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ANALYSIS *FCPOXS* GENE EXPRESSION RESPONSIBILITY ON PEROXIDASE SYNTHESIS FOR TWO FIG CULTIVARS GROWING UNDER SALT STRESS AND TREATED WITH PROLINE AND NEUTRA-SOL

Doaa Ali Kadhem AL-hadrawi[1] and Ali Saeed Atiyah AL-Janabi[2]

[1] Ministry of Agriculture, Najaf, Iraq;

[2] Horticulture and Landscape gardening Department, Faculty of Agriculture, University of Kufa, Najaf, Iraq .

* Corresponding author: phone: +946 7806205757; ali.aljanabi@uokufa.edu.iq.

Abstract

This experiment was conducted in the wood canopy of Horticulture and forest division /Najaf ,Agricultural directorate during the season 2019-2020 on the transplants of White (Adriatic) and Black (Diyala) Figs cultivars to study the response of vegetative group of white and black fig for treated with Prolin Amino acid with concentration (0,50 and 100 mg.L^{-1}) and add organic compound Neutra-sol (Salinity therapy) for soil with concentration(0,2and 4 ml.L^{-1}) and interaction among them ,add to control treatment (Spraying and add distilled water only)under the conditions of Salinity stress. The gene expression for three peroxidase genes (*FcPOXs*) were used Real time-PCR to measurements relative quantity for two fig cultivars used (GeneX) linked with (CFX Manager software)..The *FcPOX1* gene expression the gene expression were highest in control treatment for White (Adriatic) and Black (Diyala) fig cultivars reached 32.37 and 34.00 fold respectively, so the *FcPOX2* gene expression 37.5 and 33.53 fold respectively but the lowest gene expression was in proline 50mg.L^{-1} and 2ml.L^{-1} Neutra-sol reached 16.52 fold. The gene expression in White (Adriatic) and Black (Diyala) fig cultivars reached for *FcPOX3* pattern almost equal.

***Keywords*:** Peroxidase gene, Salt stress, Gene expression, Prolin

Introduction

Ficus carica belongs to the Moraceae family. It contains more than 140 cultivar categorized in 40 genera (Watson and Dallwitz, 2004). Figs have been cultivated for a long time in different places around the world as edible fruit. It originated in Western Asia and spread to the Mediterranean and some countries of the world. The most productive countries of edible figs are Egypt, Turkey, Morocco, Spain, Greece, California, Italy, Brazil, and other places characterized by mild winter and hot dry summer (Tous *et al.,* 2010). The fig tree (*F. carica*) is cultivated for its fruit in temperate zones, and has been investigated for its enzymes, organic compounds, and natural rubber (Kang *et al*., 2000). Despite its high agricultural and economical value, little attention has been given to investigate the physiological and biochemical traits of the fig tree. Salt tolerance is a complex physiological and multigenic trait. The most important problems for current agriculture is the salinity of irrigated soils. It is estimated that over 6% of the world's total surface area and about 20% of irrigated lands are affected by salinity1 and more than 75 countries are facing salinity problems (Tous *et al.,* 2010). In addition, low precipitation, irrigation with brackish water, and inadequate farming practices are causing an expansion of saline areas by about 10% per year. As water consumption increases around the world, degradation of surface and ground-water quality is inevitable (Jensen *et al*., 1990) report that about one half of the world's irrigation systems are seriously affected by salinity. Moreover, no reports to identify and characterize the genes expressed in *F. carica* have been published in the literature. Consequently, crop production with an appreciable yield becomes a challenge under these conditions. The ability of plants to modify their behavior appropriately in response to salt stress is a major factor in their adaptation to this specific constraint. To date, environmental constraints, including salinity, become more and more unfavorable especially for glycophytes such as grapevines (Hend *et al.*, 2011). Those features are frequently reported as discriminators between salt-tolerant and salt-sensitive cultivars (Daldoul *et al*., 2012). One of the strategies adopted in overcoming salinity is the use of tolerant genotypes through the characterization of local genetic resources and the selection of potential tolerant genotypes (Daldoul *et al*., 2010). Plant response to salt stress occurs at various levels: molecular, cellular and physiological (Yamaguchi-Shinozaki *et al*., 2002). Tolerance to abiotic stresses is a complex feature influenced by the coordinated and differential expression of a group of genes (Chen *et al*., 2002). In general, several modifications are expected to be activated as a response to abiotic stresses (Jain *et al*., 2001). Salinity induces osmotic stress, ionic imbalance, ion toxicity and nutrient deficiency regarding plant growth (Pardo, 2010). Also salinity is involved as an oxidative stress which produces reactive oxygen cultivar (free radicals) like superoxidase, hydroxyl radical , hydrogen peroxide and singlet oxygen that involved in promoting membrane lipid peroxidation as well as membrane leakage (Ashraf, 2004), and these reactive oxygen cultivar finally scratch chloroplast and mitochondria by distracting their cellular structures (Mittler, 2002). Furthermore, growers have mistakenly believed that date palm does not require much attention, while the successful orchard management practices are the way to high yield of good fruit quality. One of the best tools of horticultural practices is fertilization. The use of fertilizers to increase yield is an important factor in all agricultural systems (Dong *et al*., 2005).

Proline accumulation is a common physiological response to salinity and osmotic stress in many plants cultivar (Ashraf and Foolad, 2007). Proline is mainly synthesized from l-glutamic acid (Glu), which is reduced to

glutamate semialdehyde (GSA) by pyrroline-5-carboxylate synthetase (P5CS); next, GSA spontaneously cyclizes to form P5C. P5C reductase (P5CR) further reduces the P5C intermediate to Proline. This pathway is found in the cytosol and in plastids (Hu *et al*., 1992; Verbruggen and Hermans, 2008). Formation of GSA/P5C from ornithine (Orn) via orn--aminotransferase (OAT) was postulated to constitute an alternative pathway of Proline synthesis and accumulation (Roosens *et al*., 1998; Verbruggen and Hermans, 2008). There is also evidence for a pathway in which OAT does not seem to contribute to Proline biosynthesis, but generates P5C, which is used for the production of glutamate (Funck *et al*., 2008). Pro is catabolized to Glu in mitochondria by Pro dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) (Hu *et al*., 1992; Verbruggen and Hermans, 2008). Moreover, Proline is thought to be a component of the antioxidative defense system, a regulator of cellular redox potential, a stabilizer of subcellular structures and macromolecules or a component of signal transduction pathways that regulate stress-responsive genes (Szabados and Savouré, 2010; Székely *et al*., 2008). Proline accumulation during osmotic stress is due to increased synthesis and reduced degradation (Rentsch *et al*., 1996; Verbruggen and Hermans, 2008). Proline accumulation, various abiotic and biotic stresses also activate endogenous production of reactive oxygen cultivar (ROS). Although most of them induce cellular damage, it has been demonstrated that hydrogen peroxide can play an important role as a signaling molecule that triggers the acclimation to adverse environmental conditions (Rejeb *et al*., 2014). The accumulation of Proline in the course of osmotic stress is partially due to ABA and H2O2 signaling (Savouré *et al*., 1997; Rejeb *et al*., 2014; Kumar, 2019).

