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RESEARCH ARTICLE

ROLE OF HYDROGEN PEROXIDE PRETREATMENT ON DEVELOPING ANTIOXIDANT CAPACITY IN THE LEAVES OF TOMATO PLANT (Lycopersicon esculentum) GROWN UNDER SALINE STRESS

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Abstract

Salinity stress had adverse effects on biomass yield and water content (succulent value and relative water content) in the tomato leaves. Foliar hydrogen peroxide, malondialdhyde and phenolic contents as well as superoxide dismutase, catalase and guaiacol peroxidase activities significantly increased with increase salinity levels . In contrast, the activities of ascorbate peroxidase , glutathione reductase and polyphenol oxidase as well as ascorbate and glutathione fractions were significantly decreased. Pretreatment of tomato seeds with hydrogen peroxide resulted in a significant increase of non-enzymatic and enzymatic antioxidants, except superoxide dismutase, in the leaves under saline conditions. There were four stress protein bands of M.M. 88.8, 60.3 ,29.1 and 19.6 kDa appeared under salt stress, while pretreatment with H_2O_2 resulted in synthesis of only one stress protein band with M.M 17.9 kDa in the NaCl-stressed leaves . The current of this study may suggested that the primarily prominent hydrogen peroxide pretreatment appears to play a role in enhancement of scavenging the generated reactive oxygen species under saline conditions.

INTRODUCTION

Salinity is generally detrimental agent to plant growth, adversely affects plant metabolism and causes important modifications in growth (Kong-Ngern et al., 2005). One of the biochemical changes occurring when plants are subjected to harmful stress conditions is the generation and accumulation of reactive oxygen species (ROS) (Neill et al., 2002a; Ashraf and Harris, 2004). These destructive reactive compounds could cause oxidative damage to protein, DNA mutation and per oxidation of lipid structures (Ashraf and Foolad, 2005) .

Hydrogen peroxide is potentially reactive oxygen, but not a free radical. By comparison with superoxide (O2•–) and hydroxyl radical (OH•), H_2O_2 is relatively safe in the absence of transition metals (such as Fe) because it is stable and unreactive even at high concentrations (Halliwell et al., 2000). The synthesis of H_2O_2 is regulated via several routes in plant cells. Electron transport processes such as photosynthesis and respiration generate basal levels of H_2O_2, which increase in response to stress. Enzymatic sources of H_2O_2 include NADPH oxidase, cell wall peroxidases, amine oxidases and other flavin containing enzymes were shown in several plants(Neill et al., 2002a ; Desikan et al., 2003). Available information suggest that H2O2 directly regulates the expression of numerous genes, some of which are involved in plant defense and the hypersensitive response (Kovtun et al.,

2000), antioxidants, cell rescue/defense proteins and signaling proteins such as kinase, phosphatase and transcription factors (Hung et al., 2005). Murphy et al. (2002) stated that exogenous application of H_2O_2 increases chilling tolerance by enhancing the glutathione level of mung bean seedlings. Azevedo Neto et al. (2005) reported that addition of H_2O_2 to the nutrient solution induces salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in maize leaves and roots .

One of the most crucial functions of plant cells is their activity to respond to fluctuation in their environment. Salt-induced proteins have been isolated and identified in many plant species (Ali et al., 1999; Mansour, 2000). Omran (2000) reported that the polypeptides are altered by salt stress at the level of their mRNA transcription and/or translation. In addition, the disappearance of certain polypeptids may be related to increase in RNAase activity (Bewly and Oliver, 1983), to inhibit mRNA transcription and repression of their synthesis as well as to differential turn-over (Riccardi et al., 1998).

It has been reported that several peroxidase isoenzymes are quantitatively measured in numerous plant species and organs (Wu and Cao, 2008; Güçlü and Koyuncu, 2012). Gökbayrak et al. (2007) stated that peroxidase isoenzymes mediate the polymerization of cinnamic alcohols to lignin and also the bonding of lignin to carbohydrates. Cao et al. (2005) revealed that the bush plant Cucurbita moschata was a gibberellin-responsive mutant, and so differences in peroxidase levels and isoperoxidase patterns may reflect the action of gibberellic acid on development.

The aim of this study was to explore the effect of NaCl stress on some growth parameters, changes in biochemical reactions and regeneration of reactive oxygen species (ROS) which indicated by accumulation of H_2O_2 and MDA in tomato leaves. Furthermore, to evaluate the role of pretreatment by H_2O_2, as bioactive molecules, on the regulation of accumulative mechanism, including non-enzymatic , enzymatic antioxidants and the synthesis of variable protein bands and change in peroxidase isoenzyme profiels in stressed and control tomato leaves.

Materials and Methods

Sterilization and germination of seeds

Tomato seeds (Super Strain B) were selected for uniformity of size, shape and color. Prior to germination, seeds were surface sterilized by soaking for two minutes in 4% (v/v) sodium hypochlorite, then washed several times with distilled water.

