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Response of Strawberry Plants Grown in the Hydroponic System to Pretreatment with H_2O_2 before Exposure to Salinity Stress

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ABSTRACT

A hydroponic experiment was carried out to investigate the effect of immersion durations (1 and 2h) of hydrogen peroxide on roots of strawberry (*Fragaria x ananassa* Duch.) grown under NaCl stress at (0, 34 and 68 mM NaCl). Roots immersion into H_2O_2 increased plant growth, photosynthetic pigment concentration, leaf relative water content and the activity of antioxidant enzymes i.e. (catalase, peroxidase and polyphenoloxidase) as well as decrease electrolyte leakage compared to untreated plants. High NaCl salinity level, induced ultrastructural alterations in leaflet mesophyll cells such as swelling thylakoids, disintegration of grana staking, increase number of plastoglobuli and starch grains as well as increase the size and number of mitochondria and its structure, shrinkage the plasma membranes, increase the Myelin-Like, membrane vesicles formation and increase the thickness of cell wall. In addition, roots immersion into H_2O_2 led to maintain the chloroplast structure, grana staking and increase the size of chloroplast and mitochondria, decrease the number and size of starch grains and plastoglobuli, decrease the number of membrane vesicles and peroxisomes, as well as maintain the cell wall structural and reduced its thickness. Furthermore, the high NaCl level led to increase the number of stomata and stomatal density and decreased the dimensions of stomatal pore. On the contrary, roots immersion into H_2O_2 decreases the stomatal density and its number. Concerning the leaflet anatomy, it was found that low NaCl salinity level increased the dimensions of midrib region, main vascular bundle as well as the thickness of palisade parenchyma. While, high salinity level, in most cases, decreased all these parameters. In conclusion, immersed roots of strawberry plants (pre-treatment) in H_2O_2 (1.0 M) for 1h application before exposure to salinity stress increased plant resistance and mitigated the deleterious effects of NaCl on cellular organelles.

Keywords: NaCl stress; Hydrogen peroxide; strawberry; antioxidant enzymes activity; leaflet ultrastructural, stomata

INTRODUCTION

Among several abiotic stress conditions, salinity is of much greater concern that affects arrests the crop productivity based on ionic and osmotic deteriorative disorders (Kumar *et al*., 2017). Worldwide, forecasting the salinization of more than 50% of the arable land by the year 2050 due to low precipitation, expansion in saline water irrigation, seawater intrusion into rivers and coastal aquifers, climate change and the intensive use of synthetic compounds (Shrivastava and Kumar, 2015). Salinity evoked various biochemical, physiological, molecular, cellular and morphological alterations on stressed plants (Abd Elgawad *et al*., 2016).

Salinity induce the generation of reactive oxygen species (ROS) including, superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) and singlet oxygen, which can cause oxidative stress and various disruptions in the metabolic balance of plant cells (Faghih *et al*., 2017). ROS-induced oxidative stress are associated with several deteriorations in plant: (i) damage to DNA, proteins, and lipids, thus disrupting the normal functionality of plant cellular system (Das and Roychoudhury, 2014), (ii) reducing net photosynthesis due to the defective synthesis of chlorophyll and carotenoid contents, the disruption of stomatal conductance, the disturbance of transpiration rate and the imbalance of intercellular CO_2 concentration (Huang *et al*., 2015), and (iii) activating K^+-permeable non-selective cation channel, thereby accelerating K^+ leakage from cytosol, which activates caspase-like proteases and triggers programmed cell death (Maksimović *et al*., 2013). Salinity-induced oxidative stress is also responsible for several alterations in the ultrastructure of plant organelles i.e. chloroplast and mitochondria (Bejaoui *et al*., 2016). In addition, plants have developed a well-organized and sophisticated antioxidant strategy including antioxidant enzymes and non-enzymes to control ROS production and accumulation (Corpas and Barroso, 2013).

Strawberry is one of the important commercial fruit crops around the world due to its health full properties and organoleptic (aroma, flavor, color and texture) and high nutritional value (Aharoni *et al*., 2002). Moreover, it is a relevant source of antioxidants properties, bioactive compounds and phytochemicals i.e. anthocyanin, phenolic compounds and flavonoids. (Simirgiotis *et al*., 2009; Giampieri *et al*., 2012). The high demand for fresh and processed fruit has led to a considerable increase in strawberry production with an annual production of 7.73 million tons from 361.662 ha (FAO STAT, 2013) of strawberry fields. In Egypt, strawberry cultivated area is estimated as 6509 ha^{-1} with a full production of about 283471 tons and average yield of approximately 43.55 tons ha^{-1} in 2014 (FAO STAT, 2017). Strawberry exposure to salinity may cause severe morphological, physiological and biochemical changes including plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes because this plant is a sensitive to salinity (Faghih *et al*., 2018; Mozafari *et al*., 2018).

Recently, exogenous supplementation of plant protectant such as signaling molecules (nitric oxide, H_2O_2, etc.) have been found to be effective in mitigating the salt induced damage in plant. Some studies have shown that pretreatment with appropriate H_2O_2 concentrations can confer tolerance to environmental stresses by modulating physiological and biochemical process as well as multiple stress-responsive pathways including the detoxification pathways of ROS. H_2O_2 at low concentrations acts as a messenger molecule involved in acclamatory signaling, triggering tolerance against various abiotic stresses, alternatively high concentrations orchestrate programmed cell death (Vandenabeele *et al*., 2003). Additionally, H_2O_2 roots pre-treatment was found induce salinity tolerance in some plants (Wahid *et al*., 2007 and Abd El-Mageed *et al*., 2016) mainly through the regulation of the activity of antioxidant enzymes and the mitigation of lipid peroxidation. In this concern, Christou *et al*. (2014)

reported that roots pretreatment of strawberry with H_2O_2 at concentration of 10 mM before exposure to salinity stress (100 mM NaCl, 8d) led to increasing chlorophyll fluorescence, photosynthetic pigments, leaf water content and lower lipid peroxidation and electrolyte leakage as well as increase the transcript levels of enzymatic antioxidants in leaves compare to plants directly subjected to salt stress. Furthermore, Tanou *et al.* (2009) demonstrated that H_2O_2 induced systemic antioxidants activity confers tolerance to salinity stressed citrus plants.

The aim of this investigation was to study the response of strawberry plants to NaCl-salinity and roots pre-treatment with H_2O_2 on morphological, physiological parameters and leaf structure as well as the ultrastructural alterations.

MATERIALS AND METHODS

Materials

All chemicals of analytical grade including sodium chloride (NaCl), and nutrient chemicals necessary for preparing cooper's nutrient solution were purchased from Merck Chemicals. Plants of strawberry (*Fragaria* × *ananassa* Duch.) cv. Festival were obtained from Agricultural Research Center, Giza, Egypt.

Experimental design and layout

In a split plot design, a hydroponic experiment comprising two treatments: (i) salinity stress with three levels [(S_0) nutrient solution, (S_1) nutrient solution + 34.0 mM NaCl and (S_2) nutrient solution + 68.0 mM NaCl], and (ii) H_2O_2-roots immersion at (1.0 M) with three durations [(H_0), (H_1) 1 and (H_2) 2 h).

A hydroponic experiment comprising nine treatments [T_1 (S_0 H_0), T_2 (S_0 H_1), T_3 (S_0 H_2), T_4 (S_1 H_0), T_5 (S_1 H_1), T_6 (S_1 H_2), T_7 (S_2 H_0), T_8 (S_2 H_1), T_9 (S_2 H_2)] was carried out during the winter seasons of 2016 and 2017 at the experimental glasshouse of Agric. Botany Dept., Mansoura Univ. and Excellence Center and Plant Pathology and Biotechnology Lab. (certified according to ISO 9001, ISO 14001, OHSAS 18001and ISO 17025), Dept. of Agric. Botany, Fac. of Agric., Kafrelsheikh Univ., Egypt. Main treatments (salinity levels) were assigned in in three units of nutrient film technique (NFT).

For each NFT unit, two PVC pipes (4.0 m long and 10 cm diameter) were connected using plastic tubes to present salinity levels. Each PVC pipe had 20 circular slots for strawberry plants in the upper side (6 cm diameter). Each NFT hydroponic treatment was divided into 3 sets comprising H_2O_2-roots immersion durations as sub-treatments (13 plant for each). NFT units were provided with reservoirs containing 15 L of cooper's nutrient solution with the salinity levels. The nutrient solution was prepared according to Cooper (1979) with nutrients concentration (mg L^{-1}): N(200); P(60); K(300); Ca(170); Mg(50); S(69); Fe(12); Mn(2); Cu(0.1); Zn(0.1); B(0.3) and Mo(0.2). NFT units were equipped with electric pumps (Hydor Seltz (S20 II), Italy) for circulating the nutrient solution with a flow rate of 1.0 L min^{-1}.

Before starting the hydroponic experiment, uniform plants (4-5 true-leaf formation stage) were divided into three sets. The first set was immersed into distilled water; however, other sets were immersed into 1.0 M H_2O_2 for 1 or 2 h followed by rising several times with distilled water to remove the excess of H_2O_2. Thereafter, plants were transferred directly to NFT units. All treatments were supplied with Cooper's nutrient solution for a week. During this week, the EC value was adjusted to ~2.0±0.2 $dS{\cdot}m^{-1}$ using EC meter (Lutron CD-4301) and the pH value was adjusted to ~6.0 by adding either HNO_3 or KOH (0.1 M for each) using pH meter. Additionally, the nutrient solution was exchanged every week by a fresh one to ensure optimum supplementation of plant nutrients. Subsequently, the salinity levels of cooper's nutrient solution was kept constant in the control treatment 0.0 mM NaCl (~2.0±0.2 $dS{\cdot}m^{-1}$); however, it raised in the second and third NFT hydroponic units to about (34.0 and 68.0 mM NaCl) (~5.42±0.2 and ~8.42±0.2$dS{\cdot}m^{-1}$) respectively.