Organic agriculture is an ecological management system that promotes and enhances biodiversity, biological cycles and soil biological activates. It is based on the minimal use of off–farm and chemical inputs and management practices that restore maintain and enhance ecological harmony. Therefore, a great attention has been paid to using the natural source of nutrition as an alternative to the mineral fertilization, but organic fruit growers have little experience with stone fruits. However, (Zhou, *et al*., 2001). Organic fertilizers improve the physical, chemical and biological properties of nearly all soil types, adjusting soil PH, increasing soil solubility and production of the plants. Adding organic fertilizers not only increase the organic matter in the soil but also increase the available phosphorus and the exchangeable potassium, calcium, and the other micro-elements, through its effect on soil pH, encourages proliferation of soil microorganisms, increases microbial population and activity of microbial enzymes, viz. dehydrogenase, urease and nitrogenase,peroxidase and catalase (Abou Hussein *et al.,* 2002 and Zohar *et al.*, 2015).

Fruit trees usually show a greater sensitivity to salinity than annual crops. Among fruit trees of the temperate zone, Fig. (*Ficus carica* L.) is reported to be moderately resistant to salinity(Ayers and Westcost, 1976). Recent work showed that fg leaves remained healthy and green upon root exposure to 100 mM sodium chloride (NaCl) for a few weeks, and their gas exchange parameters still remained relatively high (Caruso, 2017). However, concentrations ≥200 mM NaCl resulted in extensive leaf necrosis, leaf abscission, and a dramatic decrease in leaf photosynthesis (Caruso, 2017). Fig trees are also quite resistant to drought and perform well under conditions of moderate summer deficit, mostly in semi-arid climates of the Mediterranean region, Middle East and Asia. Although rain fed cultivation is commonly practiced, growth and productivity of fig trees respond positively to irrigation (Tapia *et al*, 2003). There is currently no information on the molecular response of fig trees to salinity. Considering the relatively modest genetic improvements of current fig cultivars over natural varieties, a genomic approach may be useful to speed up breeding programmers. Recently, drafts of the genome sequence of *F. carica* were published (Barghini *et al.*, 2017 and Mori *et al.,* 2017). By analyzing the gene encoding portion of the genome, genomic DNA-derived transcriptomes were reported (Mori *et al.,* 2017; Solozano *et al*., 2017 and Usai *et al.,* 2017). Mori *et al*. (2017) provided some validation of their genomic-derived transcriptome using libraries of cDNA derived from a number of organs. De novo transcriptome sequencing has also been reported in a few other studies (Cao *et al.,* 2016; Feiman *et al.,* 2014 and Ikegami *et al.,* 2013). Despite the growing interest in this crop, related to its potential use in marginal areas and to the nutraceutical value of its fruits (Kim *et al.,* 2003), there is little information that quantifes its short or long term resistance to saline stress. Understanding the molecular basis of fig salt tolerance could be useful in addressing genetic improvement for the selection of highly tolerant genotypes. Moreover, identifying genes involved in salt tolerance in a mid-to-high tolerant tree cultivar is a prerequisite to targeting such genes at the biotechnological level (e.g., by gene editing) in order to study salt tolerance in two cultivars of fig this objective of the present study was to identify peroxedase genes of fig plants affected by salt strees of irrigation and treated with proline and Neutra-sol (organic compound Salinty therapy).

Materials and Methods

Plant material

This investigation was conducted during the growing season of 2019, the fig transplants cultivars White Adriatic and Aswad Dialah developing in orchid that affiliate for ministry of agriculture, Iraq-Najaf in plastic pots (20 cm diameter) irrigated with water salinity, its source artesian well. Soil and water salinity characteristic showed in Table1. The experimental was arranged as Randomized Complete Block Design (R.C.B.D) with tow variables, spraying proline 0, 50 and 100 $mg.L^{-1}$ and soil add Neutra-sol (organic compound Salinty therapy,) 0, 2 and 4 $ml.L^{-1}$ on two fig cultivars with three replicates/cultivars as block every block have 9 treatments and every treatments have five transplants, so each block have 45 Fig. transplants, so the total transplants for three block were 135 transplants each one cultivars, that mean 270 transplants for two cultivars.

Gene expression measurement

Total RNA extraction and cDNA synthesis : Total RNA for samples were isolated from different treatment leaves of fig cultivars by uses (SV Total RNA Isolation kit/ Bioneer. Korea). The quality of RNA was verified by demonstration of intact ribosomal bands following agarose gel electrophoresis. DNA was removed from RNA samples using the DNase I Mix/ Promega, USA (DNase I, MnCl2, yellow core buffer). First strand cDNA was synthesized from (16 µl) of total RNA using the (power cDNA Syntheses kit/IntronBio. Inc. USA) with Oligo (dT) 15 primer,

following the manufacturer's instructions and quantified using gel electrophoresis.

