American-Eurasian J. Agric. & Environ. Sci., 16 (2): 294-301, 2016
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2016.16.2.12864

Hydrogen Peroxide Alleviates Salt-Stress in Two Onion (*Allium cepa* L.) Cultivars

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Abstract: As one of the active oxygen species that is widely generated in many biological systems and mediates various physiological and biochemical processes in plants, exogenous hydrogen peroxide (H_2O_2) in very low concentrations improves salt-tolerance in some plant species. Two field experiments were conducted in 2013/14 and 2014/15 to study the effect of foliar sprays at concentrations of 1 and 2 mM H_2O_2 on growth, yield, plant water relations and osmoprotectants in two onion varieties (i.e., Giza 20 and Giza Red) grown under saline soil condition (EC_e = 7.94 - 8.81 dS /m). Exogenous H_2O_2 application enhanced salt stress tolerance in onion plants by improving the photosynthetic efficiency and plant water status as evaluated by relative water content and membrane stability index. These results were positively reflected by the increase in plant growth, productivity and water use efficiency under salt stress conditions. Results of this study suggested that H_2O_2 could activate the photosynthetic system and improve the physiological attributes in plants grown under the adverse conditions of newly-reclaimed saline soils.

Key words: *Allium cepa* · Salinity · Hydrogen peroxide · Growth and yield · Water relations · Photosynthetic efficiency

INTRODUCTION

Salt stress is considered one of the major limiting factors to plant growth and crop production in many areas, particularly in arid and semi-arid regions. Salt stress negatively affects plant morphology and physiology through osmotic and ionic stress and changes biochemical responses in plants [1]. It causes osmotic stress (a physiological drought problem), adversely affecting water relations and ion homeostasis in plants, leading to toxic-ion effects on metabolic processes [2]. This, in turn, leads to an excessive generation of reactive oxygen species (ROS), which causes damage to lipids, proteins and DNA [3]. Negative effects generated under salt stress are usually caused by elevated Na^+ and Cl^- concentrations in soil or irrigation water. Salt stress decreases photosynthetic attributes [4], plant growth and development [5] and stimulates the activity of antioxidant system [6, 7]. To overcome these salt stress effects, plants develop several mechanisms to induce their tolerance such as ion homeostasis, osmotic adjustments, stress damage control and repair and growth regulation [8].

Onion (*Allium cepa* L.) is one of the most commercially valuable vegetables grown worldwide. The average annual production in the last five years in Egypt is put at 2, 113, 749 tons [9]. Onions are rated as a salt sensitive crop, bulb yield severely declines for every unit increase in soil salinity (ECe) [10].

In recent years, a growing interest has been observed with some ROS in very low concentrations to support plant growth in stress conditions, including hydrogen peroxide (H_2O_2). H_2O_2 is a signaling molecule in plants [11] and acts as a second messenger in response to various stresses, including salt stress in plants [12, 13]. It acts as a key regulator in a wide range of physiological processes such as photosynthesis, growth and development [14, 15]. Recent works show that exogenous application of H_2O_2, at low concentrations, significantly improved the antioxidant defense system in salt-stressed plants [13, 16]. It plays a dual role in plants under both normal and stress conditions and at high concentration it initiates programmed cell death [17]. However, at low concentrations H_2O_2 acts as a signal molecule and is involved in acclamatory signaling triggering tolerance against salt stress [18, 13]. The objective of this study was to evaluate the potential of foliar H_2O_2 application to alleviation of salt stress in onion plants and study their growth, yield, photosynthetic pigments, osmoprotectants, water relations and photosynthetic system efficiency in the presence of both salinity and H_2O_2 applications.

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The hypothesis tested is that H_2O_2 will enhance the activity of the photosynthetic system and accordingly will protect the stress generated by soil salinity. In addition, H_2O_2 will help in enhancing onion growth and production under the adverse effects of soil salinity.