The sterilized tomato seeds were soaked for 8 h in distilled water or 50 µM H_2O_2 , then transferred to plastic pots (15 cm in diameter, 20 cm length with a hole at the bottom) filled with fixed amount of mixture of previously acid-washed quartz sand and clay soil in a ratio of 2:1. Five seeds were germinated in each pot and the pots were placed under natural environmental conditions (photoperiod, 16L/8D light/dark; temperature, 27±2°C light/23±2°C dark; light intensity PPFD, 23 µmol m-2s.-1) with 80% water holding capacity. The pots were irrigated using 1/10 strength modified Hoagland solution recommended by Epstein (1972) every two-day interval with distilled water. After twenty-two days from the beginning of the experiment, the pots were divided into four sets and irrigated as following: set A, soaked seeds in distilled water and irrigated with 1/10 strength modified Hoagland solution described as control; set B, soaked seeds in distilled water and irrigated with 1/10 strength modified Hoagland solution supplemented with 100 mM NaCl described as NaCl-salinized treatment; set C, soaked seeds in 50 µM H_2O_2 and irrigated with 1/10 strength modified Hoagland solution described as pretreated H_2O_2 and set D, soaked seeds in 50 µM H_2O_2 and irrigated with 1/10 strength modified Hoagland solution supplemented with 100 mM NaCl described as H_2O_2+NaCl. The irrigation with tested nutrient solution was carried out every two days to reach 80% of water holding capacity. To prevent salt accumulation, irrigation with distilled water was done every two-day interval to reach 80% water holding capacity. The homologous plants were harvested at the end of experimental period (40 days). All the seedlings were taken carefully from the pots, washed thoroughly from adhering soil particles, blotted gently; leaves were taken and saved for determination of fresh, dry biomasses and chemical analyses.

Determination of Growth Biomass

The leaves of homologous plants (three replicates) were taken and weighed for fresh biomass. The oven-dry biomass was determined after drying the samples in an oven at 60°C till constant weight. Leaf relative water content (RWC) was determined as described by Silveira et al. (2003) based on the following equation:

$$RWC = [(f.m.- d.m.) / (t.m. – d.m.)] . 100$$

Where f.m. is the leaf fresh biomass, d.m. is the dry biomass of leaves after drying at 80°C for 48 h and t.m. is the turgid mass of leaves (after soaking in water for 4 h at room temperature).

Estimation of electrolyte leakage (EL)

Determination of electrolyte leakage were occurred according to Dionisio-Sese and Tobita, (1998). The electrolyte leakage (EL) was expressed following the formula

EL = EC1/EC2 X 100

Where EL is The electrolyte leakage

EC1 is the initial electrical conductivity

EC2 is the final electrical conductivity

Extraction and determination of total phenolic content

Extraction of the total phenolic compounds were occurred according to Malusà et al.(2006) . Total phenolic content of leaves extract was determined using a modified Folin- Ciocalteau spectrophotometric method as described by (Marigo, 1973). Phenolic concentration was calculated according to a calibration curve using ferulic acid as a standard and the results were expressed as mg gallic acid eq. g-1 d.m.

Determination of hydrogen peroxide and malondialdehyde (MDA) contents

Hydrogen peroxide content in fresh control leaves and salt-stress ones was determined according to Velikova et al. (2000). The content of H_2O_2 was calculated by comparison with a standard calibration curve using different concentrations of H_2O_2 and the results were expressed as µmol H_2O_2 g-1 d.m. The level of lipid peroxidation was measured according to the thiobarbituric acid (TBA) test, which determines the MDA as the end product of the lipid peroxidation reaction (Heath and Packer, 1968). The results were expressed as µmol g-1 d.m. No significant readings were obtained without addition of the reactive TBA.

High-performance liquid chromatographic (HPLC) method for determination of ascorbate fractions

The ascorbate fractions were extracted according to the method of Ismail and Fun (2003). The ascorbate fractions were determined by HPLC according to the method of Gahler et al. (2003). The separation and quantitative estimation were carried out using a HPLC system (Perkin Elmer series 200 LC and UV/Vis detector 200 LC. USA) equipped with a 5 µm column (Spheri-5 RP-18. 220 mm × 4.6 m`m Brownlee). The solvent used was H2O (pH 2.2 using H_3PO_4) run isocratically with flow rate 0.75 ml/min and injection volume 50 µl. The detector was set at 260 nm for the integration of peak areas after calibration with the external standard ASA and the results were expressed as µg g-1 d.m.

Extraction and determination of glutathione content

Reduced glutathione (GSH) and oxidized glutathione (GSSG) contents fresh leaves of untreated and treated plants were extracted and determined according to the florescence spectroscopy method of Hissin and Hilf (1976). All results were expressed as µg g-1 d.m.

Assays of antioxidant enzymes

Enzymes extraction method

Selected fresh leaf samples of control and treated plants were taken for enzyme extraction according to Azevedo Neto et al.(2006).

Activity measurement

Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate and the result expressed as U g-1 d.m.

Catalase (CAT, EC 1.11.1.6)

Catalase activity was measured according to the method of Beers and Sizer (1952), with minor modifications as described by Azevedo Neto et al. (2006). The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 M-1 cm-1) and the results expressed as µmol H2O2 g-1 d.m. min-1.