Quantitative Real Time PCR (qRTPCR): Gene expression analyses were performed by qRT-PCR using a Mini Option's System real-time PCR and GO Taq Master Mix SYBR Green kit QPCR/ IntronBio. Inc. USA. Primers for gene specific amplification of Mulberry were designed to generate a product of 1113 bp, the primers are available in Gen Bank (www.ncbi.nih.nlm.gov/ Gen Bank/EMBL/DDBJ. the primers sequences *FcPOXs* gene responsible on peroxidase. Primers for QRT-PCR amplification were two parts *FcPOX1* (Forward 5- TGATCTGGTTGCATCTCTCGG -3) (Reverse 5-TGTCGTGTTCAATGTGGGGT-3) *FcPOX2* Forward 5-TCGCCTTCATTTCCACGACT -3) (Reverse 5-ACGTTGAAACCTCTTGCGGA -3) and *FcPOX3* Forward 5- TGATCTGGTTGCACTCTCGG -3) (Reverse 5-TGTTCAATGTGGGGTCAGGG 3) T.m (59.75-59.89) (59.82-60.18) and (59.75-59.89), GC %55. PCR reactions were carried out in duplicate in plate. Reaction mix (22.5 µl per well) contained 12.5 µl, Master Mix SYBR Green, 2.5 µl forward and reverse primers,7.5 µl DEPC-D.W and 2.5 µl of c DNA. The thermal cycling conditions consisted of an initial denaturation step of 95 ºC for 10 min, followed by 40 cycles of 95 ºC for 30 s, 60 ºC for 1 min, and 72 ºC for 30 s. The specificity of the PCR amplification was monitored by melting curve analysis following the final step of the PCR products were also checked for purity by agarose gel electrophoresis. The housekeeping F. carica gene (Fc18S) was used to normalize as endogenous reference (Forward 5-AGGTTCGTAGTGAACCGCTG -3)(Reverse 5-TTCGTTTACTCAGGGGGTGC -3), T.m (60.04-59.82), GC %55. The real-time PCR data were analysis by GeneX programs. The transcript levels of 3 genes Measured after 7 days treatments with prolin and Neutra-sol and interaction add control. Relative gene expression values were compared between non-treated samples (control) versus treated samples (prolin and Neutra-sol)under water salinity. As housekeeping genes was FcrRNA 18S sequence, which showed constitutive expression profiles. qRT-PCR was performed on 3 biological and 3 technical replicates for each treatment. Relative abundance of transcripts was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Results

Total RNA extraction and cDNA synthesis

Figure (1) showed that success of RNA extraction from leave fig cultivars for all treatments which used (Total RNA Isolation kit)were high efficiency and gave RNA concentration ranged between 77ds to 81ds with purity reached 1.9 to 2.1. The synthesis cDNA from RNA of leave fig cultivars treatments were success because used (power cDNA Syntheses kit) that was high efficiency and specific (Figure 2).

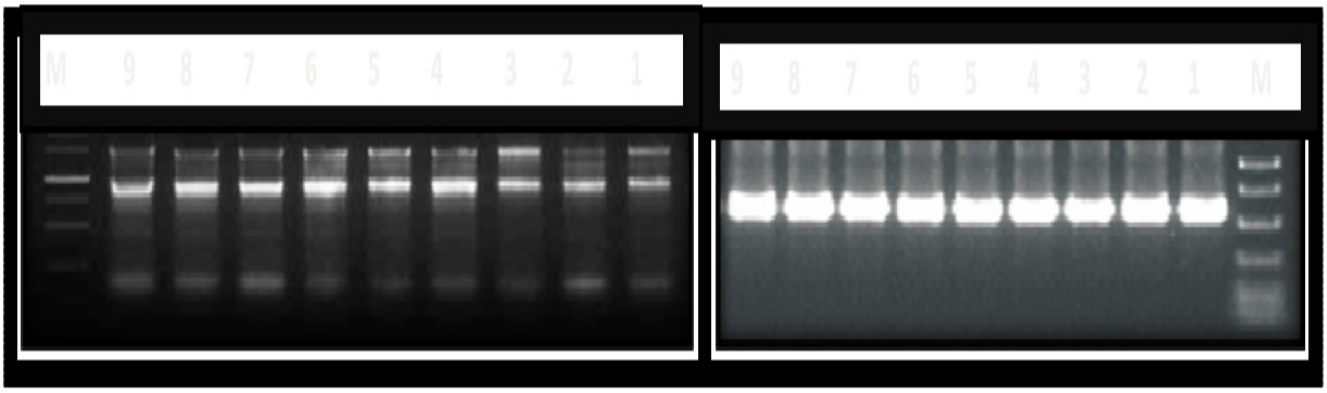

Fig. 1 : Represents extraction total RNA of the fig leave cultivars(Black right and White left) on agarose gel (1%) and voltage (100 V) for (20 minutes): M = Marker Leader : 1 = Control , 2 = proline50mg.L-1, 3 = proline100mg.L-1, 4 = neutral-sol 2ml.L-1, 5 = neutral-sol 4ml.L-1 , 6= proline50mg.L-1+ neutral-sol 2ml.L-1 , 7 = proline50mg.L-1+ neutral-sol 4ml.L-1 , 8 = proline100mg.L-1 + neutral-sol 4ml.L-1, 9 = proline100mg.L-1 + neutral-sol 4ml.L-1.

