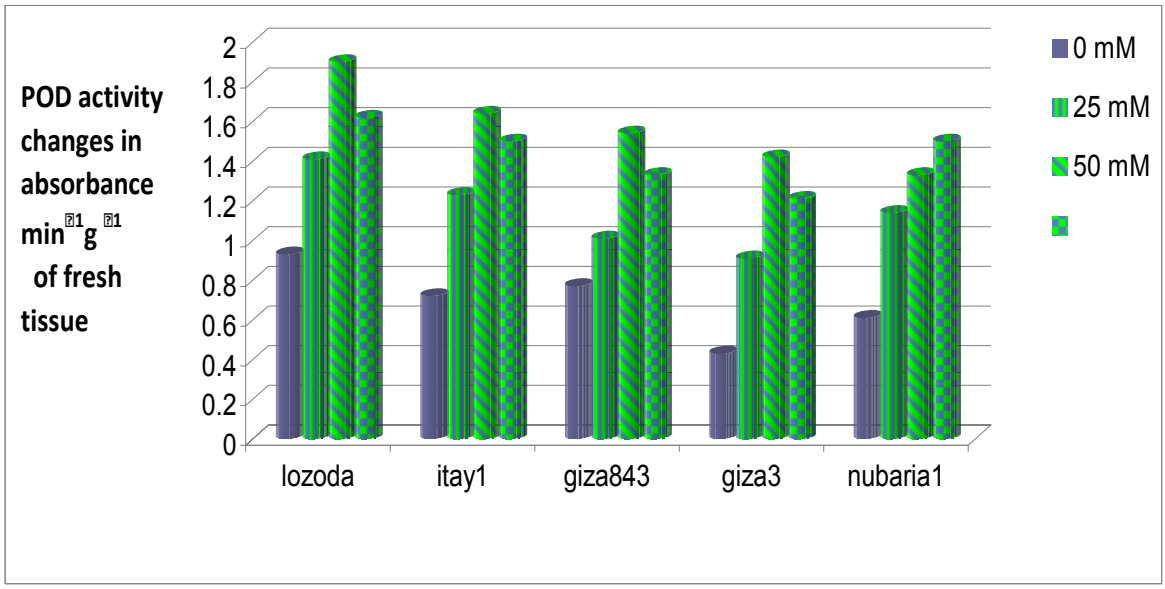

Fig. 2: Peroxidase (POD) activity in potato tubers (Kara c.v) after treatment with NaCl concentrations.

Table 4: Polyphenol oxidase (PPO) activity in of broad bean cultivars after treatment with NaCl concentrations.

Cultivars.	PPO activity (changes in Absorbance min^{-1}g^{-1} of fresh tissue)				
	NaCl (mM)				
	0	25	50	100	Mean
lozoda	0.51	1.12	1.44	1.23	1.075
Itay11	0.65	0.92	1.35	1.3	1.055
giza843	0.44	0.73	1.21	1.01	0.8475
giza3	0.45	0.92	1.32	0.9	0.8975
Nubaria11	0.34	0.82	0.98	1.04	0.795
$LSD_{0.05}$ (Treat.) = 0.0018					

*, Values are means of three replications.