Ascorbate peroxidase (APX, EC 1.11.1.11)

Ascorbate peroxidase activity was assayed according to Nakano and Asada (1981). Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 mM-1 cm-1) and the result expressed in µmol H_2O_2 g-1 d.m. min-1, taking into consideration that 2 mol ascorbate are required for reduction of 1 mol H_2O_2 (Mckersie and Leshem, 1994).

Glutathione reductase (GR, EC 1.6.4.2)

Glutathione reductase activity was assayed according to Foyer and Halliwell (1976), with minor modifications as described by Azevedo Neto et al. (2006). Enzyme activity was determined using the molar extinction coefficient for NADPH (6.2 mM-1 cm-1) and expressed as µmol NADPH g-1 d.m. min-1.

Polyphenol oxidase (PPO, EC 1.10.3.1)

The polyphenol oxidase activity (PPO) was assayed as the method described by Kumar and Khan (1982). PPO activity was expressed in U min-1 g-1 d.m. One unit (U) is defined as the amount of purpurogallin formed, which raised the absorbance by 0.1 per minute under the assay condition.

Guaiacol peroxidase (GPX, EC 1.11.1.7)

Guaiacol peroxidase activity was determined as described by Urbanek et al. (1991) . Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM-1 cm-1). The results were expressed as µmol guaiacol min-1 g-1 d.m. Taking into consideration that 4 mol H_2O_2 are reduced to produce 1 mol tetraguaiacol (Plewa et al., 1991).

Determinantion of protein pattern and preparation of SDS polyacrylamide gel.

Discontinuous polyacrylamide gel electrophoresis (SDS-PAGE) was used for protein analysis according to the method adopted by Stegman et al. (1987).While SDS polyacrylamide gel was prepared according to Laemmli (1970).

Isoenzyme analysis

A combination of agar- starch- polyvinylpyrrolidone (PVP) gel electrophoresis was carried out according to the procedures described by Sabrah and El-Metainy (1985) for peroxidase isoenzyme analysis.

Statistical Analysis

All data were expressed as means of triplicate experiments. Comparisons of means were performed using SPSS 20.0 software. Data were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared by lowest standard deviations (LSD) test. Comparisons with $P< 0.05$ were considered significantly different.

Results

Changes in growth biomasses

Exposure of tomato plants to NaCl stress exhibited a significant decrease in the fresh and dry biomasses of leaves and that was accompanied with a significant decrease of succulent value (f.m./d.m.) as well as RWC of leaves. In contrast, the leakage percentage of leaves was significantly increased under salt stress (Table 1) . Pretreatment of tomato seeds with 50 µM H_2O_2 grown under saline conditions resulted in a significant maintenance of measured growth parameters, comparing to those untreated seeds and grown under NaCl stress.

Changes in H2O2 and MDA content

There was a significant increase of $H_2O_2$and MDA contents in the leaves of NaCl-salinized plants and H_2O_2-treated tomato seeds grown under saline conditions for 40-days, compared to control (Table 2). Although pretreatment with H_2O_2 resulted in a significant increase of H_2O_2 and MDA accumulation in the leaves of tomato plants grown under non-saline conditions, the attended values were significantly less than those grown under NaCl salinity alone.

Changes in total phenolic content

Irrigation of tomato plants with salinized nutrient medium exhibited a significant increase of total phenolic compounds in the tomato leaves compared to control (Table 2). At the end of experiment, the concentration of total phenolic compounds in NaCl-stressed leaves reached to 8-fold of control. Pretreatment of tomato seeds with H_2O_2 and then grown in salinized or non-salinized nutrient media resulted in a significant enhancement of phenolic compounds accumulation, compared to control, but the attended values were less than those grown in salinized medium alone.

Changes in ascorbate fractions

Exposure of H_2O_2-pretreated and untreated tomato seeds to NaCl salinity stress , resulted in a significant decrease of ascorbate fractions in the leaves except of DHASA content in H_2O_2-pretreatedseedsandgrownundernon-salineconditions.

The decrease of total ascorbate content in the leaves of H_2O_2-pretreated and untreated seeds grown under salinized conditions was related mainly to a significant decline of both reduced ascorbate (ASA) and oxidized (DHASA) forms (Table 2). This suppression was accompanied by a significant change in ascorbate oxidation potential (ASA/DHASA) . The ASA/DHASA ratio decreased from 77 in the leaves of NaCl-untreated salinized plants to 42 in the leaves of H_2O_2 – pretreated salinized plants(Table 3) . It was , also demonstrated that the ascorbate oxidation potential (ASA/DHASA) in the foliar H_2O_2-pretreated plants grown under saline conditions was significantly lower than that of control and this may related to significant accumulation of foliar DHASA.

Changes in glutathione contents

Generally, total glutathione (TG) and both oxidized (GSG) and reduced forms(GSH) in all salinized treatments (H_2O_2-pretreated or untreated) were significantly declined, compared to control. Pretreatment with H_2O_2 resulted in a significant increase of all glutathione forms in the leaves of tomato plants grown under salt stress, compared to NaCl- salinized plants. It is clearly demonstrated that (Table 2) the decline in the foliar glutathione forms under saline condition was mainly accompanied with a significant decrease of glutathione redox potential (GSH/TG) (Table 3). It is noteworthy that, the oxidized glutathione in the leaves of H_2O_2-pretreated tomato seeds grown under non saline conditions was significant higher than the control, resulting in a significantly decrease of glutathione redox potential value .