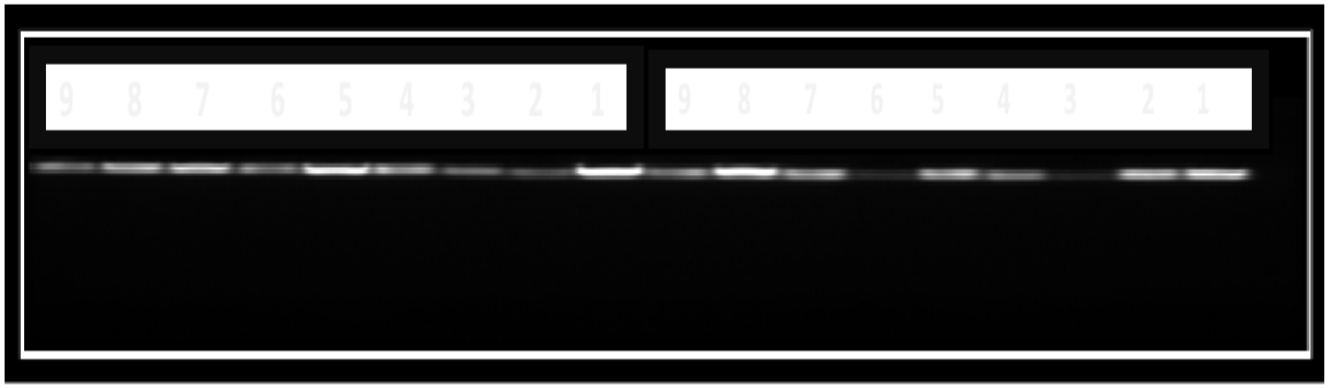

Fig. 1 : Represents cDNA from RNA of the fig leave cultivars (Black on right and White on left) on agarose gel (1%) and voltage (100 V) for (20 minutes): M = Marker Leader : 1 = Control , 2 = proline50mg.L-1, 3 = proline100mg.L-1, 4 = neutral-sol 2ml.L-1, 5 = neutral-sol 4ml.L-1 , 6= proline50mg.L-1+ neutral-sol 2ml.L-1 , 7 = proline50mg.L-1+ neutral-sol 4ml.L-1 , 8 = proline100mg.L-1 + neutral-sol 4ml.L-1, 9 = proline100mg.L-1 + neutral-sol 4ml.L-1.

Genetic expression measurement for Peroxidase1 gene (*Pox1)* The white cultivar(Adriatic) gave the highest genetic expression in the comparison transaction ("control"), reached 32.37fold followed by the same type in the treatment with proline (100 mg.L-1) at 311 The Adriatic fig was lowest genetic expression at the concentration (50mg.lner-1 + 4ml.l-1) reached (20.62fold), while the others treatments of the coefficients in the genetic expression level are graded between the highest value and the lowest value (Figure 3).

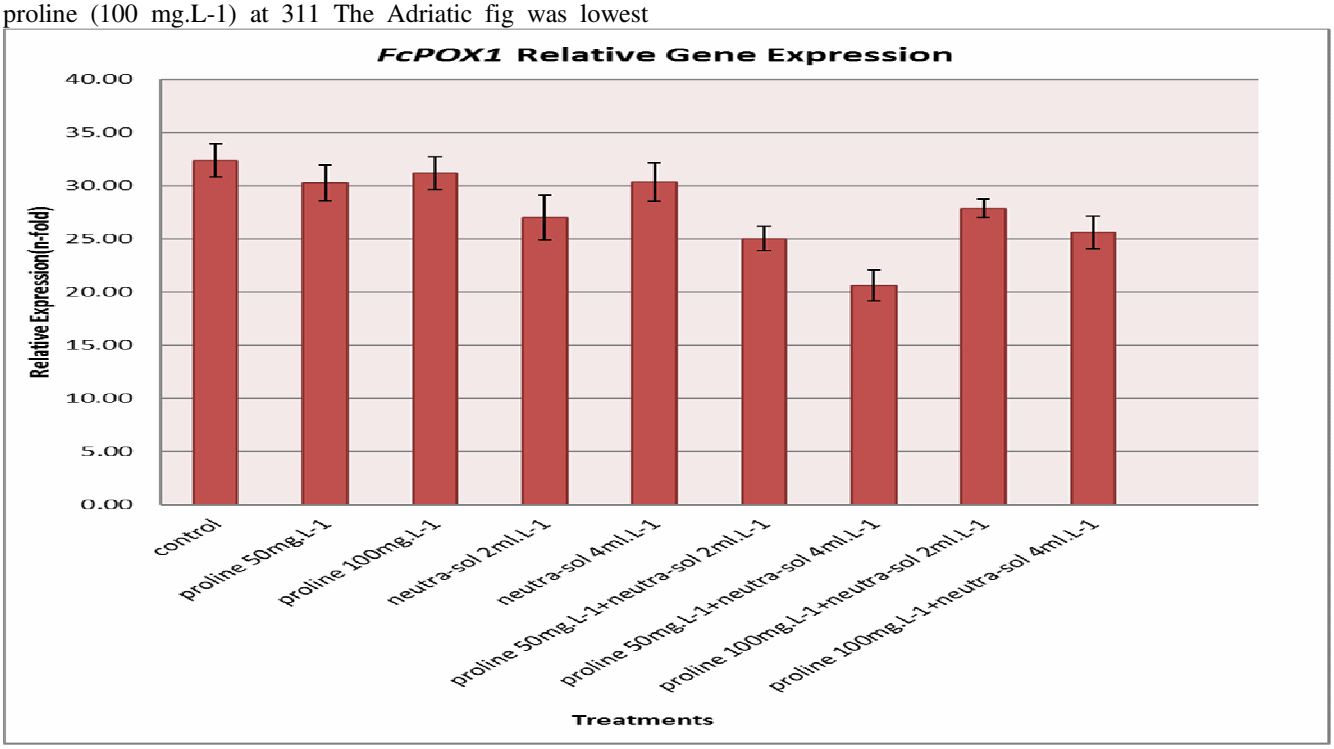

Fig. 3 : Relative gene expression of *FcPOX1*gene for white fig(Adriatic) transplants grown under salt stress and treated with proline (50 and 100 mg.L^{-1}) and Neutra-sol (2 and 4 ml.L^{-1}).

results of Figure (4) showed that an estimate of the relative expression of the Genus 1 FcPOX in the transplants of Black Fig (Diyala) cultivar under the influence of salt stress, the Black fig(Diyala) cultivar gave e highest percentage of genetic expression in control treatment reached(38.45 folds), whereas this cultivar gave the lowest genetic expression at concentration 50 mg.L^{-1} proline and 2ml Neutra-sol) (16.52 fold) The others of the coefficients at the level of gene expression were between the highest value and the lowest valu.