MATERIALS AND METHODS

Plant Material, Experimental Design and Treatments: Two field experiments were conducted in 2013/14 and 2014/15 at a private farm in Sennoris District, Fayoum, Southwest Cairo, Egypt between latitudes 29° 02´ and 29° 35´ N and longitudes 30° 23´ and 31° 05´ E. The climatic data of studied area indicate that the total rainfalls does not exceed 7.5 mm/year and the mean minimum and maximum annual temperatures are 14.5 and 31.0°C in January and June, respectively. The evaporation rates coincide with temperatures where the lowest evaporation rate (1.9 mm/day) was recorded in January while the highest value (7.3 mm/day) was recorded in June. According to the aridity index [19]; the area is located under hyper-arid climatic condition. These landforms are characterized by less than 3.5% surface slopes with an elevation vary from 49 m below sea level to 26 m above sea level.

Healthy seeds of two varieties [i.e., Giza 20 and Giza Red] of onion (*Allium cepa* L.) were sown on 30 and 25 September 2013 and 2014 respectively. The produced transplants were transported and replanted on the 7th and 10th of December respectively and harvested on 6 May for both seasons. The experimental layout was a randomized complete block design with three replications. Total surface area used for the experiment was 550 m^2 divided into 18 experimental plots of 16.5 m^2 each (1.1 m wide × 15 m long). Each experimental unites were separated by two guard rows to protect against border effects. These plots included eight planting rows placed 10 cm apart with a distance of 15 cm between plants. These plant densities are a typical of onion production in Egypt. The soil was fertilized with NPK fertilizer according to the recommendations of the Ministry of Agriculture and Land Reclamation (450 kg ha^{-1} ammonium nitrate (33.5% N), 400 kg ha^{-1} calcium super-phosphate (15.5% P_2O_5) and 150 kg ha^{-1} potassium sulphate (48% K_2O).

Onion plants were irrigated in 2 d intervals using the amounts of applied water as 100% in a drip irrigation method. The daily ETo was calculated from weather data according to FAO-PM [20].

All other recommended agricultural, disease and pest management practices were followed as recommended by the Egyptian Ministry of Agriculture and Land Reclamation.

Water and soil analyses were carried out according to [21] and [22] and the data are shown in Tables (1 & 2). Based on the EC values shown in Table (2), the soil is classed as being strongly saline according to [23]. In addition, according to [24] scale the used irrigation water lies within the second categories for salinity and sodicity levels (C_2S_1, ECiw = 0.75 – 3.00 dS/m and SAR < 6.0). The experiments were arranged in a randomized complete block design, with 3 levels of H_2O_2 (0, 1.0 and 2.0 mM) and three replicate plots.

Twenty days after transplanting (DAS), onion seedlings in each plot were sprayed to run-off with 0 (tap water as a control), 1.0 and 2.0 mM H_2O_2 and then the sprays were repeated at 40 and 60 DAS. The concentrations of H_2O_2 and the number and timing of sprays were based on results from a preliminary pot trial (data not shown). To ensure optimal penetration into leaf tissues, 0.1% (v/v) Tween-20 was added to the foliar sprays as a surfactant.

Measurement of Plant Growth Traits, Yields and Water Use Efficiency (WUE): Ninety-day-old onion plants were carefully removed from each experimental plot and the lengths of their shoots were measured using a meter scale. Numbers of leaves per plant were counted. Leaf areas were measured manually using a graph sheet, where the squares covered by the leaf were counted to note the leaf area. The shoots of plants were weighed to record their fresh weights and then placed in an oven at 70ºC till the constant weight to record their dry weights. At the end of experiments, all onion plant in each experimental plot were removed to estimate the bulb yield in 3 sizes; < 5.0 cm, 5 – 7.5 cm and >7.5 cm, which were then collected together to measure the total bulb yield.