Superoxide dismutase (SOD) activity

Salinity stress with 100 mM NaCl treatment resulted in a significant increase of SOD activity in tomato leaves of H_2O_2 - pretreated and untreated tomato seeds. In contrast, pretreatment of tomato seeds with H_2O_2 and grown in absence of salinity caused an insignificant change of SOD activity, compared to control leaves (Table 4).

Catalase (CAT) activity

Similar to SOD enzyme, foliar CAT of tomato plants was significantly increased during the exposure to NaCl stress (Table 4). It is clearly demonstrated that, unsimilar to SOD activity, H_2O_2 pretreatment resulted in a significant increase of CAT activity in the leaves of tomato plants grown in non-saline medium compared to control.

Ascorbate peroxidase (APX) activity

Dissimilar to SOD and CAT, APX activity in the leaves of tomato seeds grown under 100mM NaCl condition was significantly decreased compared to control leaves . In contrast, the foliar APX activity of H_2O_2-pretreated tomato plants grown under saline or non-saline conditions was significantly higher than those of control leaves (Table 4).

Glutathione reductase (GR) activity

There was a significant suppression of foliar GR activity in the leaves of tomato seeds grown under salinity level alone; it reached to 35% of the control. Similar to APX activity , previously treated with H_2O_2 resulted in a significant increase of foliar GR activity of tomato seeds grown under saline or non saline conditions.

Guaiacol peroxidase (GPX) activity

Foliar GPX activity in tomato leaves of H_2O_2-pretreated or untreated tomato seeds grown under 100mM NaCl level was significantly increased compared to control plants (Table 4). Pretreatment with H_2O_2 resulted in an enhancement of GPX activity in the leaves of tomato plants grown in non-saline conditions .

Polyphenol oxidase (PPO) activity

Dissimilar to GPX, foliar PPO activity in tomato leaves was significantly decreased under NaCl treatment (Table 4). On the other hand, PPO activity was significantly increased in the leaves of H_2O_2-pretreated tomato seeds grown under non-saline or saline conditions. These results indicate that a defense mechanism might be enhanced by H_2O_2 via increasing the GPX and PPO activities for scavenging the generated ROS using phenolic compounds as reductant agents.

Changes in protein pattern and peroxidase isoenzymes.

In Figure 1 there are five distinct protein bands in control leaves with M.M. of 100, 50, 40, 27.3 and 25 kDa (Lane 1). Under application of salinity stress, four new stress polypeptide bands with molecular masses (M.M.) of 88.8, 60.3, 29.1 and 19.6 kDa were markedly appeared (Lane 2).

It is noteworthy that H_2O_2- pretreatment had an insignificant effect on the synthesis of protein bands of tomato leaves (Lane 4), compared to control. On the other hand, one stress protein with M.M. of 17.9 kDa appeared in salinized-H_2O_2- pretreated leaves (Lane 3), with complete disappearance of all stress proteins specific for NaCl stress (Lane 2).

Peroxidase system profiles

The zymogram (Fig 2) demonstrated that all of treatments expressed peroxidase isoenzymes at the POD-2c and POD-1a loci , while The POD-3c locus was also expressed, except in H_2O_2-pretreatment (H_2O_2) . As for POD-2a isoenzymes were completely disappeared in all treatments. The POD-3a locus was expressed only in NaCl treated leaves.

Discussion

Exposure of tomato plants to 100 mM NaCl resulted in a significant decrease of foliar fresh and dry biomasses, succulent values and relative water content (RWC) and increase of leakage value of leaves. These results might

reveal the reduction of water absorption, and disorder of plasma membrane integrity resulting in low maintenance of water content as well as inhibition of biochemical and metabolic reactions. Similarly, several studies (Rajpar et al., 2006 , Li , 2009 and Amirjani, 2010) have been reported that salt stress inhibits the growth of various plant species. Garsia-Sanchez et al. (2002) reported that the reduction in leaf growth might be due to ion toxicity, ion deficiency, ion imbalance, change in growth regulators and/or other processes such as hardening of cell wall and plasma membranes that limit cell expansion and increase leakage. Data in Table 2 show an enhancement of lipid peroxidation (as indicated by increase of MDA content) of fatty acids of plasma membranes under NaCl stress, leading to an increase of plasma membranes hardness (loss of plasma membranes integrity) and hence an increase of ion leakage and decrease of water absorption as well as retarted the growth of tomato leaves. Many authors (Parida et al ., 2002 ; Wahid et al . , 2007 ; Stoeva and Kaymakanova , 2008) reported that salt stress induces the production of ROS that is accompanied with an increase of lipid peroxidation which led to reduce membrane fluidity and selectively as well as increase of electrolyte leakage . Conversely to the inhibitory effect of salinity on growth parameters of tomato leaves, pretreatment with H_2O_2 resulted in a significant increase of fresh and dry biomasses as well as succulent values of tomato leaves under salinized conditions. This enhancement was accompanied with a marked decrease of leakage percentage of H_2O_2- pretreated tomato seeds grown under saline or unsaline conditions indicating the protective role of H_2O_2 on plasma membrane integrity via depretion or activation of antisystems under saline conditions. Murphy et al. (2002) stated that exogenous application of H_2O_2 increases chilling tolerance by enhancing the glutathione level of mung bean seedlings, while Azevedo Neto et al. (2005) reported that addition of H_2O_2 to the nutrient solution induces salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots of maize.