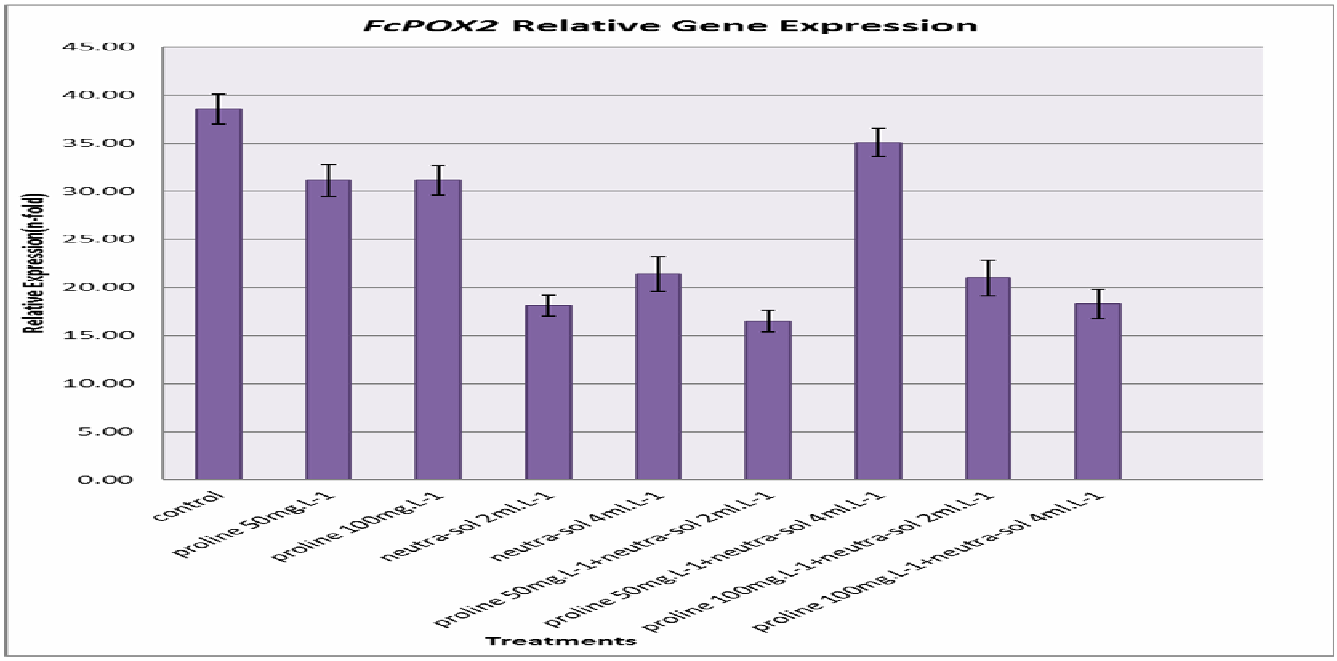

Fig. 4 : Relative gene expression of *FcPOX1*gene for black Fig. (Diyala) transplants grown under salt stress and treated with proline (50 and 100 mg.L^{-1}) and Neutra-sol (2 and 4 ml.L^{-1}).

Genetic expression measurement for Peroxidase2 gene (*Pox2)*: The results of figure (5) note that estimation of the relative expression of the *FcPOX*2 in white Fig (Adriatic) leaf under the influence of salt stress was given at a transaction (control) the highest of genetic expression, reaching (34.00 fold), followed by the same cultivar at the treatment with proline (50 mg.L^{-1}) reached (53 fold) whereas the additive gave the lowest genetic expression at the concentration of 100 mg.L^{-1}, reached 22.66 fold, the others treatments of the coefficients at the level of the genetic expression between the highest and lowest value.

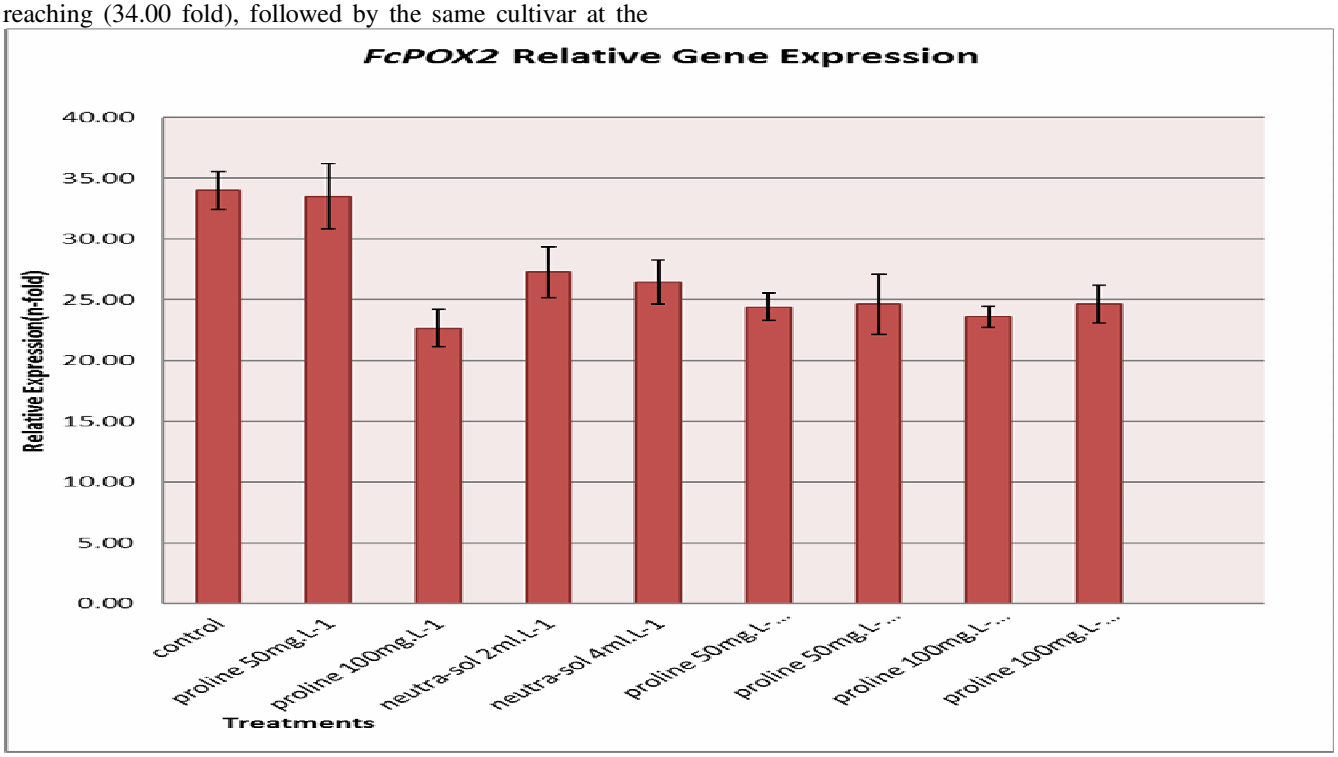

Fig. 5 : Relative gene expression of *FcPOX2*gene for white Fig. (Adriatic) transplants grown under salt stress and treated with proline (50 and 100 mg.L^{-1}) and Neutra-sol (2 and 4 ml.L^{-1})

The results of figure (6) show that the relative expression of the *FcPOX2* gene in the transplants of black fig (Diyala) cultivar under the influence of salt stress, the cultivar gives the highest genetic expression in the control, at 33.57fold, while the FcPOP2 gene has the lowest genetic expression at concentration (100 mg.L^{-1} Proline + 4 ml.L^{-1} Neutra-sol reached 17.11 fold, while the others of the treatments at the level of gene expression have been graded between the highest and lowest value .