Plant analytical procedures

Morphological parameters

After four weeks from transplanting, three representative plants were taken from each treatment to measure plant height, root length, number of leaves, leaf area $plant^{-1}$ and number of adventitious roots. Samples were divided into shoots and roots, washed gently with distilled water before measuring fresh weight roots, shoots and leaves. Afterwards, samples were dried in hot-air oven at 70 °C until constant weight to obtain the dry weight (~72 h).

Physiological measurements

Photosynthetic pigments

Fresh samples (0.05 g) obtained from the terminal leaflet of the 6th leaf were extracted for 24 h using methanol (10 ml) and traces from sodium carbonate (Wellburn, 1994). Consequently, chlorophylls a, b, and total carotenoids were calorimetrically determined using T60 UV/VIS Spectrophotometer, PG Instruments Limited, Uk at wavelengths of 452.5, 650 and 665 nm for chlorophylls a, b, and total carotenoids, respectively and expressed as mg g FW^{-1}.

Relative water content (RWC)

RWC was estimated according to Sánchez *et al.* (2004). After obtaining fresh weight (FW), representative samples were floated in closed petri dishes within distilled water and kept in darkness for 24 h at 4.0 °C to obtain turgid weight (TW). Thereafter, samples were placed in an oven at ~ 80 °C until reaching the constant dry weight (DW).

RWC was calculated using the following equation:

$$\mathbf{RWC\ (\%)} = \frac{(FW-DW)}{(TW-DW)} \times \mathbf{100}.$$

Electrolyte leakage (EL)

Leaf membrane damage was evaluated following the data of electrolyte leakage (EL %) as described by Dionisio-Sese and Tobita (1998). Fresh chopped leaf samples of strawberry plants (10 g weight) were placed into plastic tubes containing 25 ml DI water, then covered, and placed into water bath at 32°C. The initial electrical conductivity value (EC_1) was determined by EC meter (Lutron CD-4301). Thereafter, plant tissues were boiled for 30 min to destroy plant tissues for releasing internal electrolytes before measuring the subsequent EC value (EC_2). Electrolyte leakage (EL%) was calculated as

$$\mathbf{EL=EC_1/EC_2\times 100.}$$

Activity of enzymatic antioxidants

To determine the activity of antioxidant enzymes, 0.5 g of fresh leaf samples were homogenized in 50 mM TRIS buffer (pH 7.8) containing 7.5% polyvinylpyrrolidone and 1

mM EDTA-Na_2 at 0-4˚C. Subsequently, samples were centrifuged at 12,000 rpm at 4˚C for 20 min. Enzyme activities in the supernatant were determined calorimetrically at 25˚C using a UV-160A spectrophotometer (Shimadzu, Japan). Catalase activity (CAT, E.C. 1.11.1.6) was determined according to Aebi (1984). Peroxidase activity (POD, E.C. 1.11.1.7) was determined based on the technique of Hammerschmidt *et al*. (1982). Polyphenol oxidase activity (PPO, E.C. 1.14.18.1) was determined according to Malik and Singh (1980).

Transmission (TEM) and Scanning (SEM) Electron Microscopy:

For TEM investigation, about 10 mm^2 specimens of some selected treatments were obtained from the terminal leaflet of the 6th leaf of strawberry plants. Specimens were fixed in in 2.5% glutaraladehyde and 1% osmium tetroxide, dehydrated in a graded acetone series and infiltrated with epoxy resin. Using LKB ultratone III microtome, ultrathin sections of about 50-100µ were prepared. These ultrathin sections were double stained using saturated uranyl acetate and Reynolds' lead citrate (15 min for each solution). Prepared sections were investigated and visualized using JEOL 100s TEM.

Other specimens (about 25 mm) were prepared for SEM investigation. Specimens were fixed in 4% (v/v) glutaraldehyde in 0.1 mol L^{-1} phosphate buffer (pH 6.8) for 36 h at 4 °C and dehydrated in ethanol series. Dehydrated specimens were vacuum-dried using EM-CPD-030 critical point dryer (Leica, Heidelberg, Germany) for 40-60 min and coated with gold sputter. Visualized specimens were photographed at 10 kV accelerating voltage using a JSM-6400 LV SEM (JEOL Ltd., Tokyo, Japan). Five random SEM micrographs were investigated to obtain cell wall thickness (µm), number of stomata (field = 0.19 mm^2), stomatal density per mm^2 and dimensions (length and width of stomatal pore) as mentioned by Balasooriya *et al*. (2009).

Leaf blade structure

Leaf specimens (0.5 cm) were obtained from the middle leaflet of the 6th leaf for the anatomical investigation of leaf blade. Specimens were fixed in FAA solution (Formalin: Acetic acid: ethyl Alcohol) for 48 h, dehydrated in a series of ethanol and embedded in paraffin wax (52-54 ˚C melting point). Sections (10-15 µm thickness) were prepared using rotary microtome, stained with toluidine blue and mounted in Canada balsam (Ruzin, 1999). Selected sections were examined by light microscope (Nikon, Japan) and visualized using a digital camera (TUCSEN, USB2, H Series) to obtain dimension of midrib and main vascular bundle and, thickness of leaflet blade (palisade and spongy parenchyma's), thickness of mesophyll as well as thickness of upper and lower epidermis.

Statistical analysis

Statistical analysis was carried out by CoStat (Version 6.303, CoHort, USA, 1998-2004) using Duncan's multiple range test based on analysis of variance (ANOVA) at a significance level of 0.05.

RESULTS

Morphological parameters

Morphological growth parameters of salinity stressed strawberry plants and H_2O_2-immersion durations are presented in Table (1). The vegetative growth parameters include plant height, length and number of adventitious roots, leaf number and area per plant as well as fresh and dry weights of shoot and roots. It is realized from Table (1) that all morphological parameters were significantly decreased under salinity stress. The highest reduction was observed on plants grown under the concentration of 68.0 mM NaCl as compared to control.

Table 1. Effect of salinity and H_2O_2 and their interactions on morphological characters of strawberry plants average of two seasons (2016/2017).