WUE values as kg yield per m^3 of applied water were calculated for different treatments after harvest according to the following equation [25]:

$$\text{WUE} = \frac{\text{Bulb yield (Kg ha}^{-1})}{\text{water aplied (m}^3\text{ ha}^{-1})}$$

Determination of Leaf Photosynthetic Pigments and Chlorophyll Fluorescence: Total chlorophyll and carotenoids were extracted and determined (in mg g^{-1} FW) following the procedure given by [26].

Table 1: Chemical composition of irrigation water

Ionic concentration (Meq/l)										
CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{++}	Mg^{2+}	Na^+	K^+	EC[a] (dS/m)	pH	SAR[b]
2013/14										
0.00	4.35	16.73	6.82	7.34	6.84	12.4	1.32	2.67	7.46	5.38
2014/15										
0.00	4.21	15.32	6.41	6.42	5.39	12.71	1.42	2.53	7.41	7.40

[a]EC means the average electrical conductivity, [b]SAR means sodium adsorption ratio.

Table 2: Some initial chemical and physical properties of the experimental soil

	Value	
Chemical properties	2013/14	2014/15
pH [at a soil: water(w/v) ratio of 1:2.5]	7.75	7.86
ECe (dS/m; soil - paste extract)	7.94	8.81
$CaCO_3$ (%)	4.83	4.81
Organic matter (%)	1.20	1.10
N (%)	0.005	0.004
P (mg/kg soil)	530.20	523.80
K (mg/kg soil)	71.20	69.90
Fe (mg/kg soil)	3.60	3.40
Mn (mg/kg soil)	10.64	10.60
Zn (mg/kg soil)	0.72	0.70
Cu (mg/kg soil)	0.53	0.50
Physical properties		
Sand %	79.2	78.27
Silt %	10.0	9.80
Clay %	10.8	10.55
Texture class	Loamy sand	Loamy sand

Fresh leaf samples (0.2 g) were homogenized in 50 ml 80% (v/v) acetone and then centrifuged at 10, 000 × *g* for 10 min. The absorbance of the acetone extract was measured at 663, 645 and 470 nm using a UV-160A UV-visible recording spectrometer (Shimadzu, Kyoto, Japan).

On two different sunny days, chlorophyll fluorescence was measured using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK). One fully expanded mature leaf was chosen per plant to conduct the fluorescence measurements. Maximum quantum yield of PS II F_v/F_m was calculated using the formulae; $F_v/F_m = (F_m - F_0) / F_m$ [27]. F_v/F_0 reflects the efficiency of electron donation to the PSII RCs and the rate of photosynthetic quantum conversion at PSII RCs. F_v/F_0 was calculated using the formulae; $F_v/F_0 = (F_m - F_0) / F_0$ [28]. Performance index of photosynthesis based on equal absorption (PI_{ABS}) was calculated as reported by [29].

Measurement of Free Proline and Total Soluble Sugars (TSS): Proline was extracted and determined (in mg per g of leaf DW) using the method described by [30]. A dried leaf sample (0.5 g) of leaf tissue was ground in 10 ml of 3% (v/v) sulphosalicylic acid and the mixture was then centrifuged at 10, 000 × *g* for 10 min. Two-ml of freshly prepared acid-ninhydrin solution was added to 2 ml of the supernatant in a test-tube and the tube was incubated in a water bath at 90ºC for 30 min. The reaction was terminated in an ice-bath and the reaction mixture was then extracted with 5 ml of toluene and vortex-mixed for 15 s. The tube was allowed to stand for at least 20 min in the dark at room temperature to separate the toluene and aqueous phases and the toluene phase was then collected carefully into a test tube. The absorbance of the toluene phase was read at 520 nm and the concentration of proline was determined from a standard curve prepared using analytical grade proline and expressed as mg per g of leaf DW.

