The Investigation on Growth and Some Antioxidative Enzymes of the Maize (Zea mays L.) Plant under Salt and Water Stress

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Abstract

The present study was carried out to determine interactive effects of salinity and drought stress on growth and antioxidative enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POX), and polyphenol oxidase (PPO) in hydroponically grown plants of maize, *Zea mays* L.cv DKC647. Plants were treated two salt (NaCl) concentrations and PEG 6000 to create water stress The results obtained from this experiment show that high salinity reduced shoot and root dry and fresh weight but PEG treatment had no significant effect on this parameters. SOD, POX and PPO activities were increased with the increase in the intensity of NaCl stress, but PEG treatment in addition to NaCl had more significant effect on this enzyme activity. However, the interactive effects of salinity and water stress induced highest SOD, POX, and PPO activities compared to the other treatments in maize plants. These results suggest that maize plants may be increased the activity of antioxidant enzymes to have a better protection against reactive oxygen species (ROS) under salt and water stress.

Key words: Maize, Salinity and Water Stress, PEG 6000, Growth, Antioxidant enzymes (SOD, POX, PPO).

INTRODUCTION

Plants are immobile and therefore unable to escape stressful environments. Abiotic stresses such as salt excess (NaCl) and drought are among factors most limiting to plant productivity (Bohnert et al., 1995). In higher plants, exposure to abiotic stresses, *e.g.* water stress and high salinity, often results in different damages such as oxidative injury (Smirnoff, 1993, Fadzilla et al. 1997).

Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world (Tester and Davenport, 2003, Ashraf and Foolad, 2005). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect, or a combination of these factors (Ashraf and Haris, 2004). On the other hand, water stress, one of the most common environmental limitations affecting growth and productivity of plants, causes many metabolic, mechanic and oxidative changes in plants (Kalefetoğlu and Ekmekçi, 2005).

Abiotic stresses such as salt excess (NaCl) and drought are among factors most limiting to plant productivity (Bohnert et al., 1995). Greenway and Munns (1980) suggested that reasons of growth reduction in plants under salt stress may be described as following i) Salinity can induce water stress as it increases the osmotic pressure of the soil solution, ii) High salinity may also result in too high an internal ion concentration (ion excess) and thus cause growth reduction. It is often difficult to assess the relative importance of ion excess and water stress as growth limiting factors (Marcelis and

Hooijdonk, 1999). Steduto et al., (2000) also suggested that the decline in productivity observed for many plant species subjected to excess salinity is often associated with a reduction in photosynthetic capacity. Salt may affect growth indirectly by decreasing the rate of photosynthesis (Meloni et al., 2003).

Many crop species are sensitive to high concentrations of salt with negative impacts on agricultural production. Maize (*Zea mays* L.) is considered a moderately salt-sensitive plant (Maas and Hoffman, 1977). In general, salinity tolerance mechanisms are described as cellular, organizational, and whole plant adaptations. But other additional means include cellular osmotic protection due to adjusting of internal osmotic balance by accumulation of compatible solutes and protection against consequences of endogenous oxidative stress (Levinsh, 2006).

Salt and water stress, like other abiotic stresses, also leads to oxidative stress through an increase in reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) (Alscher et al., 1997; Mittler, 2002; Neill et al., 2002). ROS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Rout and Shaw, 2001). Fortunately plants possess a number of antioxidant enzymes and antioxidants that protect them against the damaging effects of activated oxygen species (Dionisio-Sese and Tobita, 1998). In plant cells, one such protective mechanism is an antioxidant system, composed of both non-enzymatic and enzymatic antioxidants (Foyer et al., 1994). Plants have evolved mechanisms to protect cell and subcellular systems from the effects of these reactive oxygen radicals by using enzymes such as superoxide dismutase, catalase, peroxidase, glutathione reductase, polyphenol oxidase and non-enzymic ascorbate and glutathione (Agarwal and Pandey, 2004).