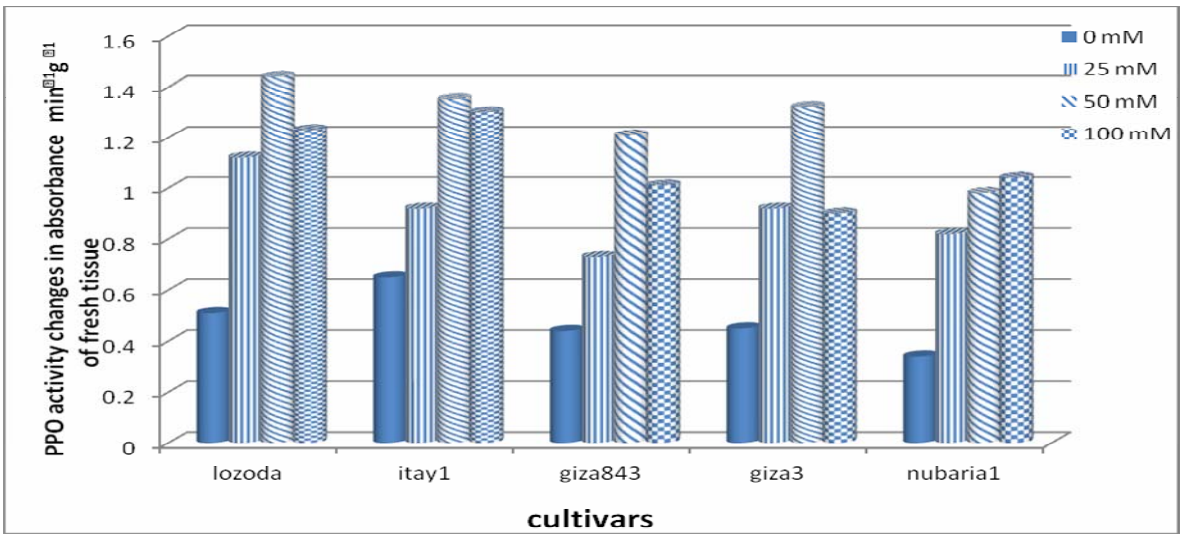

Fig. 3: Polyphenol oxidase (PPO) activity in potato tubers (Kara c.v) after treatment with NaCl concentrations.

3. Determine the optimum NaCl concentration for POD and PPO activities throw the Addition of NaCl concentration in reaction of enzyme

The addition of sodium chloride increases the POD and PPO activities over that for the enzyme alone. The optimum NaCl concentration for POD and PPO activities were at 25 mM and 0 mM in all cultivars accept Nubaria1 the high activity was at 50mM. A higher concentration of NaCl decreased the relative activity (Fig 4and 5)

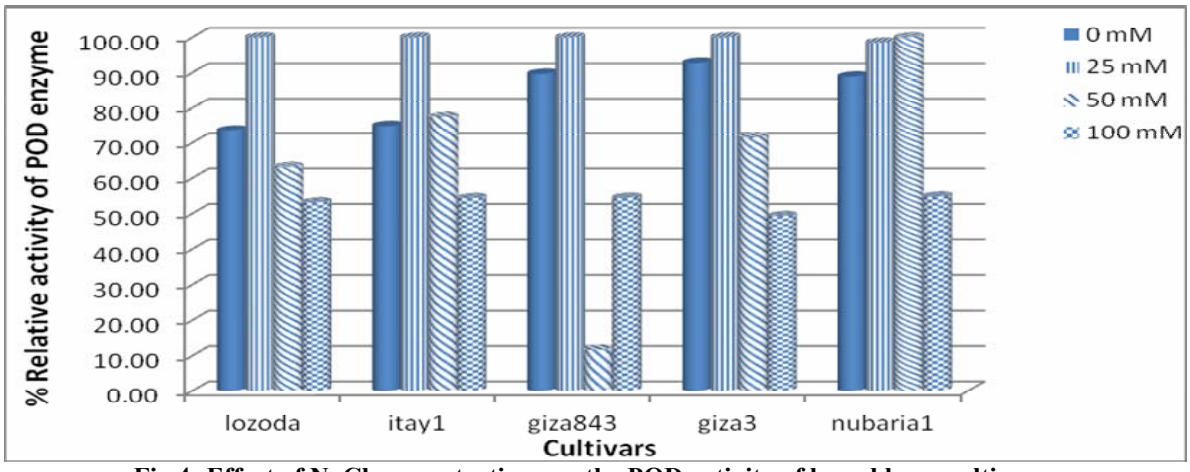

Fig 4: Effect of NaCl concentrations on the POD activity of broad bean cultivars.