There was a significant accumulation of foliar H_2O_2 and MDA content of tomato plants exposed to NaCl stress revealing the dramatic oxidative damage of plasma membranes by generated ROS. These observation are in agreement with other results in several plant species including tomato (Shalata and Tal , 1998) ,wheat (Bartoli et al ., 2004) and soybean (Hu et al ., 2005). In contrast, foliar H_2O_2 content in the leaves of H_2O_2 – pretreated tomato seeds grown under unsalinized or salinized conditions was significantly lower than NaCl –salinized plants. Similarly, Wahid et al . (2007) showed that the level of H_2O_2 in wheat seedling arising from H_2O_2 – treated seeds grown under salinity was markedly lower than salinized controls. They concluded that H_2O_2 , a seed signal molecule , was evaluated as seed treatment to produce the metabolic changes regulate the expression of numerous genes and antioxidants which could lead to improve salt. Therefore, it can be concluded that H_2O_2 may act as priming regulators for a number of genes causing several changes in antioxidant systems in H_2O_2-pretreated tomato seeds, under this study , and exist in the plants to offset the inhibitory effect of oxidative stress generated by NaCl treatment . Ashraf and Foolad (2005) reported that various priming strategies including osmopriming , halopriming , hormonal priming or hydro priming …..etc , involving treatment of seeds to induce pre-germination changes for developing a defense mechanism against the exposure to environmental stresses.

It is clearly noted that a significant increase of foliar phenolic compounds of H_2O_2 –pretreated tomato plants grown under salinized and unsalinized conditions , compared to control , but the attained values were significantly lower than those recorded in NaCl – salinized plants . At the same time , there was a significant decrease of H_2O_2 and MDA contents associated with an increase of GPX and PPO activity(Table 4). Ashraf et al . (2010) and Sorour (2010) reported that phenolics involved in the mechanism of tolerance as scavenger of ROS which resulted in reduction of membrane damage and hence improved the growth of wheat and Zea plants . Therefor, the increase of phenolics accumulation in the leaves of H_2O_2-pretreated tomato seeds grown under saline or unsaline conditions of this study might act as ROS scavengers by a direct reduction of endogenous or generated free oxygen radicals in which GPX and PPO used the phenolic compounds as reductants. The accumulation of phenolic compounds under biotic or abiotic stress has been reported in several plant species including Morus alba (Agastian et al ., 2000) , Crataegus (Kirakosyan et al ., 2004) , Cakile maritime (Ksouri et al ., 2007) , two maize varieties (Hichem et al ., 2009) and two wheat cultivars (Ashraf et al ., 2010).

Total ascorbate , glutathione and their fractions in the leaves of NaCl-salinized tomato plants were significantly decreased compared to control plants. This suppression was accompanied with a significant increase in ASA/DHASA ratio and decrease of GSH/TG as well as APX and GR activities. These observations might explain the increase of foliar H_2O_2 of tomato plants grown under NaCl level (Table 2) and that due to inhibition of ascorbate –glutathione cycle for providing the reductant agent of generated H_2O_2. Gossett et al . (1994) showed a decline of APX activity in Gossypium hirsutum grown under salt stress and Meneguzzo and Navarilzzo (1999) showed a decrease in GR activity in wheat roots . Conversely, pretreatment of tomato seeds with H_2O_2 resulted in a significante decrease of ascorbate and glutathione fractions, ASA/DHASA and GSH/GT ratios