TSS were extracted and determined according to [31]. A dried leaf sample (0.2 g) was homogenized in 5 ml of 96% (v/v) ethanol and then washed with 5 ml 70% (v/v) ethanol. The extract was centrifuged at 3500 × g for 10 min and the supernatant was stored at 4°C prior to determination. The reaction mixture of 0.1 ml of the ethanolic extract and 3 ml of freshly-prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] was placed in a boiling water bath for 10 min and was then cooled. The absorbance of the mixture was recorded at 625 nm using a Bauschand Lomb-2000 Spectronic Spectrophotometer.

Determination of Membrane Stability Index (MSI) and Relative Water Content (RWC): The MSI was determined using duplicate 0.2 g samples of fully-expanded leaf tissue [6]. The leaf sample was placed in a test-tube containing 10 ml of double-distilled water. The content of the test-tube was heated at 40°C in a water bath for 30 min and the electrical conductivity (C_1) of the solution was recorded using a conductivity bridge. A second sample was boiled at 100°C for 10 min and the conductivity was measured (C_2). The MSI was calculated using the formula:

$$MSI(\%) = \left[1 - \left(\frac{C1}{C2}\right)\right] \times 100$$

RWC was estimated using 2 cm-diameter fully-expanded leaf discs [32]. The discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes for 24 h, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry mass (DM) was recorded after dehydrating the discs at 70°C until the constant weight. The RWC was then calculated using the following formula:

$$RWC(\%) = \left[\frac{(FM - DM)}{(TM - DM)}\right] \times 100$$

Statistical Analysis: The statistical analysis of the experimental data was carried out using ANOVA procedures in GenStat statistical package (version 11) (VSN International Ltd, Oxford, UK). Combined analysis of data of the two seasons was conducted and difference between means was compared using least significant difference test (LSD) at 5% level ($p \leq 0.05$).

RESULTS

Growth Traits of Onion Plants as Affected by H_2O_2 under Saline Soil Conditions: Significant reduction ($P < 0.05$) was observed in growth parameters [i.e., shoot length, number of leaves, leaf area, shoot fresh weight (FW) and shoot dry weight (DW)] of the two varieties of onion plants under salt stress condition. However, exogenous H_2O_2 remarkably promoted onion plants growth under salt stress (Table 3). The level of 1 mM H_2O_2 was found to be more effective, increasing shoot length, number of leaves, leaf area, shoot FW and shoot DW by 16.6 and 63.7%, 17.1 and 45.7%, 42.2 and 146.0%, 44.6 and 65.2% and 46.7 and 68.7% in the Giza Red and Giza 20 varieties, respectively compared to their controls. The data also indicate that the Giza 20 variety was more sensitive to soil salinity, while it was more responsive to the foliar application with 1 mM H_2O_2.

Bulb Yields and Water Use Efficiency (WUE) of Onion Plants as Affected by H_2O_2 under Saline Soil Conditions: Table 4 shows the weights of the different sizes of bulb yields, total bulb yield and WUE of the two varieties under saline soil conditions (EC_e = 7.94 - 8.81). Bulb yield, particularly the size of 5.0 – 7.5 cm that consumer preferences, total yield and WUE of the two varieties of onion plants were significantly increased by the foliar application of H_2O_2 compared to untreated control plants. The applied level of 1 mM H_2O_2 was noticed to be more effective, significantly increasing the 5.0 – 7.5 cm-size bulb yield, total yield and WUE by 118.8 and 112.1%, 74.9 and 30.7% and 75.2 and 30.7% in the Giza Red and Giza 20 varieties, respectively compared to their controls. The data also show that the Giza Red variety had more yield and WUE than the Giza 20 variety which was more sensitive to soil salinity.