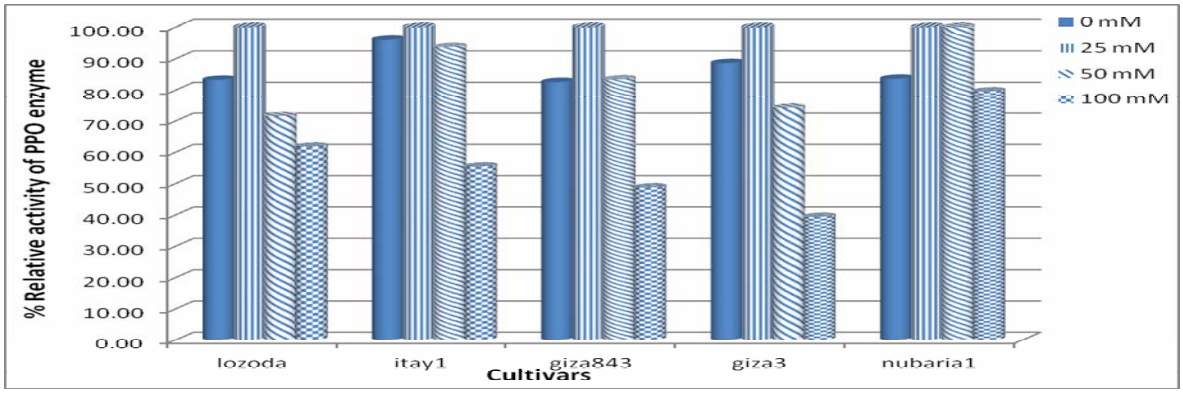

Fig 5: Effect of NaCl concentrations on the PPO activity of broad bean cultivars.

4. Determination of POD and PPO genes expression using RT-qPCR

The level expression of POD gene in potato leaves of lozoda and Nubaria1 were high gene expression at 50 Mm followed by 25mM,while giza843 and giza3 were high gene expression at 25 Mm followed by 50mM. Itay1 was high gene expression at 25 Mm followed by 100mM. lozoda and Itay1 were high gene expression, while giza843 and giza3 were moderately gene expression. Nobaria1 was low POD gene expression.

The level expression of PPO gene in brood been leaves of lozoda and giza3 were high gene expression at 25 Mm followed by 50mM,while giza843 and Nubaria1 were high gene expression at 50 Mm followed by 100mM. Itay1 was high gene expression at 100 Mm followed by 25mM. lozoda and Itay1 were high gene expression, while giza843 and giza3 were moderately gene expression. Nobaria1 was low POD gene expression

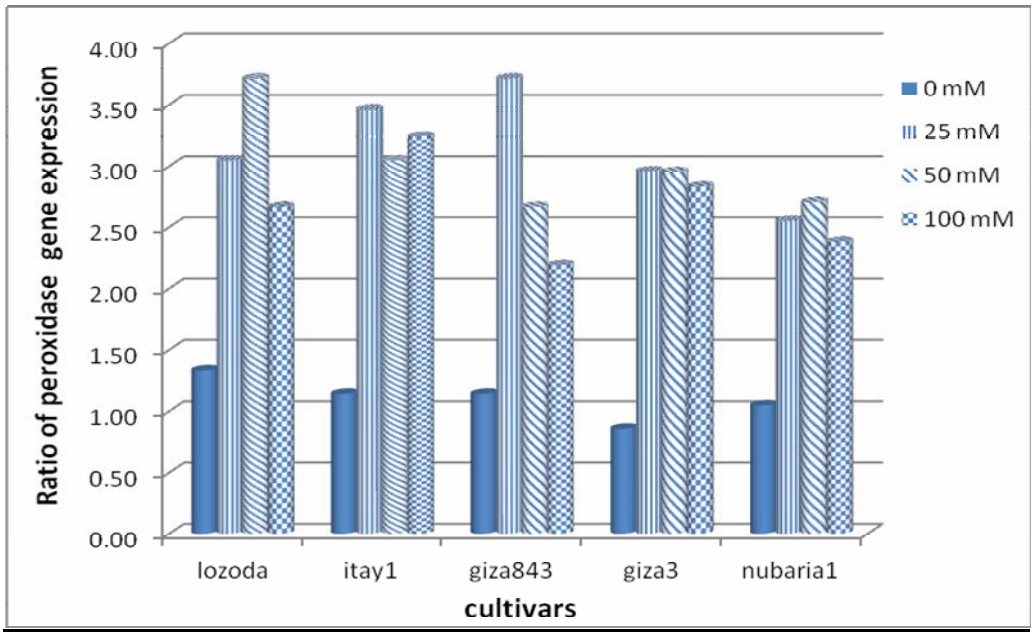

Fig. 6: Peroxidase (POD) gene expression in broad bean cultivars after treatment with NaCl concentrations.