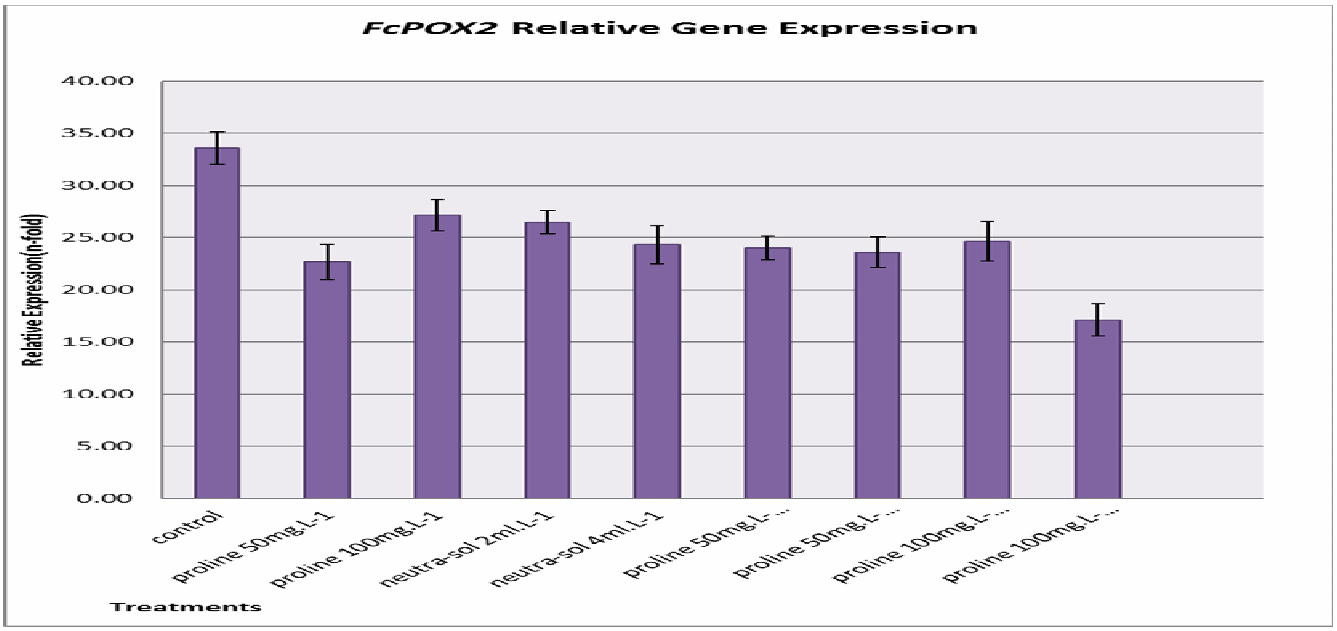

Fig. 6 : Relative gene expression of *FcPOX1*gene for black Fig. (Diyala) transplants grown under salt stress and treated with proline (50 and 100 mg.L^{-1}) and Neutra-sol (2 and 4 ml.L^{-1}).

Genetic expression measurement for Peroxidase3 gene (*Pox3)*:The analysis of the relative quantity of the *FcPOX3* gene in the transplants of white fig(Adriatec)cultivar under the influence of salt stress shows if the overlap transaction is given the highs genetic expression on a control treatment reached 23.53 fold, whereas the gene *FcPOX3* gave the lowest genetic expression at concentration of 100 mg.L^{-1}Proline and +4 ml. L^{-1}Neutra-sol) reached 13.17fold, while the others treatments level of genetic expression have graded the highest and lowest value and form indicating the relative quantity levels of genetic expression for the *FcPOX3* gene(Figure 7).