Leaf Photosynthetic Pigments and Chlorophyll Fluorescence of Onion Plants as Affected by H_2O_2 under Saline Soil Conditions: Changes in photosynthetic pigment contents and the photosynthetic system efficiency were evaluated 90 days after transplanting (Table 5). Foliar application of H_2O_2 significantly increased total chlorophyll, total carotenoids, *Fv/Fm*, Fv/F_0 and PI of the two varieties of onion plants compared to those of the control plants that received no exogenous H_2O_2. The applied level of 1 mM H_2O_2 was more effective, increasing the above attributes by 40.0 and 28.0%, 19.2 and 24.0% and 9.5 and 11.0%, 93.9 and 85.3% and 151.2 and 134.0% in the Giza Red and Giza 20 varieties, respectively compared to their controls.

Osmoprotectants, Membrane Stability Index (MSI) and Relative Water Content (RWC) as Affected by H_2O_2 under Saline Soil Conditions: Data introduced in Table 6 showed that the application of H_2O_2 significantly reduced the concentrations of free proline and total soluble sugars (TSS) and significantly increased MSI and RWC in the

Table 3: Effects of spray treatments with H_2O_2 (mM) on growth traits of two varieties of onion plants under saline soil condition

Treatment						
Variety	H_2O_2	Shoot length (m)	Number of leaves/plant	Leaf area/ plant (dm^2)	Shoot fresh weight/plant (g)	Shoot dry weight/ plant (g)
Giza Red	0	0.72 ± 0.04b[#]	8.2 ± 0.4c	16.1 ± 0.8b	72.6 ± 4.3c	7.5 ± 0.4b
	1	0.84 ± 0.05a	9.6 ± 0.5ab	23.7 ± 1.2a	105.0 ± 5.8ab	11.0 ± 0.6a
	2	0.81 ± 0.04a	9.2 ± 0.5b	22.5 ± 1.3a	100.7 ± 5.5b	10.6 ± 0.5a
Giza 20	0	0.52 ± 0.03c	7.0 ± 0.3d	10.0 ± 0.6c	64.3 ± 1.5d	6.7 ± 0.2c
	1	0.85 ± 0.04a	10.2 ± 0.7a	24.6 ± 1.5a	106.2 ± 5.7ab	11.3 ± 0.7a
	2	0.78 ± 0.07a	9.6 ± 0.6ab	22.1 ± 1.6a	114.3 ± 6.4a	10.6 ± 0.6a

[#]Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by least-significant difference test (LSD) at $P \leq 0.05$.

Table 4: Effects of spray treatments with H_2O_2 (mM) on yields and water use efficiency (WUE) of two varieties of onion plants under saline soil condition

Treatments		Yield (t/ha) of different sizes				
Variety	H_2O_2	< 50 mm (ton/ha)	50 - 75 mm (ton/ha)	>75 mm (ton/ha)	Total yield (ton/ha)	WUE (kg bulbs/m^3 of water)
Giza Red	0	2.0 ± 0.1d[#]	13.8 ± 0.6d	14.9 ± 0.7c	30.7 ± 0.9e	3.51 ± 0.12e
	1	2.5 ± 0.2cd	30.2 ± 1.8a	21.0 ± 1.1a	53.7 ± 1.5a	6.15 ± 0.20a
	2	2.2 ± 0.2cd	28.3 ± 1.7a	18.3 ± 0.9b	48.8 ± 1.2b	5.56 ± 0.18b
Giza 20	0	6.8 ± 0.4a	11.6 ± 0.5d	18.4 ± 0.9b	36.8 ± 1.0d	4.20 ± 0.14d
	1	3.7 ± 0.3b	24.6 ± 1.3b	19.8 ± 1.0ab	48.1 ± 1.2b	5.49 ± 0.17b
	2	2.8 ± 0.3c	22.5 ± 1.2c	15.8 ± 0.8c	41.1 ± 1.1c	4.69 ± 0.15c

[#]Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by least-significant difference test (LSD) at $P \leq 0.05$.