Treatment	Plant height (cm)	Root length (cm)	No. of adventitious roots	leaf number plant^{-1}	Leaf area Plant (cm)	Root FW (g/plant^{-1})	Leaves FW (g/plant^{-1})	Shoot FW (g/plant^{-1})	Root DW (g/plant^{-1})	Leaf DW (g/plant^{-1})	Shoot DW (g/plant^{-1})
Salinity (S)											
S_0	16.69^{a} ±0.42	14.39^{b} ±0.40	22.44^{a} ± 1.57	11.56^{a} ± 0.50	173.0^{a} ±9.89	7.36 a ± 0.50	7.51 a ± 0.45	8.37 a ± 0.52	0.87^{a} ± 0.045	1.61^{a} ± 0.078	1.81^{a} ± 0.093
S_1	12.83^{b} ±0.33	15.56^{a} ±0.68	23.33^{a} ± 0.97	12.11^{a} ± 0.77	134.0^{b} ±5.02	7.19 a ± 0.28	6.19 b ± 0.30	7.26 b ±0.39	0.76^{b} ± 0.032	1.20^{b} ± 0.045	1.32^{b} ± 0.048
S_2	11.56^{c} ±0.27	12.00^{c} ±0.46	13.22^{b} ± 0.66	7.22^{b} ± 0.40	80.3^{c} ± 7.88	4.10 b ± 0.40	3.42 c ± 0.26	3.95 c ± 0.29	0.45^{c} ± 0.024	0.73^{c} ± 0.073	0.82^{c} ± 0.077
H_2O_2 (H)											
H_0	12.83^{c} ±0.75	13.17^{b} ± 0.46	17.78^{b} ± 1.76	9.33^{b} ± 0.80	103.2^{c} ±12.96	5.42^{b} ± 0.45	4.94 ± 0.61^{c}	5.57 ± 0.66^{c}	0.59^{c} ± 0.054	0.94^{c} ± 0.133	1.05^{c} ± 0.140
H_1	14.92^{a} ±0.87	15.89^{a} ± 0.67	23.33^{a} ± 2.06	12.33^{a} ± 0.97	153.9^{a} ±14.37	7.66^{a} ± 0.57	6.78 ± 0.67^{a}	7.89 ± 0.76^{a}	0.81^{a} ± 0.073	1.38^{a} ± 0.136	1.54^{a} ± 0.156
H_2	13.33^{b} ±0.74	12.89^{b} ± 0.55	17.89^{b} ± 1.35	9.22^{b} ± 0.66	130.1^{b} ±3.82	5.58^{b} ± 0.64	5.40 ± 0.63^{b}	6.12 ± 0.68^{b}	0.68^{b} ± 0.065	1.22^{b} ± 0.121	1.36^{b} ± 0.136
Salinity * H_2O_2											
S_0 H_0	15.67^{b} ±0.44	13.50^{c} ±0.29	18.00^{d} ± 0.58	11.00^{c} ± 0.58	136.1^{d} ±0.27	5.94^{de} ± 0.23	6.03^{cd} ± 0.56	6.66^{cd} ± 0.55	0.72^{c} ± 0.006	1.35^{c} ± 0.017	1.48^{c} ± 0.017
S_0 H_1	18.25^{a} ±0.15	15.83^{b} ±0.17	28.33^{a} ± 0.88	13.33^{b} ± 0.33	203.6^{a} ±0.32	9.20^{a} ± 0.36	8.88^{a} ± 0.06	10.02^{a} ± 0.09	1.03^{a} ± 0.026	1.87^{a} ± 0.003	2.11^{a} ± 0.006
S_0 H_2	16.17^{b} ±0.17	13.83^{c} ±0.44	21.00^{c} ± 0.58	10.33^{c} ± 0.33	179.2^{b} ±1.67	6.95^{c} ± 0.07	7.62^{b} ± 0.30	8.43^{b} ± 0.34	0.87^{b} ± 0.003	1.62^{b} ± 0.081	1.84^{b} ± 0.061
S_1 H_0	12.00^{de} ±0.29	14.50^{c} ±0.29	23.67^{b} ± 0.33	10.67^{c} ± 0.67	121.5^{e} ±2.06	6.61^{cd} ± 0.10	6.19^{c} ± 0.29	6.99^{c} ± 0.29	0.67^{d} ± 0.012	1.04^{e} ± 0.009	1.14^{e} ± 0.006
S_1 H_1	14.00^{c} ±0.29	18.17^{a} ±0.44	26.33^{a} ± 0.88	15.00^{a} ± 0.58	153.2^{c} ±1.61	8.21^{b} ± 0.27	7.15^{b} ± 0.28	8.65^{b} ± 0.27	0.88^{b} ± 0.026	1.35^{c} ± 0.003	1.47^{c} ± 0.006
S_1 H_2	12.50^{d} ±0.29	14.00^{c} ±0.29	20.00^{cd} ± 0.58	10.67^{c} ± 0.33	127.3^{e} ±3.13	6.75^{cd} ± 0.30	5.24^{d} ± 0.12	6.14^{d} ± 0.16	0.74^{c} ± 0.003	1.23^{d} ± 0.012	1.35^{d} ± 0.012
S_2 H_0	10.83^{f} ±0.17	11.50^{d} ±0.29	11.67^{f} ± 0.88	6.33^{e} ± 0.33	52.2^{h} ± 1.92	3.70^{f} ± 0.28	2.60^{f} ± 0.11	3.05^{f} ± 0.09	0.38^{g} ± 0.003	0.45^{h} ± 0.020	0.52^{h} ± 0.020
S_2 H_1	12.50^{d} ±0.29	13.67^{c} ±0.17	15.33^{e} ± 0.67	8.67^{d} ± 0.33	104.8^{f} ±6.07	5.56^{e} ± 0.29	4.31^{e} ± 0.16	5.00^{e} ± 0.15	0.54^{e} ± 0.003	0.93^{f} ± 0.023	1.04^{f} ± 0.020
S_2 H_2	11.33^{ef} ±0.17	10.83^{d} ±0.44	12.67^{f} ±0.67	6.67^{e} ± 0.33	83.9^{g} ± 1.25	3.03^{f} ± 0.06	3.35^{f} ± 0.08	3.80^{f} ± 0.08	0.43^{f} ± 0.003	0.80^{g} ± 0.006	0.90^{g} ± 0.003

Means within columns followed by different letters are significantly different ($p < 0.05$); Means (±SE) were calculated from three replicates for each treatment. FW, fresh weight; DW, dry weight. S_0, (nutrient solution), S_1 (nutrient solution + 34.0 mM NaCl), S_2 (nutrient solution + 68.0 mM NaCl), H_0 (non-immersion roots into H_2O_2), H_1 (roots immersion into H_2O_2 for 1h) and H_2 (roots immersion into H_2O_2 for 2h).

On the other hand, immersion plant roots in H_2O_2 significantly minimized these dramatic reductions. Roots immersion for 1h was more effective in this respect. The interaction effect between salinity stress and immersion durations was significant on the growth parameters. The heights growth parameters were recorded under S_0 H_1; however, the lowest growth parameters were observed with plants grown under S_2 H_0. On the other hand, roots immersion into H_2O_2 for 2 h caused a slight inhibition on growth parameters of plants grown under both salinity levels as compared to untreated plants. Generally, H_2O_2 led to counteracting the harmful effect of salinity stress thereafter decreased with increasing immersion time.

Physiological measurements

Photosynthetic pigment concentration

As shown in Table (2) increasing NaCl in nutrient solutions decreased photosynthetic pigments (Chl a, Chl b, total chlorophyll and carotenoids). The highest inhibition of these parameters was observed under salinity level of 68.0 mM NaCl. On the contrary, immersion roots into H_2O_2 for 1h or 2h recorded a significant increase on photosynthetic pigments of strawberry plants compared to control plants. Meanwhile, the roots immersion into H_2O_2 for 1 h was more effective in this respect. The significant interaction between treatments was able to overcome the harmful effect of salinity stress. S_0 H_1 was the most efficient treatment for improving photosynthetic pigments concentration. Meanwhile, S_2 T_0 treatment was the most stressful treatment in this regard.

Electrolyte leakage (EL %)

Electrolyte leakage of strawberry leaves was increased significantly with the increasing of NaCl levels in the nutrient solutions (Table 2). The maximum electrolyte leakage was recorded at 68.0 mM NaCl level (36.21%) compared to the control plants. Immersion plants roots into H_2O_2 caused a significant reduction of EL%. However, the lowest leaf electrolyte leakage (30.40%) was obtained with plants pretreated with H_2O_2 for 1h before exposure to high salinity level (68.0 mM NaCl) compared to untreated plants under high salinity level. Furthermore, immersion plant roots into H_2O_2 and subsequent exposure to salinity resulted in the preservation of membrane integrity and the alleviation of cellular damages, as indicated by decrease percentages of EL compared with NaCl alone treated plants.

Relative water content (RWC %)

In general, the reduction in RWC % was considered as direct indicator of salinity stress. RWC of strawberry plants statistically decreased under salinity stress compared to control treatment (Table 2). The high salinity level (S_2) in the nutrient solutions was the most effective in this concern as compared to control. On the contrary, immersion roots into H_2O_2 caused a significant increase in RWC values. In addition, immersion plant roots into H_2O_2 for 1h proved to be more effective than 2h to counteract the harmful effect of salinity on RWC %. Immersion plant roots into H_2O_2 mediated the reduction of RWC values in strawberry plants exposed to low salinity level (34.0 mM NaCl).

Activity of enzymatic antioxidants

Regarding the effect of salinity and H_2O_2-immersion durations on enzymes activity, data presented in Fig. 1 indicated that salinity levels and H_2O_2 treatments significant increases CAT and POD enzyme activities of stressed strawberry plants compared with control plants (T_1). However, the increase in PPO was not significant under T_4 and T_5 treatments. The maximum activity of CAT was recorded with the high salinity level 68.0 mM NaCl (T_7) followed by T_9 treatments (Fig. 1-A). Likewise, POD activity increased significantly with T_9 (Fig. 1-B) in the plants exposure to salinity compared with other treatments and control plant. The obtained data in Fig. (1-C) showed that there are significant differences in PPO activity between stressed plants and control (T_1). The high level of PPO activity was observed in T_9 in this regard.

Table 2. Effect of salinity and H_2O_2 and their interactions on Electrolyte leakage and relative water content as well as photosynthetic pigments (mg g^{-1} FW) in the leaves of strawberry plants (season 2017).