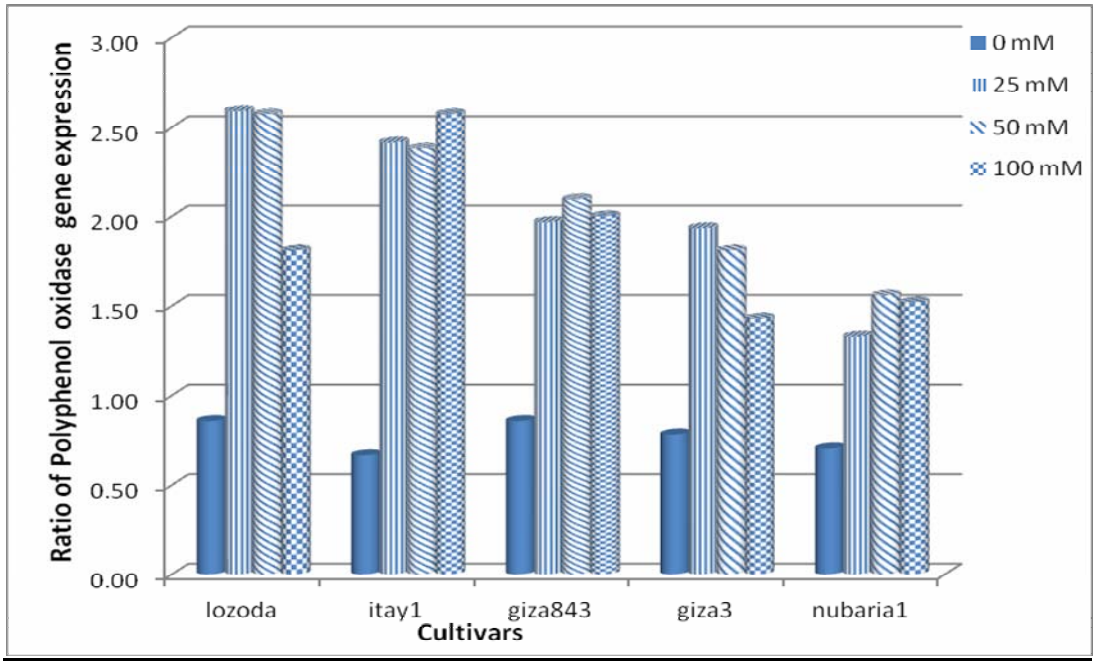

Fig. 7: Polyphenol oxidase (PPO) gene expression in broad bean cultivars after treatment with NaCl concentrations

DISCUSSION

Plants differ greatly in their tolerance of salinity, as reflected in their different growth responses. The variation of salinity tolerance in dicotyledonous species is greater than of monocotyledonous species. Most legume species are sensitive to salinity (Munns et al., 1999). The increase of salt concentration in the growth media decreased the whole plant fresh weight of all broad bean cultivars. These results agree with many studies have pointed to the negative effect of salt stress on fresh and dry weight. These include a study by Jamil et al. (2007) on radish plants Raphanus sativus L., a study by Ha et al. (2008) on Kyllinigia peruviana L., a study by Rui et al. (2009) on Bruguiera gymnorrhiza L., and finally a study by Memon et al. (2010) on Brassica campestris L.

Salt stress in V. faba plants induced activation of antioxidant enzymes, such as SOD, POD and Ascorbate peroxidases (APX), in the leaves. These results are in agreement with those of Hassanein *et al.* (2009), who observed that salt stress increased the activities antioxidant enzymes in leaves of Zea mays plants. Increased activity of these antioxidant enzymes is considered to be a salt-tolerance mechanism in most plants (Ashraf, 2009; Hu *et al.,* 2012). Previous studies showed that salt-tolerant cultivars generally have enhanced or higher constitutive antioxidant enzyme activity under salt stress compared with those of salt-sensitive cultivars. Such a trend has been demonstrated in numerous plant species, such as tomato (Mittova et al., 2004), Crithmum maritimum (Amor *et al.,* 2006), Azolla (Masood et al., 2006), and Medicago truncatula (Mhadhbi *et al.,* 2011). However, the present results showed that salt stress caused a increase in POD and PPO activities with increasing NaCl concentrations ,while the activity decrease at high NaCl concentration in all broad bean cultivars except Nubaria1 cultivar. The salt-tolerant cultivars (lozoda and Itay1) generally have enhanced or higher constitutive antioxidant enzyme activity under salt stress compared with those of salt-sensitive cultivars (Nubaria1)