Table 5: Effects of spray treatments with H_2O_2 (mM) on leaf photosynthetic pigments and chlorophyll fluorescence of two varieties of onion plants under saline soil condition

Treatments						
Variety	H_2O_2	Total chlorophyll (mg/g FW)	Total carotenoid (mg/g FW)	F_v/F_m	F_v/F_0	PI
Giza Red	0	2.5 ± 0.1c[#]	0.26 ± 0.01b	0.74 ± 0.02b	2.29 ± 0.11c	2.09 ± 0.60d
	1	3.5 ± 0.2a	0.31 ± 0.02a	0.81 ± 0.03a	4.44 ± 0.23a	5.25 ± 0.83a
	2	3.1 ± 0.2b	0.30 ± 0.02a	0.80 ± 0.03a	4.18 ± 0.21a	5.22 ± 0.69a
Giza 20	0	2.5 ± 0.1c	0.25 ± 0.01b	0.73 ± 0.02b	2.31 ± 0.11c	2.03 ± 1.01d
	1	3.2 ± 0.2b	0.31 ± 0.02a	0.81 ± 0.03a	4.28 ± 0.21a	4.75 ± 0.53b
	2	2.6 ± 0.1c	0.26 ± 0.01b	0.80 ± 0.03a	3.60 ± 0.17b	3.96 ± 0.44c

[#]Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by least-significant difference test (LSD) at $P \leq 0.05$.

Table 6: Effects of spray treatments with H_2O_2 (mM) on leaf free proline, total soluble sugars (TSS), membrane stability index (MSI) and relative water content (RWC) of two varieties of onion plants under saline soil condition

Treatments					
Variety	H_2O_2	Free proline (mg/g DW)	TSS (mg/g DW)	MSI (%)	RWC (%)
Giza Red	0	0.14 ± 0.01b	3.65 ± 0.12a	62.68 ± 1.93c	70.45 ± 1.20b
	1	0.11 ± 0.01d	2.30 ± 0.08d	75.78 ± 2.26a	77.96 ± 1.58a
	2	0.12 ± 0.01c	2.61 ± 0.09c	70.51 ± 2.08b	77.45 ± 3.91a
Giza 20	0	0.17 ± 0.01a	3.87 ± 0.13a	60.41 ± 1.81b	71.91 ± 0.55b
	1	0.12 ± 0.01c	2.95 ± 0.10b	72.11 ± 2.18ab	79.93 ± 2.57a
	2	0.14 ± 0.01b	3.09 ± 0.10b	70.29 ± 2.09b	78.85 ± 1.10a

[#]Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by least-significant difference test (LSD) at $P \leq 0.05$

two varieties of onion plants compared to untreated control plants. The applied level of 1 mM H_2O_2 was found to be more effective, reducing the concentrations of free proline and TSS by 21.4 and 29.4% and 37.0 and 23.8% and increasing MSI and RWC by 20.9 and 19.4% and 10.7 and 11.2% in the Giza Red and Giza 20 varieties, respectively compared to their controls.

DISCUSSION

By stimulating the overproduction of reactive oxygen species (ROS) through various organelles and enzymes, salt stress negatively affects different processes during seed germination, growth and flowering that negatively reflects in plant productivity [5]. To avoid effects of salt stress, plants adopt several strategies such as ion homeostasis, osmotic adjustment and improvement of antioxidant defense system [33]. As recently reported, exogenous application of H_2O_2 increases tolerance to many environmental stresses including salt stress [13, 15, 16]. H_2O_2 has also been reported to promote plant defense system that improves growth and photosynthetic ability of plants under salt stress [15], which agreed with our findings (Tables 3 & 5). [34] reported a dual role of H_2O_2 during biotic and abiotic stresses. As an element of oxidative stress, it deleteriously affects cell components on the excess accumulation and simultaneously it induces protective mechanisms, particularly at the early stage of

plant stress response. H_2O_2 can act as a signaling molecule for stress adaptation and plant application with H_2O_2 can lead to programmed cell death and regulate plant development [34].