Treatment	*EL%*	*RWC %*	*Chl. a*	*Chl. b*	*Total Chl.*	*Car.*
			Salinity (S)			
S_0	24.4 ^{c} ± 0.646	86.9 ^{a} ± 1.250	2.740 ^{a} ± 0.044	1.193 ^{a} ± 0.052	3.933 ^{a} ± 0.073	0.577 ^{a} ± 0.022
S_1	31.2 ^{b} ± 0.770	80.8 ^{b} ± 1.605	2.508 ^{b} ± 0.032	0.753 ^{b} ± 0.025	3.262 ^{b} ± 0.046	0.573 ^{a} ± 0.029
S_2	34.0 ^{a} ± 0.937	71.7 ^{c} ± 1.282	2.294 ^{c} ± 0.098	0.528 ^{c} ± 0.064	2.821 ^{c} ± 0.157	0.362 ^{b} ± 0.046
			H_2O_2 (H)			
H_0	32.1 ^{a} ± 1.483	76.3 ^{c} ± 2.551	2.358 ^{c} ± 0.119	0.703 ^{b} ± 0.109	3.061 ^{c} ± 0.223	0.436 ^{b} ± 0.048
H_1	27.1 ^{c} ± 1.266	84.6 ^{a} ± 2.421	2.675 ^{a} ± 0.056	0.915 ^{a} ± 0.101	3.590 ^{a} ± 0.153	0.609 ^{a} ± 0.027
H_2	30.4 ^{b} ± 1.589	78.5 ^{b} ± 1.853	2.509 ^{b} ± 0.038	0.856 ^{a} ± 0.105	3.366 ^{b} ± 0.139	0.468 ^{b} ± 0.045
			Salinity * H_2O_2			
S_0 H_0	26.5 ^{e} ± 0.361	84.9 ^{b} ± 0.485	2.712 ^{b} ± 0.054	1.034 ^{b} ± 0.090	3.746 ^{b} ± 0.039	0.541 ^{b} ± 0.044
S_0 H_1	22.1 ^{g} ± 0.185	91.3 ^{a} ± 1.811	2.884 ^{a} ± 0.023	1.303 ^{a} ± 0.069	4.187 ^{a} ± 0.076	0.616 ^{ab} ± 0.040
S_0 H_2	24.6 ^{f} ± 0.315	84.5 ^{b} ± 0.768	2.624 ^{bc} ± 0.053	1.242 ^{a} ± 0.013	3.867 ^{b} ± 0.066	0.572 ^{b} ± 0.024
S_1 H_0	33.7 ^{b} ± 0.174	76.7 ^{c} ± 0.664	2.447 ^{d} ± 0.038	0.748 ^{c} ± 0.051	3.195 ^{cd} ± 0.067	0.516 ^{b} ± 0.006
S_1 H_1	28.7 ^{d} ± 0.188	87.0 ^{b} ± 0.829	2.605 ^{bc} ± 0.023	0.762 ^{c} ± 0.040	3.367 ^{c} ± 0.017	0.676 ^{a} ± 0.036
S_1 H_2	31.3 ^{c} ± 0.931	78.8 ^{c} ± 0.196	2.473 ^{cd} ± 0.055	0.751 ^{c} ± 0.057	3.224 ^{cd} ± 0.111	0.528 ^{b} ± 0.030
S_2 H_0	36.2 ^{a} ± 0.784	67.3 ^{e} ± 0.699	1.915 ^{e} ± 0.041	0.328 ^{d} ± 0.078	2.242 ^{e} ± 0.119	0.250 ^{c} ± 0.016
S_2 H_1	30.4 ^{c} ± 0.072	75.6 ^{cd} ± 0.445	2.536 ^{cd} ± 0.050	0.681 ^{c} ± 0.022	3.216 ^{cd} ± 0.042	0.533 ^{b} ± 0.023
S_2 H_2	35.3 ^{a} ± 0.379	72.1 ^{d} ± 1.400	2.430 ^{d} ± 0.034	0.575 ^{c} ± 0.095	3.006 ^{d} ± 0.125	0.302 ^{c} ± 0.046

Means within columns followed by different letters are significantly different ($p < 0.05$); Means (±SE) were calculated from three replicates for each treatment. EL, electrolyte leakage; RWC, relative water content; *Chl. a,* chlorophyll a; *Chl. b,* chlorophyll b; *Total Chl.*, total chlorophyll; *Car.*, carotenoids. S_0, (nutrient solution), S_1 (nutrient solution + 34.0 mM NaCl), S_2 (nutrient solution + 68.0 mM NaCl), H_0 (non-immersion roots into H_2O_2), H_1 (roots immersion into H_2O_2 for 1h) and H_2 (roots immersion into H_2O_2 for 2h).

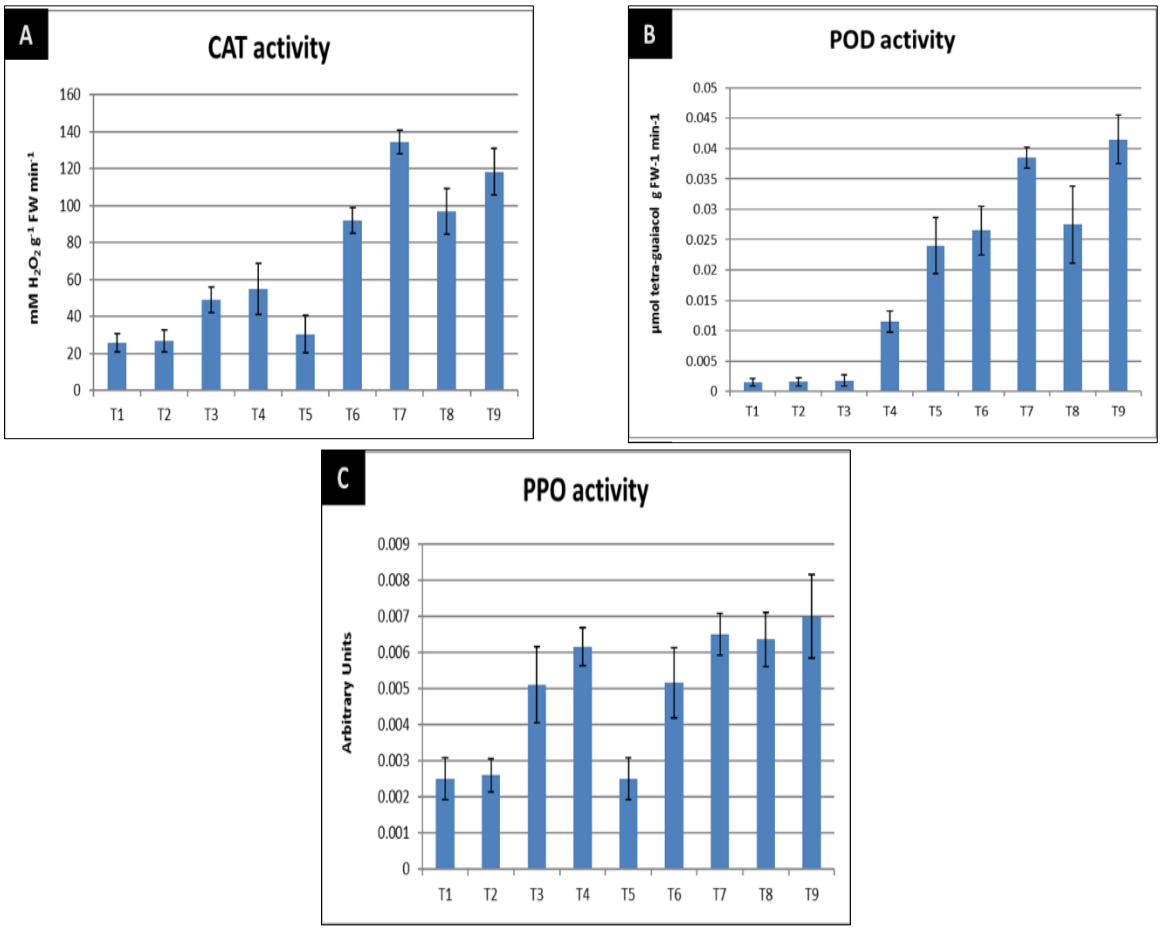

Figure 1. Effect of sodium chloride stress (0, 34.0 and 68.0 mM) and roots immersion into H_2O_2 1.0 M for 1 or 2 h as well as their interactions on antioxidant enzymes activities of strawberry plant season 2017. CAT, catalase; POD, peroxidase; PPO, polyphenoloxidase. [T_1 (S_0 H_0), T_2 (S_0 H_1), T_3 (S_0 H_2), T_4 (S_1 H_0), T_5 (S_1 H_1), T_6 (S_1 H_2), T_7 (S_2 H_0), T_8 (S_2 H_1), T_9 (S_2 H_2)]; S_0, (nutrient solution), S_1 (nutrient solution + 34.0 mM NaCl), S_2 (nutrient solution + 68.0 mM NaCl), H_0 (non-immersion roots into H_2O_2), H_1 (roots immersion into H_2O_2 for 1h) and H_2 (roots immersion into H_2O_2 for 2h).

Ultrastructural characterization of Leaflet mesophyll cells by TEM

Chloroplasts

The ultrastructure investigations of chloroplast in plants grown under normal condition (control) showed an obvious internal with compactly arranged grana stacks contained a few small starch grains and plastoglobuli. Grana and stromal lamellae with unbroken compacted thylakoids generally orientated parallel to the chloroplasts long axes (Plate 1-a). On the contrary, under high salinity level (68.0 mM NaCl) a clear change in the chloroplast structure was obvious i.e. decrease the number and size of chloroplasts and the shape of chloroplasts became rounded. In addition, the internal membranes were still intact and lamellar system was disoriented and a wavy configuration, swelling of thylakoids, as well as destruction the grana staking. Also, the stroma contained a large starch grains and increase in its number (Plate 1-b). In some chloroplasts, the starch grains converted from spiral shape to a rounded shape as compared to control and the number of plastoglobuli showed a substantial increase (Plate 1, 2-b). Roots immersion into H_2O_2 before exposure to saline conditions led to increase the chloroplast size, maintained the chloroplast structure and grana staking, reduced the number and size of starch grains as well as plastoglobuli (Plate 1-c, -f) in leaflet plants exposure to high salinity level.

Nucleus

The nuclei of mesophyll cells of control plants appeared normal, and the nuclear envelope, nucleolus, and nuclear chromatin were intact and uniformly distributed comparatively (Plate 1-d). While, the nucleus of mesophyll cells of plants grown under high salinity level (68.0 mM NaCl) were smaller with irregularity in shape as well as the nuclear chromatin was condensed, and the nucleolus has vanished in some cells (Plate 1-e). Whereas, immersion plant roots into H_2O_2 for 1h before exposure to saline conditions led to improve the plant tolerance as the nucleus appeared normal with nucleolus but the nuclear envelope was shrinkage in some positions (Plate 1-f).