in the leaves under salinized or unsalinized conditions compared to control plants . These observations were associated with a significant increase in the activities of both APX and GR
suggesting the detoxification of endogenous or generated H_2O_2 through enhancement the ascorbate-glutathione cycle . Dat et al . (1998) reported that , in mustard seedling, both ASA and DHASA forms responded dramatically to head acclimation causing substantial decrease of ASA to DHASA ratio . Similar change have been reported for wheat seedlings and Zea plants grown at super optimal temperature (Paolacci et al ., 1997 ; Sorour , 2010) . Molina et al . (2002) reported that the changes in glutathione redox potential might be involved in acclamatory stress signaling . Therefor , the increase of foliar GR activity in H_2O_2 – pretreated tomato plants grown under saline or nonsaline conditions maintains the glutathione pool in the form of GSH (high GSH/TG) allowing GSH to be used as a reductant of DHASA to ASA . Hence the decrease in ASA/DHASA ratio mainly controlled by APX activity using ASA as a reductant agent of endogenous or generated H_2O_2 in the tomato leaves , meaning that an induction of the ascorbate glutathione cycle for salt acclimation or salt avoidance .
The SDS-PAGE analysis (Fig 1) demonstrates that protein profiles in tomato leaves were markedly different according to the treatments, but some similarity could be detected. Also, the protein band intensities were different under treatment conditions. In the comparison of leaf proteins, NaCl stress induced four newly synthesized polypeptides (salt-induced proteins) with M.M. of 88.8, 60.3, 29.1 and 19.6 kDa. However, H_2O_2-pretreatment had similar protein bands of the control (Lane 4), a specific protein band with M.M. of 17.9 kDa was expressed in the salinized- H_2O_2-pretreated leaves (Lane 3). Tamas et al. (2001) found that four polypeptides with M.M. of 61, 51, 39 and 29 kDa were synthesized in the roots of NaCl-stressed maize plants. Valeria et al. (2005) reported up-regulation of a 26 kDa germin-like protein in tobacco plants under salt stress. Moreover, Zhou et al. (2009) identified several salt-induced and salt-suppressed proteins in tomato leaves under NaCl stress. They concluded that these identified proteins affect cellular activities for antioxidant, stress protection, carbon fixation and carbohydrate partitioning.
Passardi et al. 2005 concluded that POD isoenzymes are known to occur in a variety of plant tissues. The expression pattern of these isoenzymes varies in different tissues of healthy plants and is developmentally regulated or influenced by environmental factors .
The zymogram (Fig. 2) showed that peroxidase isoenzymes at the POD-2c and POD-1a loci were expressed under salinized and unsalinized treatments . Furthermore, there was one isoenzyme at POD-3a expressed under NaCl stress. These observations are associated with the variation of peroxidase activity revealing that a qualitative and quantitative difference in isoenzymes reflects peroxidase profiles.
Rahnama and Ebrahimzadeh (2006) indicated that POD isoenzyme profiles at 100 mM NaCl were different from that of the control. These differences were quantitative and were expressed more in terms of increased or decreased isoenzymes activities. Gao et al., 2008 reported that increased POD activities might enable plants to protect themselves against salt stress. Valizadeh et al ., 2013 evaluate the effect of relatively high salinity (9±0.2 ds m-1) on some alfalfa yield related traits along with analysis of antioxidant and non-antioxidant enzymes by native polyacrylamide gel electrophoresis showed that significant correlations exist only between changes on antioxidant isoenzymes activities and variation of yield characteristics, which are consistent with previous findings in several plants.
In conclusion , the pretreatment of tomato seeds (Lycopersicon esculentum) in this study with H_2O_2 resulted in acclimatize or adaptive the ability of tomato leaves to 100 mM NaCl stress. In addition, acquisition of tomato plants to salt acclimation may be related to a consequence resistance to generated ROS and oxidative stress through increased the activities of antioxidant enzymes ,as well as enhanced ascorbate –glutathione cycle and influence of different polypeptide bands and peroxidase isenozyme profiles.

Table 1: Changes in fresh and dry biomasses (mg 10 plant^{-1}) , succulence , relative water content (RWC) and electrolyte leakage of leaves of tomato plants as a result of presoaking of seeds for 8 h either in water or 50 µM hydrogen peroxide (H_2O_2) followed by irrigation with 1/10 Hoagland solution alone (Control, H_2O_2) or supplemented with 100 mM NaCl (NaCl , H_2O_2+NaCl).

Treatment	Time/day				
	40				
	f.m.	d.m.	Succulence	RWC	Leakage %
Control	285.66±29.2[a]	93.02±7.98[a]	3.07±0.22[a]	80.8±6.98[a]	30
NaCl	70.38±6.22[c]	38.24±3.22[c]	1.84±0.162[b]	29.1±2.74[c]	89
H_2O_2	279.88±25.1[a]	92.90±8.21[a]	3.01±0.23[a]	83.7±6.98[a]	40
H_2O_2 + NaCl	96.63±8.2[b]	48.18±3.98[b]	2.00±0.22[c]	37.2±4.12[b]	72
ANOVA	22.1	16.25	6.85	12.4	
p	0.001*	0.0013*	0.015*	0.0051*	

Table 2: Changes in total phenolic content, hydrogen peroxide content, malondialdhyde content and ascorbate and glutathione fractions of leaves of tomato plants as a result of presoaking of seeds for 8 h either in water or 50 µM hydrogen peroxide (H_2O_2) followed by irrigation with 1/10 Hoagland solution alone (Control, H_2O_2) or supplemented with 100 mM NaCl (NaCl, H_2O_2+NaCl).

Treatment	Total phenolic content	H2O2 content	MDA content	Ascorbate fractions			Glutathione fractions		
	40 days								
	mg gallic acid eq. g-1 d.m.	µmol H2O2 g-1 d.m.	µmol g-1 d.m.	µg g-1 d.m.			µg g-1 d.m.		
				TASA	ASA	DHASA	TG	GSSG	GSH
Control	276 ±23.5d	31.61 ±2.85d	14.93 ±1.23d	418.07 ±39.8b	163.59 ±17.2a	254.48 ±26.8b	612.60 ±62.5a	241.51 ±25.1b	371.09 ±36.2a
NaCl	2206 ±21.5a	171.91 ±16.9a	139.34 ±12.8a	245.06 ±25.6d	106.32 ±9.85c	138.74 ±14.21d	152.22 ±16.8d	130.94 ±14.2d	21.28 ±2.56d
H_2O_2	674 ±71.2c	76.47 ±7.25c	41.81 ±4.01c	401.58 ±41.6a	122.62 ±12.6b	278.96 ±28.6a	579.19 ±58.2b	331.24 ±32.8a	247.95 ±25.1b
H_2O_2+NaCl	2153 ±105.3b	129.37 ±13.9b	116.76 ±12.6b	286.67 ±29.8c	85.17 ±9.21d	201.50 ±21.6c	205.12 ±21.3c	149.89 ±15.2c	55.23 ±5.6c
ANOVA	82.98	65.6	101.3	16.52	8.98	11.6	9.12	18.9	68.5
p	0.0001*	0.001*	0.0001*	0.0016 *	0.026*	0.015*	0.019*	0.003*	0.001*