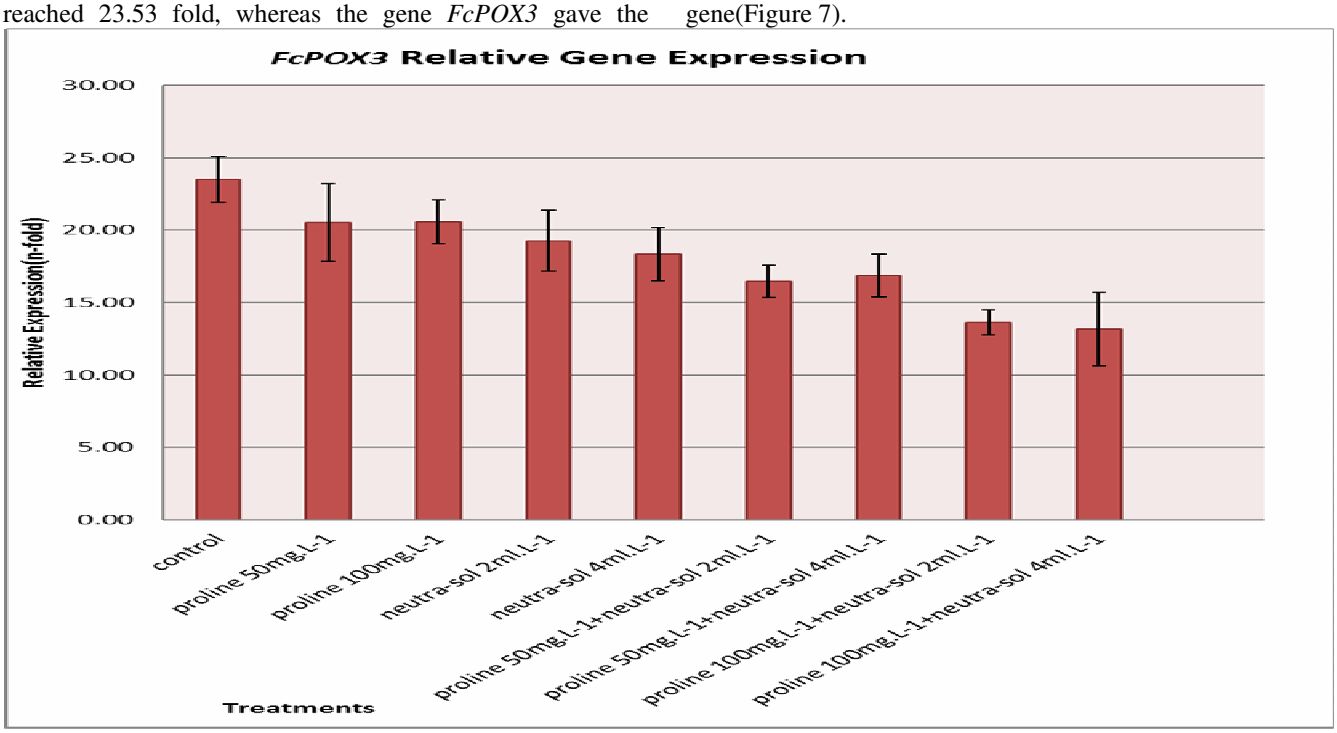

Fig. 7 : Relative gene expression of Figure(3)Relative gene expression of *FcPOX2*gene for white Fig. (Adriatic) transplants grown under salt stress and treated with proline (50 and 100 mg.L^{-1}) and Neutra-sol (2 and 4 ml.L^{-1})

The analysis of the relative quantity of the *FcPOX3* gene in the transplants of black fig (Diyala)cultivar under the effects of salt stress(Figure 8) shows that the highest genetic expression in a control treatment were given 11.53 fold followed by the same gene at a concentration of 50mg.L^{-1}proline, reached 11.51 fold, while the gene expression was lowest genetic expression at concentration (100mg.L^{-1} Proline+4.ml.L^{-1}Neutra-sol) reached 7.17fold, while the others of the treatments at the level of genetic expression are graded between the highest value and lowest value and Figure (8) indicating the relative levels of the gene expression of the *FcPOX3* gene.

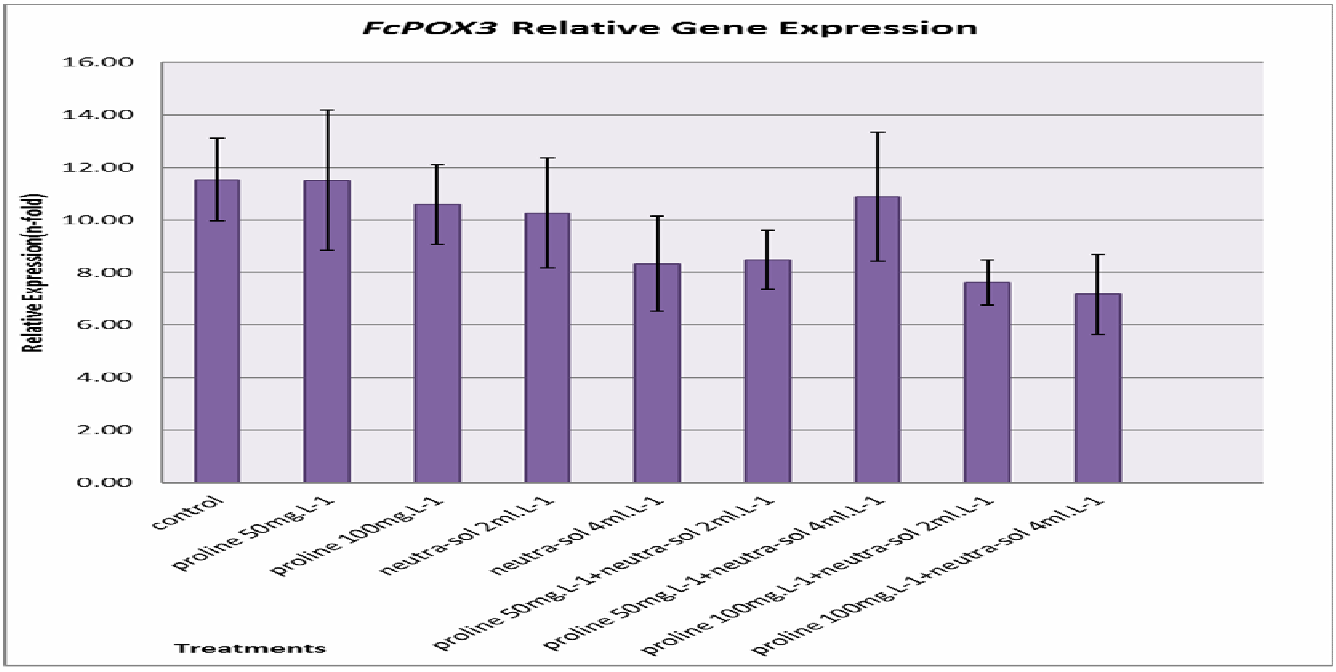

Fig. 8 : Relative gene expression of *FcPOX1*gene for black Fig. (Diyala) transplants grown under salt stress and treated with proline (50 and 100 mg.L^{-1}) and Neutra-sol (2 and 4 ml.L^{-1}).

Discussion

Plants can evolve a complex defensive mechanism to counteract the salinity effects (Fan, 2013), which includes activation of numerous signaling sensors that conclusively excites various transcription factors (TFs) to induce stress-responsive genes, which enable plants to nurture and transcend the adverse conditions. In salinity, factors involved in signaling are: (i) discerning accretion or elimination of ions to stabilize the K^+/Na^+ balance and other ion levels via salt-inducible enzyme Na^+/H^+ antiporter (V-ATPase or PPase) and K^+ and Na^+ transporters (SOS family); (ii) biosynthesis of congenial solutes to adjust the vacuolar ionic balance and restore water in the biochemical reaction (Like polyols and mannitol); (iii) adjust the cell membrane structure; (iv) synthesis of multiple resistance-oriented proteins like ROS and pathogenesis-related proteins (PR family); and (v) induction of plant hormones (ABA, JA and IAA). These biological pathways improve the inclination of salt tolerance are likely to collaborate and may have the synergistic effect ((Tester and Davenpart, 2003 and Wang *et al.,* 2018). Besides, various transcription factors (TFs), such as HD-Zip, ERF, WRKY, bHLH are known to play a vital role in regulating salt resistance mechanism in plants (Jaffar *et al*., 2016)

The physiological and morphological strategies whereby plants cope with saline stress vary across cultivar. However, in most crops high saline concentrations cause osmotic and ionic stresses, both at the cellular and whole plant level. Salt decreases water and nutrient absorption, reduces CO_2 availability due to diffusional and photochemical limitations, and modifies carbohydrate partitioning and metabolism (Niinemets and Keenan, 2014).