[13] observed that exogenous H_2O_2 treatment reduced the harmful effects of salt stress on growth of wheat and [35] suggested the multiple positive effects of H_2O_2 on root system, leaf and coleoptiles growth of wheat seedlings. In our study, salt stress caused a significant reduction in all growth parameters of Giza Red and Giza 20 varieties (Table 3), but the spray applications of H_2O_2, particularly the lower level (1 mM) significantly increased the growth of these varieties, which reflected in the final yields (Table 4).

Although H_2O_2 is known as the central signaling molecule in stress responses, little information explains how it affects the photosynthetic machinery [36]. Salt stress partially inhibited photosynthesis by the reduction in photosynthetic pigments and chlorophyll fluorescence (*Fv/Fm*, Fv/F_0 and PI; Table 5), but H_2O_2 application could be associated with the H_2O_2-mediated increase in ascorbic acid (AsA) and glutathione (GSH) concentrations, which act as an antioxidants and photosynthetic machinery protector from salt-induced ROS. It has been shown that pre-treatment of seeds with H_2O_2 increase the net photosynthetic rate in wheat seedlings [15, 37]. In the present study, chlorophyll and carotenoid contents and chlorophyll fluorescence decreased under salt stress through the decrease in intermediates of chlorophyll biosynthesis [38], leading to a decreased absorption of light by the chloroplast, indirectly impairing photosynthesis [15]. The reduction in chlorophyll content might have been due to salt induced increase in the activity of chlorophyll degrading enzyme chlorophyllase [39].

The F_v/F_m, F_v/F_0 and PI were used as a noninvasive method to determine the functional state of the photosynthetic machinery. The F_v/F_m, F_v/F_0 and PI were significantly reduced by salt stress, but H_2O_2 application significantly improved these components in leaves of salt-stressed plants (Table 5). The highest F_v/F_m, F_v/F_0 and PI were observed in the leaves of salt-stressed plants sprayed with 1 mM H_2O_2. However, H_2O_2 application was a remedy for plants under salt stress effects. The reduction in the F_v/F_m, F_v/F_0 and PI provides an indicator of photo-inhibitory damage caused by the incident photon flux density when plants are subjected to a wide range of environmental stresses [40].

The reduction in growth and yield of onion plants (Giza Red and Giza 20) grown under salt stress was associated with a reduction in water potential, decreasing the water use efficiency (WUE; Table 4), but the application with H_2O_2 reversed these effects and increased WUE, which may be related to the increase in relative water content (RWC; Table 6). H_2O_2 treatments enabled the leaf to maintain a high level of RWC by regulating the osmolality in the leaf, alleviating the effects of salt stress. The increase in water potential and osmotic potential might help stabilization of proteins and increase photosynthesis [15]. Under salt stress, osmotic stress is triggered by an excess of salt in the soil and ionic stress is caused by the over-accumulation of salt in plant cells. These stresses individually affect the physiological status of plant [41, 42]. Exogenous application of H_2O_2 showed amelioration of the salt effects and increased membrane stability index (MSI) and RWC, maintaining turgid cells for healthy metabolic processes and membrane integrity.

[43] suggested that H_2O_2 application induces the accumulation of compatible-solutes (i.e., polyols, sugars and amino acids including proline) in stressed plants to allow the maintenance of water uptake and cell turgor under drought induced by salt stress. The reduction in free proline and soluble sugars by H_2O_2 application in this study (Table 6) may be attributed to the crucial role of H_2O_2 in mitigating the negative salt effects.

CONCLUSION

This study suggests that spraying onion plants (either Giza Red or Giza 20 variety) with 1 mM H_2O_2 improves the response of onion plants to salt stress (7.94 - 8.81 dS/m) through the increased efficiency of the photosynthetic system, increased plant water relations and reduced free proline and soluble sugars concentrations. Therefore, H_2O_2 may be considered beneficial in onion production to help plants to overcome the harmful effects of salt stress conditions.

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