Table 3: Changes in ASA/DHASA and GSH/TG ratios of leaves of tomato plants as a result of presoaking of seeds for 8 h either in water or 50 µM hydrogen peroxide (H_2O_2) followed by irrigation with 1/10 Hoagland solution alone (Control, H_2O_2) or supplemented with 100 mM NaCl (NaCl , H_2O_2+NaCl).

Treatment	ASA/ DHASA %	GSH/ TG
	40 days	
Control	64±5.11	0.61±0.036
NaCl	77±6.28	0.14±0.011
H_2O_2	44±5.58	0.43±0.032
H_2O_2+NaCl	42±4.98	0.27±0.018
ANOVA	2.36	2.06
p	0.112	0.225

Table 4 : Changes in SOD , CAT , APX , GR , GPX and PPO of leaves of tomato plants as a result of presoaking of seeds for 8 h either in water or 50 µM hydrogen peroxide (H_2O_2) followed by irrigation with 1/10 Hoagland solution alone (Control, H_2O_2) or supplemented with 100 mM NaCl (NaCl, H_2O_2+NaCl).

Treatment	SOD	CAT	APX	GR	GPX	PPO
	40 days					
	U g-1 d.m.	µmol H_2O_2 g-1 d.m. min-1	µmol H_2O_2 g-1 d.m. min-1	µmol NADPH g-1 d.m. min-1	µmol guaiacol min-1 g-1 d.m.	U min-1 g-1 d.m.
Control	25.56 ±3.1c	33.93 ±d	35.85 ±3.65b	34.15 ±2.98d	29.67 ±2.51d	0.121 ±0.01b
NaCl	103.03 ±8.52a	99.99 ±c	11.70 ±1.08c	11.83 ±1.02c	41.35 ±4.01c	0.022 ±0.003c
H_2O_2	24.76 ±2.61c	114.16 ±10.8b	96.38 ±8.74a	86.71 ±8.7b	96.72 ±8.9b	1.204 ±0.107a
H_2O_2+NaCl	88.59 ±7.98b	122.94 ±12.3a	106.22 ±10.7a	114.25 ±10.6a	107.04 ±9.82a	1.265 ±0.11a
ANOVA	32.65	25.1	19.8	16.8	17.65	6.98
p	0.001*	0.001*	0.003*	0.004*	0.001*	0.023*