Photosynthetic responses to salinity include stomata closure (Chaves *et al*., 2009) and biochemical limitations that decrease mesophyll conductance, strongly limiting CO_2 diffusion into the chloroplasts (Niinemets and Keenan, 2014. and Centritto *et al*., 2003) Changes in stomata conductance and transpiration are common responses in cultivar of medium tolerance to salinity as they limit salt accumulation into the leaves (Tattini, 1995). Excluding Na^+ and Cl^- at the root level is often the main mechanism whereby plants prevent the accumulation of toxic ions in shoots, leaves, and meristems (Chen *et al*., 2018) Cells in the xylem parenchyma, cortex and pericycle can all be involved in the exclusion mechanism, but the Casparian strip in the endodermis effectively blocks the transport of Na^+ into the aerial organs, thus controlling the distribution of ions within the plant 12. When salts accumulate in plant organs they lower the osmotic potential (Gucci and Tattini, 1997). In addition, changes in carbon (C) and nitrogen (N) partitioning under salinity stress lead to increased concentrations of carbohydrates, amino acids and other metabolites, that can actively contribute to osmoregulation and protection (Gucci and Tattini 1997; Nejad and Shekafandeh, 2014).

Under saline conditions, plants have to activate different mechanisms in order to cope with the resulting stress. Such mechanisms include changes in morphology, anatomy, water relations, photosynthesis, the hormonal profile, toxic ion distribution and biochemical adaptation (such as the antioxidative metabolism response). Prevents the entry of salts into the vascular system. Na^+ and Cl exclusion by roots ensures that Na^+ and Cl does not accumulate to toxic concentrations in leaves.

Salt stress is considered as most severe abiotic stress, which impairs all principal physiological functions, including photosynthesis, lipid metabolism and synthesis of proteins (Mandhania *et al*., 2008). To confront the stress, plants are compelled to initiate protective responses, like restoring cellular ion concentrations and reducing the toxicity of ions like Na^+/H^+, K^+ and Cl^-. Moreover, the accretion of osmoprotectants and hydrophilic proteins, such as sugars, polyols, proline, glycine betaine (GB), amino acids (AA) and amines are crucial for governing the osmotic potential pressure. Also, the accumulation of ROS enzymes and antioxidants is vital to prevent tissue damage by eliminating the free radicals induced by salt stress (Carillo *et al*., 2011 and Horie, 2012).

Thus, the equilibrium between ROS production and quenching is critical under salt stress. Plants can unfold a complex antioxidative defense system to limit the oxidative damage, which mainly comprised of enzymatic antioxidant (SOD, CAT, POD and GST) and non-enzymatic antioxidants (AsA, GSH, proline and phenolic compounds) (Mandhania *et al*., 2008 and Abogadallah, 2010). In the present study, the antioxidative defense system was activated in salt-treated leaves, although SOD-related transcripts were down-regulated, while CAT, POD and GST-related transcripts were significantly up-regulated. Similar research on Pyrus pyrifolia (Liu *et al*., 2013) and Fagopyrum tataricum (Wu *et al*., 2017) depicted that over-expression of GST transcripts significantly enhances salt stress tolerance. Moreover, the enhanced activities of ROS enzymes (CAT, POD and GST) are consistent with the transcriptomic data, which symbolize their vital functions in ROS detoxification. However, CAT activity increased significantly in our findings till 36 h of salt stress but drastically decrease at 48 h, which is in agreement with the down-regulation of SOD-related transcripts in grapevine under salt stress. Zhang *et al*. and (Yan *et al*., 2015) reported that salt stress up-regulates the expression of CAT, POD and GST and increases the corresponding enzymes activities. Similarly, the complex accumulation pattern of antioxidant enzyme activities was observed in our findings, which is consistent with the findings of grapevine (Baneh *et al*., 2013) and soybean (Weisany *et al*., 2012) under salt stress.

Salinity tolerance is positively correlated with the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPX), ascorbate peroxidise (APX), and glutathione reductase (GR) and with the accumulation of nonenzymatic antioxidant compounds salt stress induces the increased activities of SOD, CAT, Ascorbate peroxidase and GR in Karna khatta having higher antioxidant capacity to NaCl (Yan *et al*., 2015).

Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt. Salt tolerance is a complex trait involving several interacting properties. When salts accumulate in plant organs they lower the osmotic potential (Gucci *et al*., 1997). In addition, changes in carbon (C) and nitrogen (N) partitioning under salinity stress lead to increased concentrations of carbohydrates, amin oacids and other metabolites, that can actively contribute to

osmoregulation and protection (Gucci and Tattini, 1997; Gucci *et al*., 1997 and Nejad, R.A. and Shekafandeh, 2014).

In most tree cultivar, root growth is inhibited as salt reduces water uptake and inhibits the absorption of K^+, Ca^{2+} and NO^{3-} by roots. These primary stresses induce the generation of reactive-oxygen-cultivar (ROS) in the plant (Gill and Tuteja, 2010 and Meloni *et al*., 2003) may cause hormonal changes (Munns, 2002) and result in alterations in carbohydrate metabolism (Gao *et al*.,1998). The consequences of these metabolic modifications are a decrease in cell division and the acceleration of cell death (Hasegawa *et al*., 1998). Osmotic stress also reduces the expansion of radical tips, growth and expansion of new leaves, and induces stomatal closure (Munns and Tester, 2008). In halophytes and other tolerant cultivar salts can be extruded out of the leaf tissue via specialized structures such as glands and trichomes (Gravano *et al*., 2008).

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