Mitochondria and peroxisomes

The ultrastructural investigation showed substantial alterations in the mitochondria size and shape in strawberry plants grown under 68.0 mM NaCl compared to the control treatment. In control plants, the mitochondria appeared normal with clear double membranes as well as a normal distribution of cristae (Plate 1-g). A conspicuous feature of these experiment was the increase in the number of mitochondrial profiles

per leaf cell under salinity as compared to control plants (Plate 1-b, -e). Results in Plate (1-h), showed that an increase the number of mitochondria meanwhile, its size decreased irregularly as well as the cristae was absent or often very short and the distribution of cristae was indistinct or abnormal. In addition, the number of peroxisomes exhibits a substantial increase (Plate 1-h). While, immersion plant roots into H_2O_2 for 1h before exposure to saline condition 68.0 mM NaCl led to increasing the size of mitochondria, while, the number of peroxisomes was decrease, but its size was increased (Plate 1-i).

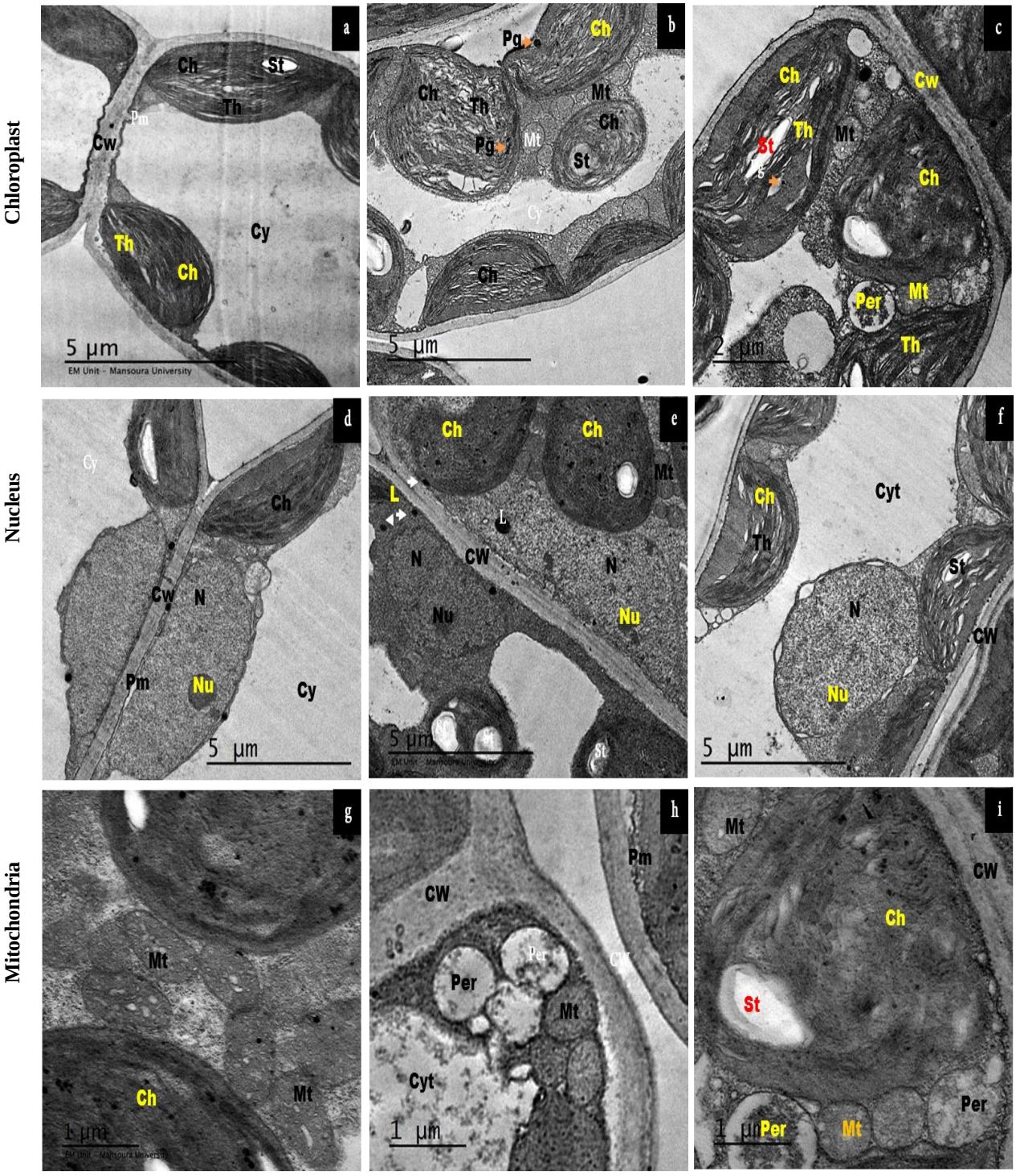

Plate 1. TEM micrographs of mesophyll cells of strawberry leaflet showing alterations in the ultrastructure of cell organelles i.e. chloroplasts, nucleus and mitochondria: a, d and g (control); b, e and h (68.0 mM NaCl); c, f and i (68.0 mM NaCl + roots immersion into H_2O_2 for 1h). Ch, chloroplast; CW, cell wall; Cyt, cytoplasm; L, lipid droplets; Mt, mitochondria; MV, membrane vesicles; Nu, nucleus; Nul, nucleolus; Per, peroxisomes; Pg, plastoglobuli; Pm, plasma membrane; St, starch grain; V, vacuole; Th, thylakoid. Scale Bar = (a-f) 5 µm and (g-i) 1 µm.

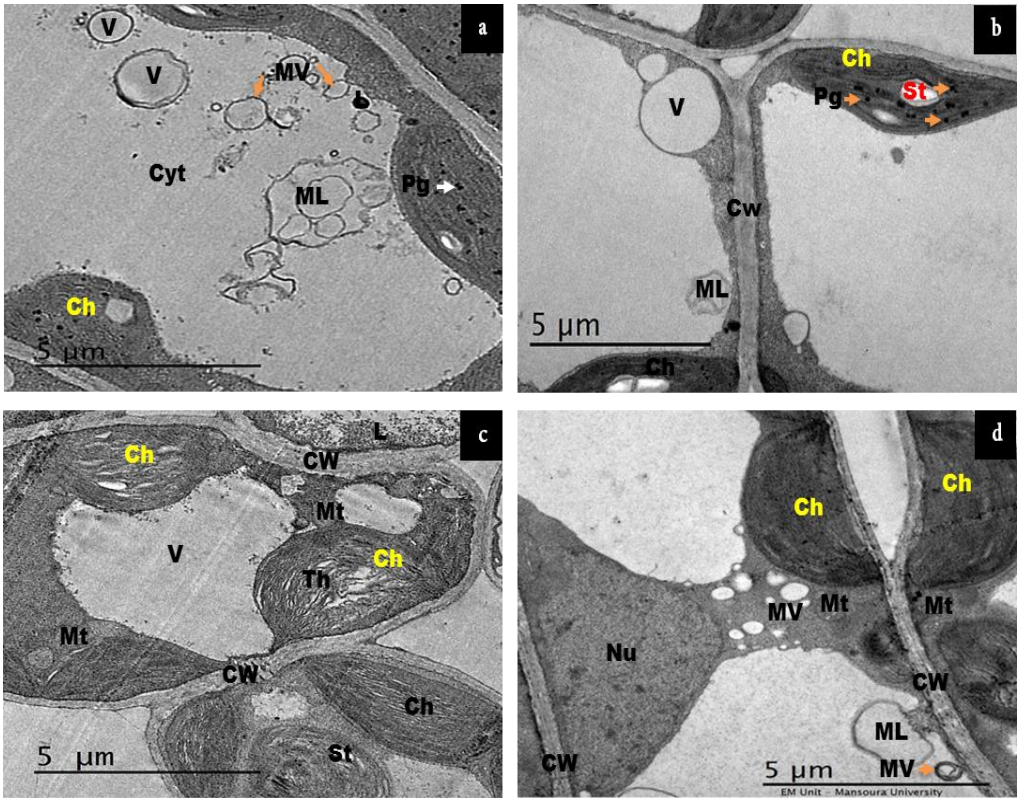

Plate 2. TEM micrographs of mesophyll cells of strawberry leaflet showing alterations in the ultrastructure of cell organelles i.e. chloroplasts, nucleus, cell wall and plasma membranes: a- d (68.0 mM NaCl). Ch, chloroplast; CW, cell wall; Cyt., cytoplasm; L, lipid droplets; ML, Myelin-Likes; Mt, mitochondria; MV, membrane vesicles; Nu, nucleus; Nul, nucleolus; Per, peroxisomes; Pg, plastoglobuli; Pm, plasma membrane; St, starch grain; V, vacuole; Th, thylakoid. Scale Bar = 5 µm.