Salt tolerance is a complex trait involving the function of many genes (Flowers, 2004; Foolad, 2004). In fact, the exploitation of natural genetic variations and the generation of transgenic plants introducing novel genes or altering expression levels of the existing genes are being used to improve salt tolerance (Austin, 1993; Jain and Selvaraj, 1997; Yeo, 1998; Hasegawa *et al.,* 2000; Park *et al.,* 2001; Xiong and Yang, 2003; Guo *et al.,* 2004; Davletova *et al.,* 2005; Dana *et al.,* 2006; Hong and Hwang, 2006. The constitutive high expression of certain stress response genes in plants able to thrive in a particular stress environment emerges as a possibly widespread adaptive mechanism. This would imply that rather than the expression of particular species-specific stresstolerance genes, it is the altered regulation of conserved genes that enables certain plants to survive in harsh environments. The present results showed that increase of POD and PPO gene expression with increasing NaCl concentrations but decrease at high concentrations of salt stress in all broad bean cultivars. POD and PPO gene expression were high in lozoda and Itay1 cultivars, while giza843 and giza3 were moderately POD and PPO gene expression. Nubaria1 was low of POD and PPO gene expression under salt stress.

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الملخص العربى

تأثير الملوحة على التعبير الجينى ونشاط الانزيمين POD وPPO على اصناف الفول

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تعتبر الملوحة من اكثر الاجهادات الغير الحيوية التى لها تاثير على المحاصيل النباتية وخاصة البقوليات.تم دراسة تأثير تركيزات مختلفة من كلوريد الصوديوم(٠، ٢٥، ٥٠ و ١٠٠ مليمول) على خمسة اصناف من الفول على الوزن الجاف والطرى للمجموع الخضري والجذري. أشارت النتائج أن زيادة تركيز الملح ادى الى انخفاض الوزن الطرى والجاف للمجموع الخضري والجذري،

وبدراسة تأثير التركيزات المختلفة من كلوريد الصوديوم على انشطة انزيمى POD و PPO في اصناف الفول. وجد ان اعلى نشاط لانزيمى ال POD و PPO كان على تركيز ٥٠ مليمول بينما انخفض النشاط بارتفاع تركيز الملوحة في جميع أصناف الفول باستثناء الصنف .Nubaria1

وبتكرار التجربة بجعل تركيز مكونات التفاعل لكلا الانزيمين نفس التركيزات وجد ان التركيز المثالى الذى يعطى اعلى نشاط انزيمى هو٠ و٢٥ مليمول فى كل الاصناف ماعدا صنف Nubaria1 كان اعلى نشاط له على تركيز ٥٠ مليمول

وبقياس مستوى التعبير الجينى للانزيمين كان اعلى مستوى تعبير جينى للانزيم POD للصنفين lozoda وNubaria1 على تركيز ٥٠ مليمول في حين كان اعلى تعبير جينى للصنفين giza843 وgiza3 على تركيز ٢٥ مليمول. وكان كان اعلى تعبير جينى للصنف Itay1 على تركيز ٢٥ ملي.

بينما كان اعلى مستوى تعبير جينى للانزيم PPO في الصنفين lozoda و giza3 على تركيز ٢٥ مليمول، في حين كان اعلى تعبير جينى للصنفين giza843 و Nubaria1 على تركيز ٥٠ مليمول. وكان اعلى تعبير جينى للصنف Itay1 على تركيز ١٠٠ مليمول. فكان الصنفين lozoda و Itay1أ على مستوى تعبير جينى في حين كانت giza843 و giza3 متوسطة التعبير الجيني. بينما الصنف Nobaria1 كان منخفض فى التعبير الجيني.