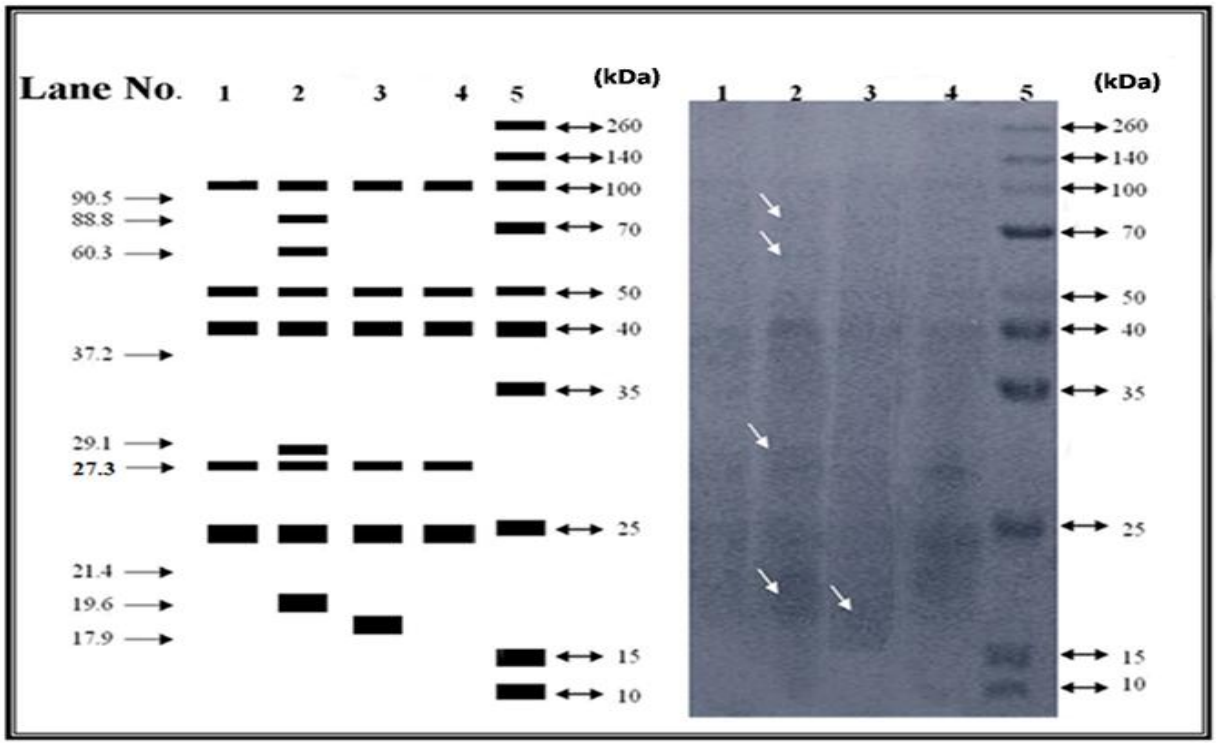

Fig. 1: SDS- PAGE electrophoretic profile of proteins among the four treatments (Lane 1: Control, Lane 2: NaCl, Lane 3: H_2O_2+NaCl, Lane 4: H_2O_2 and Lane 5: M (protein marker).

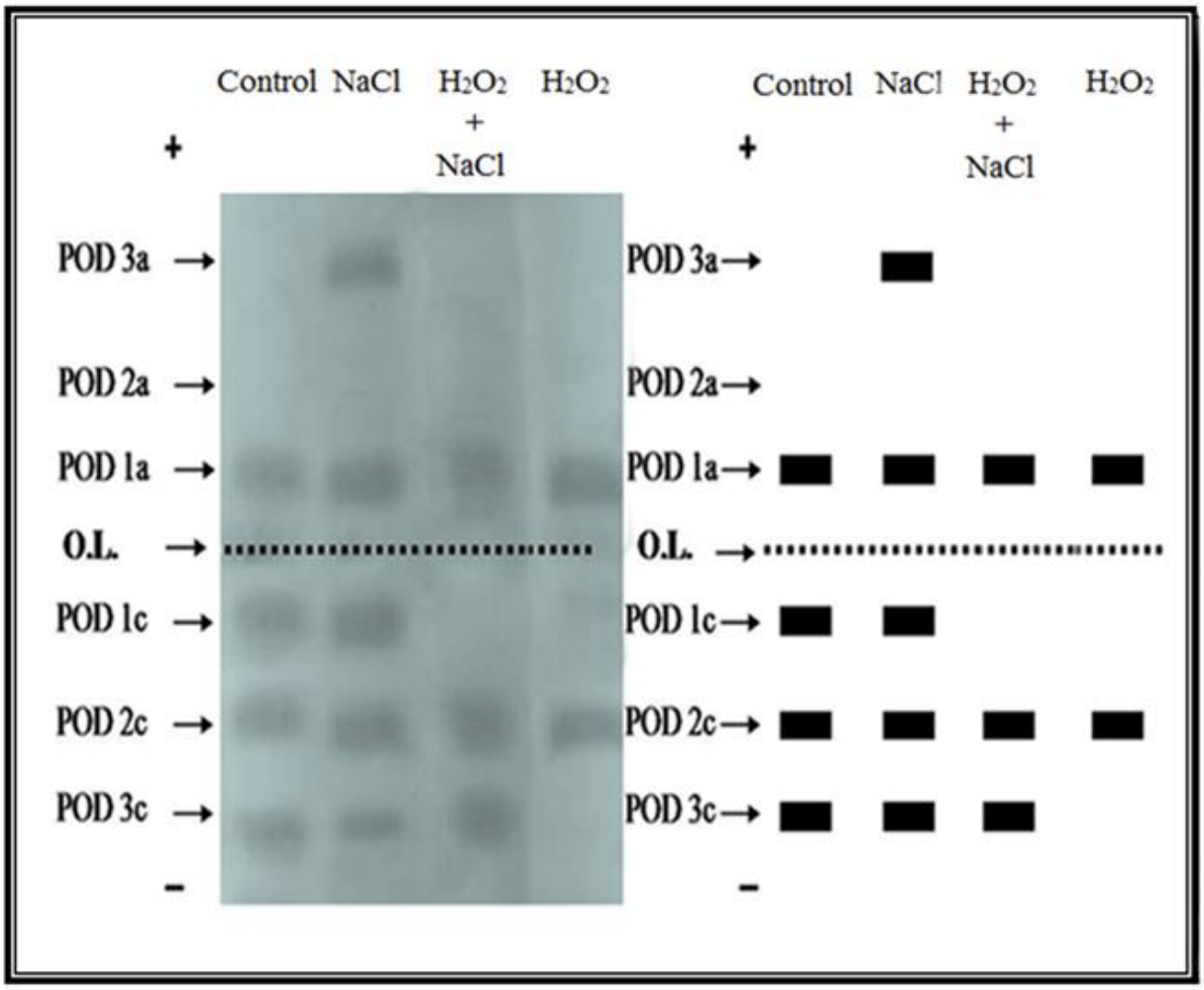

Fig. 2: Zymogram showing the electrophoretic profile of peroxidase isoenzymes among the four treatments.
O.L.: refers to original line.
a: symbol refers to anodal region.
c: symbol refers to cathodal region.

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