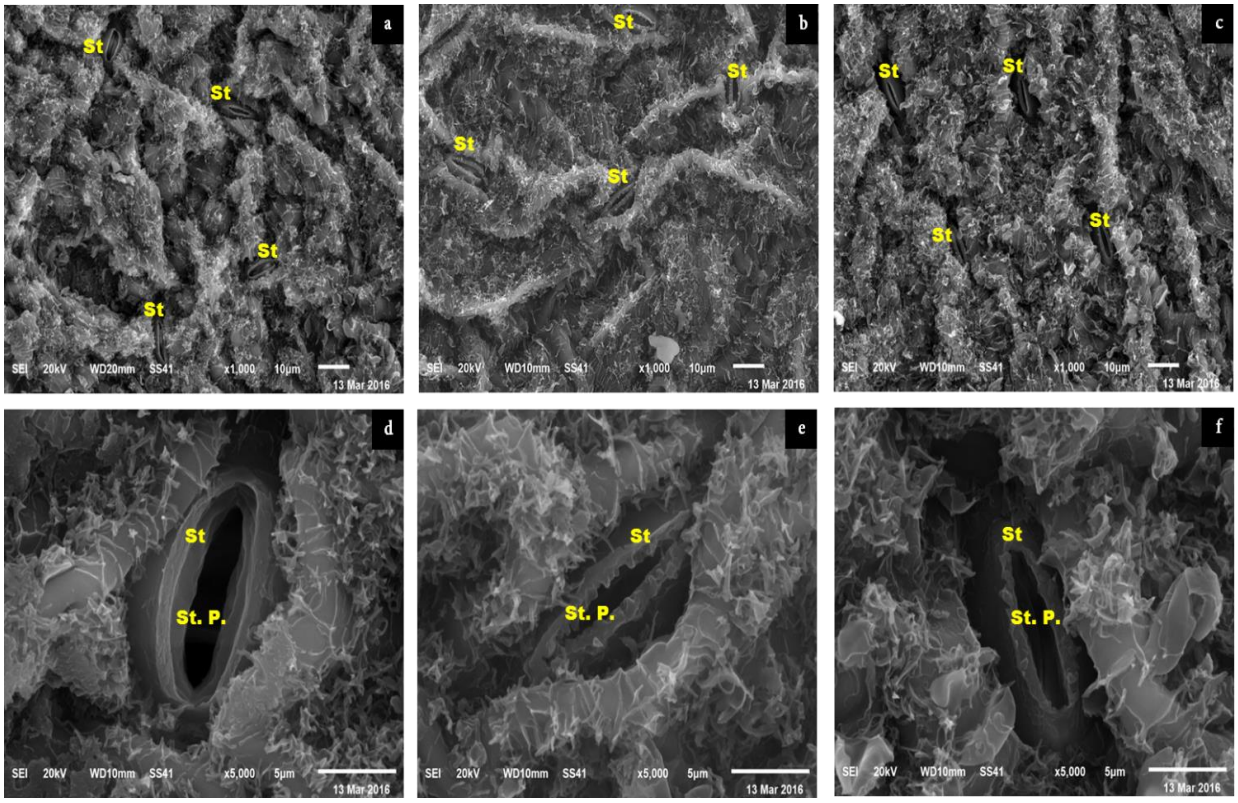

Plate 3. SEM micrographs of abaxial surface of strawberry leaflet showing alterations in the stomata (St) number, density and distribution as well as formations of cuticle wax: a, d (control); b, e (68.0 mM NaCl); c, f (68.0 mM NaCl + roots immersion into H_2O_2 for 1h). St, stomata; St.P. stomatal pore. Scale Bar = (a-c) 10 µm and (d-f) 5 µm.

Table 3. Effect of severe salinity stress (68.0 mM NaCl) with or without H_2O_2 for 1h on the thickness of cell wall, number of stomata, stomatal density and dimensions on the abaxial surface of strawberry leaves (season 2017).

Treatments	Thickness of cell wall		Stomata on abaxial surface				Dimensions of stomatal pore			
			Number (field 0.19mm^2)		Stomatal density (mm^2)		Length		width	
	µm	%	Mean	%	Mean	%	µm	%	µm	%
S_0 H_0	0.430	100.0	14.3	100.0	75.4	100.0	9.410	100.0	2.149	100.0
S_0 H_1	0.520	120.9	15.0	104.7	78.9	104.7	9.348	99.3	2.064	96.0
S_2 H_0	0.827	192.2	19.3	134.9	101.8	134.9	8.922	94.8	1.081	50.3
S_2 H_1	0.663	154.3	17.3	121.0	91.2	120.9	8.807	93.6	1.590	74.0

S_0, (nutrient solution), S_2 (nutrient solution + 68.0 mM NaCl), H_0 (non-immersion roots into H_2O_2) and H_1 (roots immersion into H_2O_2 for 1h).

Cell wall and plasma membrane

In control plant the cell wall of mesophyll was thin (0.430 µm) (Table 4). On the other hand, the cell wall in NaCl treatment 68.0 mM appeared thick (0.827 µm) (Plate 2-a, -d), and the plasma membrane was partly detected of the cell wall, increased the plasmolysis of plasma membranes, which led to increase the cytoplasmic vesiculation (membrane vesicles) from plasma membranes and fragmentation of tonoplast (Plate 2-a, -b) which absent in control (Plate 1-a). In addition, the Myelin-Likes were found in the plasma membranes (Plate 2-a, -d) with an accumulation of lipid droplets in cytoplasm (Plates 1-e and 2-a). On the other hand, roots immersion into H_2O_2 for 1h before exposure to saline condition (68.0 mM NaCl), maintained the cell wall structural and reduced its thickness (0.663 µm), and reduced the number of membrane vesicles (Plate 1-c -f) as compared to the NaCl sole treatment.

Ultrastructural characterization of abaxial leaflet surface by SEM

The SEM micrographs showed numerous variations in the stomatal number, stomatal density and the dimensions of stomatal pore (length and width) as indicated in Table (3). In general, the high salinity level (68.0 mM NaCl) resulted in a significant increase in the number of stomata (14.33 vs. 19.33 for control and high salinity level, respectively). The stomatal density increased also by about 34.9% in high salinity treatment comparing with the control treatment. Dimensions of stomatal pore (particularly stomata width) decreased sharply due to the negative effect of NaCl treatment (5.2 and 49.7 % reduction for dimensions of stomatal pore length and width, respectively). Most of the stomata on the abaxial (lower) surface of strawberry leaves were open in control treatment, meanwhile appeared closed and deep in high salinity level (68.0 mM NaCl) (Plates 3-b, -e). Besides, the condensing of the cuticle layers on the abaxial epidermis showed an increase comparing with plants grown under normal conditions (Plate 3-b, -e). Under saline conditions, H_2O_2 recorded, to some extent, an ameliorative effect against NaCl-stress as the stomata showed a partial opening although and the cuticle layers remained thick (Plates 3-c, -f). In addition, the number and the density of stomata increased only by about 21.0% comparing with 34.9% with high salinity level.

Leaflet blade structure

Data of the influence of H_2O_2 immersion on the anatomical structure of leaflet blade of strawberry plants grown under salinity stress are illustrated in Table (4) and Plate (4). It is worthily noting that, the thinner leaflets produced under high salinity level (68.0 mM NaCl) could be attributed mainly to the observed reduction in thickness of spongy parenchyma and thickness of midrib region due to the decrements induced in size of the main vascular bundle. Meanwhile, the low salinity level of NaCl (34.0 mM) led to increase the width of midrib region due to increasing the width of the main vascular bundle. In addition, the thickness of leaflet blade decreased due to the corresponding reduction in the spongy parenchyma and intercellular space. It is also obvious that roots immersion into H_2O_2 for 1h or 2h led to increase the dimensions of midrib region and main vascular bundle. Furthermore, H_2O_2 treatment for 1h led to increase the thickness of leaflet blade but H_2O_2 for 2h reduced it. Concerning the effects of the interaction between treatments, H_2O_2 caused an increase in the thickness of midrib region, dimensions of the main vascular bundle (length and width) as well as leaflet blade thickness corresponding to an increase in the thickness of palisade parenchyma. However, the thickness of spongy parenchyma showed a reduction on plants grown under low salinity level. Under high salinity level, roots immersion into H_2O_2 for 1h at (1.0 M) helps to sustain salinity effect on the anatomical structure of strawberry leaflets as compared to untreated plants under saline conditions.

Table 4. Effect of sodium chloride salinity (0, 34.0 and 68.0 mM) and immersed plant roots into H_2O_2 1.0 M for 1 or 2 hours as well as their interactions on strawberry leaflet structure (season 2017).

Treatments	Dimension of midrib				Dimension of main vascular bundle				Thickness of leaflet blade		Thickness of mesophyll				Thickness of upper Epi.		Thickness of lower Epi.	
	length		width		length		width				Palsied parenchyma		Spongy parenchyma					
	µm	%	µm	%	µm	%	µm	%	µm	%	µm	%	µm	%	µm	%	µm	%
S_0 H_0	54.0	100.0	56.3	100.0	20.3	100.0	28.3	100.0	24.5	100.0	6.8	100.0	13.5	100.0	2.0	100.0	2.5	100.0
S_0 H_1	64.0	118.5	70.0	124.4	23.8	117.3	38.5	136.3	23.3	95.0	8.2	121.8	11.6	86.2	2.9	145.5	2.4	96.1
S_0 H_2	41.0	75.9	46.5	82.7	16.5	81.5	19.8	69.9	24.0	97.8	9.5	140.6	7.8	57.6	3.6	180.3	3.1	124.0
S_1 H_0	55.3	102.3	63.5	112.9	21.3	104.9	26.8	94.7	25.5	104.1	7.8	114.8	12.6	93.5	2.6	131.3	3.0	120.0
S_1 H_1	61.5	113.9	60.8	108.0	25.0	123.5	30.5	108.0	19.2	78.2	5.4	79.4	8.9	65.9	3.0	148.2	2.0	78.2
S_1 H_2	67.3	124.5	71.8	127.6	28.8	142.0	39.0	138.1	28.0	114.1	10.1	149.9	10.6	78.7	3.4	170.8	3.2	126.5
S_2 H_0	61.3	113.4	75.3	133.8	25.3	124.7	38.8	137.2	19.5	79.8	6.8	101.2	7.4	54.8	2.9	145.5	2.4	96.1
S_2 H_1	63.3	117.1	61.0	108.4	21.5	106.2	27.0	95.6	21.7	88.6	6.6	97.5	9.0	66.5	3.5	177.1	2.7	108.8
S_2 H_2	44.8	82.9	51.5	91.6	17.3	85.2	18.0	63.7	23.6	96.3	7.0	103.1	11.3	83.9	3.5	173.9	1.9	75.9

S_0, (nutrient solution), S_1 (nutrient solution + 34.0 mM NaCl), S_2 (nutrient solution + 68.0 mM NaCl), H_0 (non-immersion roots into H_2O_2), H_1 (roots immersion into H_2O_2 for 1h) and H_2 (roots immersion into H_2O_2 for 2h).

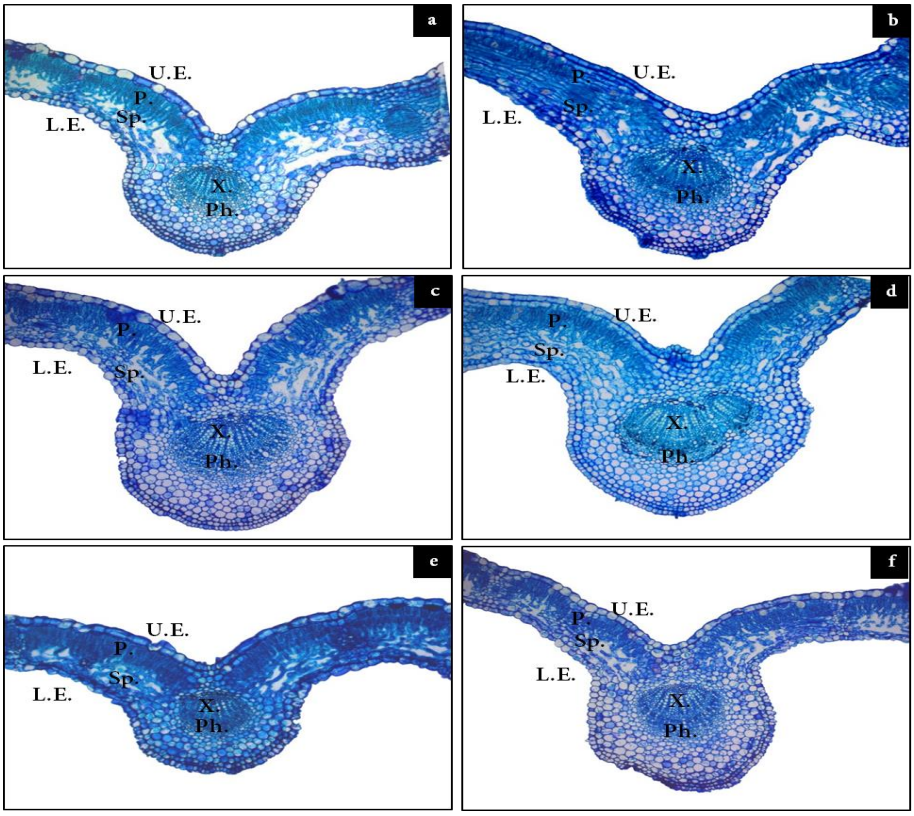

Plate 4. Cross sections micrographs of strawberry leaflet: a (control), b (roots immersion into H_2O_2 for 1h under normal condition), c (34.0 mM NaCl), d (34.0 mM NaCl + roots immersion into H_2O_2 for 1h), e (68.0 mM NaCl) and f (68.0 mM NaCl + roots immersion into H_2O_2 for 1h). U.E., upper epidermis; P., palisade parenchyma; Sp., spongy parenchyma; X., xylem; Ph., phloem; L.E., lower epidermis. (Obj.×10 * Oc.×10).

DISCUSSION

Salinity causes a multitude of biochemical and physiological changes, thereby affecting plant growth and development. Salinity reduced the plant growth resulted from ionic and osmotic imbalances and/or disturbances in water balance, which reduced availability and uptake of water and essential nutrients, internal hormonal imbalance (Schmidt, 2005) such as increase ABA and ethylene concentration and decreased level of IAA, GA3 and auxin content in plant tissue (Fricke *et al*., 2004), which lead to decreases in cell turgor. Roots immersion into H_2O_2, in most cases, resulted in a significant increase in strawberry growth parameters under normal or salinity stressed conditions. In addition, the treatment H_2O_2 for 1 h was more effective in this respect (Table 1). The stimulating effect of pre-treatment plant roots with H_2O_2 on plant growth parameters may be attributed to its physiological role on cell wall development (Carol and Dolan, 2006), formation of adventitious roots (Dunand *et al*., 2007), stomatal movement, cell growth and development (Deng *et al*., 2012) and stomatal aperture regulation as well as thereby photosynthetic pathways (Jarvis *et al*., 1999; Ge *et al*., 2015). Furthermore, H_2O_2 enhanced photosynthetic pigments and leaf relative water content (Table 2), stimulating several plant hormones (Barba-Espin *et al*., 2010). H_2O_2 pre-treatment compensated the negative effects of salinity and led to higher growth parameters and improvements, in most cases, all growth parameters compared to the control plants. In this respect, Uchida *et al*. (2002) found that pretreating rice seedlings with low level of H_2O_2 (<10 µM) led to increase salt tolerance by enhancing active oxygen scavenging enzyme activities and enhancing expression of transcripts for stress-related genes including sucrose-phosphate synthase and Δ´-pyrroline-5-carboxylate synthase.

The decline in chlorophyll concentration under saline stress are commonly phenomenon and it may be due to different reasons like an inhibition of chlorophyll biosynthesis from an activation of the chlorophylls (Hanafy *et al*., 2002) and/or decrease the rate of chlorophyll biosynthesis (Mitsuya *et al*., 2003) and/or increase ABA content resulting in promoting chlorophyll breakdown (Hatung, 2004) and/or the interference of Na^+ and Cl^- with the activity enzymes associated with chlorophyll biosynthesis or a disturbance in the integration of the chlorophyll molecules in stable complex (Husaini and Abdin, 2008) and/or a disturbed chloroplast structure, number and size (Arafa *et al*., 2009). The results of this study suggest that the immersion plant roots into H_2O_2 might compensate for the negative effect of salt stress on concentrations of chlorophylls and carotenoids pigment. Similar results were obtained by Khan *et al*. (2015) and Hasan *et al*. (2016).

Electrolyte leakage percentage was raised with increasing salinity levels in nutrient solutions as compared to the control (Table 2). Similarly, the same increasing trend of electrolyte leakage was observed by Faghih *et al*. (2018). Mitigation role of H_2O_2 pre-treatment on salt-induced cell damage also been reported previously in maize by Azevedo Neto *et al*. (2005) and strawberry by Christou *et al*. (2014),

they revealed that application of H_2O_2 to salt-subjected plants caused a significant decrease in EL%.

Relative water content is an important physiological parameter for determining the water status of the plants. In this study, the relative water content of strawberry plants decreased linearly with increasing salinity levels in nutrient solutions. This decline can be attributed to reduce the plant roots their ability to water uptake through a reduction of the absorbing surface (Yildirim *et al*., 2008), or the increase salt concentration in the external environment (Khan *et al*., 2015). Furthermore, the presence of high salinity levels in growth media is also responsible for several deteriorations for cell walls through (i) Na^+ displacement with Ca^+ from the binding sites, thereby reducing pectin crosslinking and retarding cell elongation, (ii) alterations in the chemical composition of root cell walls (e.g. pectin content), thus losing its normal functionality, (iii) changes in root diffusion barriers, (iv) modulating functionalities of cell wall proteins (Byrt *et al*., 2018). However, roots immresion in H_2O_2 has an ameliorative impact on RWC% of strawberry plants under salt stress, this effect may be due to the higher concentration of osmoregulation in tissues and cells, increase the osmotic adjustment as well as increase the relative water content inside the cells, this results in a greater membrane stability index (Ghadakchiasl *et al*., 2017 and Kholghi *et al*., 2018). Therefore, such an ameliorative effect on RWC% may have been due to the role of H_2O_2 in osmotic adjustment and ensuring the accumulation of compatible solutes under salinity stress conditions.

Salt stress causes diversified adverse effects in plants. Of them, trigger a higher production of ROS is a common phenomenon which leads to oxidative stress. In addition, plants can scavenge/detoxify ROS by producing different types of enzymatic and non-enzymatic antioxidants (Corpas and Barroso, 2013). The activation of certain antioxidative enzymes such as CAT, POD, PPO, and SOD, leading to scavenge ROS (Abdelaal *et al*., 2018). CAT is thus well-positioned to remove excess H_2O_2 before it can leak out into other parts of the cell, (Ali and Alqurainy, 2006). In addition, Jaleel et at. (2007) found that the antioxidant enzymes POD and CAT were increased under NaCl salinity treatment. Furthermore, Li (2009) revealed that, the CAT activity increased less than 100 mM NaCl, but decreased less than 200-300 mM. While, the POD activity decreased gradually at the concentration 100-300 mM NaCl on tomato seedling. In this study, increases within the activity of antioxidants enzymes by H_2O_2 treatment could play vital roles in alleviating the toxicity of ROS induced by NaCl. These results suggest that H_2O_2 is involved in regulating of antioxidant defence pathway and thus alleviates oxidative damage under stress conditions (Abdelaal *et al*., 2017 and 2018). Under salinity stress conditions, CAT induced by H_2O_2 plays an important role in the enhancement plant defense system. These findings indicate that the activation of these antioxidant enzymes (CAT, POD and PPO) were increased because of NaCl salinity exposure and H_2O_2 treatment, thereby providing enhanced tolerance against salinity stress.

From TEM images of strawberry cell structure, showed alterations in plants subjected to saline stress, especially chloroplasts. The swelling of thylakoids membranes is probably due to change in the ionic composition of stroma liquid (Yamane *et al*., 2003) and osmotic imbalance between stroma and cytoplasm (Naeem *et al*., 2012). In addition, the degradation of the membranes of the chloroplasts is associated with salinity may be induced by overproduction of $O_2^{\cdot-}$ ion, which causes oxidative stress on ultrastructural (Hernández *et al*., 1995). In the present study, there are various ultrastructural changes associated with such as the chloroplasts contained a large starch grains (Plate 1-b, -e). Rahman *et al*. (2000) verified that, the increase of starch grains accumulation in chloroplast under saline conditions is due to the damage of enzymes involved in starch degradation via changes in the ionic compositions in the chloroplast and/or the damage of the sucrose phosphate biosynthesis in the cytosol leading the triose phosphate pathways toward starch biosynthesis. The increase in the number and size of plastoglobuli in chloroplast in plant cells exposed to NaCl salinity observed in the present study (Plate 2-b), agree with Rahman *et al*. (2000) and Bejaoui *et al*. (2016). On the other hand, pre-treatment with H_2O_2 for 1h led to decrease the deleterious effects of NaCl stress in the structure of cell organelles of strawberry plants. In this respect, Uchida *et al*. (2002) suggested that superoxide radicals are the toxic byproducts of oxidative metabolism that can interact with H_2O_2 to form highly reactive hydroxyl radical, which are thought to be primarily responsible for oxygen toxicity in the cells.

The damaged elicited by NaCl-treatment in mitochondria are probably indication of salt associated alteration in mitochondria energy status resulting in decline ATP levels (Pareek *et al*., 1997). Other reports refer this damage to a high accumulation of ions responsible for salinity stress (Na^+ and Cl^-) taking into consideration the high sensitivity of mitochondria to the accumulation of these ions in mesophyll cells (Rahman *et al*., 2002). The presence of membrane vesicles in the strawberry cells exposed to NaCl stress agrees with results obtained by Rahman *et al*. (2000). These membrane vesicles are considered as adaptive strategy for Na^+ ions sequestration to alleviate their hazardous effect to cytoplasm and cell organelles (Rahman *et al*., 2002). Myelin-Likes, which considered as artifacts formed during the double fixation with glutaraldehyde and osmium tetroxide, were only existed in NaCl-stressed plants. These artifacts, however, were not observed in control treatment as an indication to the ultrastructure alterations in the membrane of NaCl-stressed plants (Bowers and Maser, 1988; Rahman *et al*., 2001). In addition, the presence of lipid droplets in the cytoplasm (Plates 1-e and 2-a) may be considered as a reserve of energy to be used by the cell to cover the increased demand in metabolic energy required to salinity tolerant (Rahman *et al*., 2000). In this concern, Li *et al*. (2008) suggested that abiotic stress generally affects membrane lipid by increasing the production ROS, which accelerate the peroxidation of membrane lipids and leads to a loss membranes integrity.

In the present study, stomatal density (SD) and the dimensions of stomatal pore varied among different treatments. Therefore, SD and size can be considered as common characteristics for understanding the adaptation or response of plant species to changing environmental conditions at large spatial scales (Wang *et al*., 2014). The SD is strongly influenced by salinity stress. Under severe salinity stress, the SD and the dimensions of stomatal pore at the abaxial surface of leaves of the strawberry plants showed an obvious increase. These changes contribute to optimizing the use of assimilates and water use efficiency in periods when water availability is decreased. As mentioned above, high SD

provides the capacity for rapid increase in the leaf stomatal conductance that maximizes CO_2 diffusion into the leaf during favorable conditions for photosynthesis. Moreover, under high salinity treatment, the most of stomata appeared closed and deep (plate 3-b, -e) as a common tolerance strategy against NaCl-induced stress and drought-avoidance arising from salinity stress because it is minimizing water loss by decreasing transpiration because of stomatal closing under water-stressed conditions (Klamkowski and Treder, 2006).

In this study, the morphological alterations and the variations in mesophyll tissues as well as vascular tissues are common response associated with the anatomical and ultrastructural alterations of leaf plant cells. The functional adaptation at the morphological level seemed to act on the leaf size and thickness (Toscano *et al*., 2018). High salinity level (68.0 mM NaCl) led to increase the thickness of palisade parenchyma as compare to control treatment. Toscano *et al*. (2018) suggested that the increase in palisade tissue thickness are related with an increase in chloroplasts number as well as a decrease in the thickness of spongy tissue, which facilitate CO_2 reaching to chloroplasts in the palisade parenchyma. These anatomical alterations could be an adaptation strategy to facilitate photosynthesis process under saline stress conditions. The hazard effect of high salinity on leaflet structure could be related to: (i) vascular elements growth inhibition, which correlated with procambial activity inhibition (Rashid *et al*., 2004), (ii) inhibition effects on the activity of the initial cells forming the leaflet blade following cell division and enlargement (Khafagy *et al*., 2009), and (iii) reduction in the dimensions of the main vascular bundle (Table 4). The positive effect of low salinity level on palisade tissue of strawberry leaflet may be due to developments of large cells and multilayer of palisade parenchyma (Plate 4).

In conclusion, roots immersion of strawberry plants into H_2O_2 for 1h proved to be useful in enhancing plant growth and physiological parameters under normal or salinity conditions. Based on the results of this study, it can be concluded that the growth increment under H_2O_2 pre-treatment was found to be associated with increasing photosynthetic pigment concentration and relative water content, and reduced electrolyte leakage percentage, as well as ameliorative the salinity injuries by enhancing the antioxidants enzymes activity which is involved in scavenging the ROS produced during salinity stress as well as improved anatomical and ultrastructural alterations in cell organelles i.e. chloroplast, mitochondria, nucleus and plasma membranes of plants that increased plant resistance to salinity stress.

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استجابة نباتات الفراولة النامية في نظام الهيدروبونيك للمعاملة المسبقة بفوق أكسيد الهيدروجين قبل التعرض للإجهاد الملحي

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اجريت تجربة الهيدروبونيك لدراسة تأثير فترات غمس جذور نباتات الفراولة في فوق أكسيد الهيدروجين لمدة ساعة أو ساعتين قبل التعرض لملوحة كلوريد الصوديوم (0، 34و 68 ملليمول). ادي غمس جذور النباتات في فوق أكسيد الهيدروجين قبل التعرض لظروف ملوحة كلوريد الصوديوم الي زيادة نمو النباتات وتركيز صبغات البناء الضوئي والمحتوي المائي النسبي في الاوراق ونشاط الانزيمات المضادة للأكسدة مثل (الكاتاليز، البيروكسديز والبولي فينول اكسديز) وكذلك إنخفاض نفاذية الأغشية للمواد الذائبة بالمقارنة بالنباتات الغير معاملة. أدي مستوي الملوحة المرتفع من كلوريد الصوديوم إلي حدوث تغييرات في التركيب الدقيق لخلايا النسيج المتوسط للوريقات مثل انتفاخ أغشية الصفائح، واختلال ترتيب اقراص الجرانا، زيادة عدد البلاستوجلوبيول وحبيبات النشا، وكذلك زيادة عدد وحجم البيروكسوسوم والميتوكونريا وتغير تركيبها، تدهور التركيب الدقيق للأغشية البلازمية وانكماشها وزيادة تكوين أشكال ميلين والحويصلات الغشائية، وزيادة سمك جدار الخلية. بالإضافة إلى ذلك ، ادي غمس جذور النباتات في فوق أكسيد الهيدروجين قبل التعرض لظروف لملوحة كلوريد الصوديوم إلى الحفاظ على التركيب الدقيق للبلاستيدات الخضراء، تراص أقراص الجرانا، وتضخم البلاستيدات الخضراء والميتوكوندريا، وانخفاض عدد وحجم حبيبات النشا والبلاستوجلوبيول، وانخفاض عدد الحويصلات الغشائية والبيروكسوسوم ، وكذلك الحفاظ على تركيب جدار الخلية ونقص سمكه. وعلاوة على ذلك ، أدى ارتفاع مستوى كلوريد الصوديوم إلى زيادة عدد الثغور والكثافة الثغريه وانخفاض أبعاد فتحة الثغر. على العكس من ذلك ، ادي غمس جذور النباتات في فوق اكسيد الهيدروجين إلي نقص الكثافة الثغرية وعدد الثغور.فيما يتعلق بتشريح الوريقات، أدي التركيز المنخفض من كلوريد الصوديوم إلي زيادة أبعاد العرق الوسطي والحزمة الوعائية الرئيسية وكذلك سمك البارنكيما العمادية. بينما أدي تركيز الملوحة المرتفع في معظم الحالات إلي نقص جميع هذه الصفات التشريحية.

الخلاصة، ادي غمس جذور النباتات (المعاملة المسبقة) في فوق أكسيد الهيدروجين بتركيز 1 مول لمدة ساعة قبل التعرض لملوحة كلوريد الصوديوم الي تقليل الاثار الضارة الناتجة عن الإجهاد الملحي.