Superoxide dismutases (SOD, EC 1.15.1.1) since discovered by McCord and Fridovich (1969), attracted the attention of many researchers because they are essential component in an organism's defense mechanism (Badawi et al., 2004). The SOD (E.C. 1.15.1.1) is the first enzyme involved in the antioxidative process (Lee et al., 2001, Rubio et al., 2002). This enzyme converts superoxide radical to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Mhadhbi et al, 2004). Hydrogen peroxide can be removed by "non-specific" peroxidases (POX, E.C. 1.11.1.7) which use H_2O_2 as electron donor to metabolise phenolic compounds. These latter enzymes are ubiquitous and are involved in various processes such as cell growth control and tolerance to environmental stress (Quiroga et al., 2000). Polyphenol oxidase (PPO; E.C.1.10.3.1) is generally used as an indicator enzyme for the adequacy of heat treatment of fruit purees (Pointing et al., 1954; Williams et al., 1986). However, it is also used as an indicator for the salinity stress. For example, Agarwal and Pandey (2004) in senna and Demir and Kocaliskan (2001) in been seedlings studied the effect of salinity stress on this enzyme activity.

We hypothesized that increased activity of antioxidant enzymes; SOD, POX and PPO contributes to the protection of maize plants from salt and water stress. Therefore, the aim of this work was to evaluate the effects of salt and water stress on the activity of antioxidative enzymes, and dry and fresh weight in maize plants, in order to better understand salt and water stress effects and plant responses.

MATERIALS and METHODS

Plant Culture and Treatment Conditions

The experiment was conducted under greenhouse conditions in Mugla (Turkey) with maize (*Zea mays* L. cv., DK 647 F1). In the maize plant, grown at hydrophonic environment and under the conditions of greenhouse (at 25/20°C, 16/8h, 75±5%, and 300 µmol m^2 s^{-1} the mean temperature, day/night lenght, relative humidity, photoperiod and photosynthetic photon flux density were maintained, respectively); in this research where the stress conditions' effects on the development and accumulation, there formed settings including control, low-rate salt (5 dS m^{-1} NaCl) and high-rate salt (10 dS m^{-1}). These 3 settings each have been divided to two applications within themselves as normal watering and PEG6000. In order to create water stress, a certain amount of PEG6000 is used enough to create -1 MPa preassure. As a means of control, irrigation water and nutrition solution. In the experimentation conducted as a 3-repetation application, the accumulation levels of proline on the leaves were analysed and the changes occuring in the plants facing stress conditions were directly observed, and examined how they effected the development.

The basic nutrient solution used in this experiment was a modified Hoagland and Arnon formulation. All chemicals used were of analytical reagent grade, and composition of nutrient solution was (mg L^{-1}): 270 N, 31 P, 234 K, 200 Ca, 64 S, 48 Mg, 2.8 Fe, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn and 0.01 Mo. The pH of nutrient solution was adjusted each time to 6.5 with 0.1 mM KOH. Each treatment was replicated three times in a randomised block design and each replicate included 6 plants (i.e., 18 plants per treatment).

Thirty day after germination different treatments were initiated. Treatments were: i) control (C) plant receiving nutrient solution, ii) low salinity treatment (C+S_L): plant receiving nutrient solution plus 5 dS m^{-1} NaCl, iii) high salinity treatment (C+S_H): plant receiving nutrient solution plus 10 dS m^{-1} NaCl, iv) PEG 6000 treatment (C+PEG): plant receiving nutrient solution plus PEG 6000 (a certain amount of PEG6000 is used enough to create -1 MPa preassure), v) low salinity and PEG treatment (S_L+PEG): plant receiving nutrient solution plus 5 ds/m NaCl plus PEG 6000 (a certain amount of PEG6000 is used enough to create -1 MPa preassure), vi) high salinity and PEG treatment (S_H+PEG): plant receiving nutrient solution plus 10 dS m^{-1} NaCl plus PEG 6000 (a certain amount of PEG6000 is used enough to create -1 MPa preassure). Each treatment was replicated three times and each replicated included 3 pots (i.e. 9 pots per treatment). The pH of the nutrient solution was adjusted to

6.5 with 0.1 m*M* KOH during the entire growing period. Plants were harvested 90 days after seedling emergence.

Experimental

In the analyses after the experiments, SOD, POX, PPO activities from antioxidative enzymes, and some plant growth parameters were determined.

Protein Content

Protein content in the enzyme extracts was determined according to Bradford (1976) using Bovine Serum Albumin V as a standard.

Enzyme Determination

Leaves (0.5 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrolidine (PVP). The homogenate was centrifuged at 20,000 *g* for 15 min at 4° C and the supernatant used for assays of the activities of POX and SOD. The activity of SOD was assayed by monitoring its ability to inhibit the photochemical reduction of NBT (Beauchamp and Fridovich, 1971). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of cytochrome c by 50%. The activity of POX was assayed by adding aliquot of the tissue extract (100 µl) to 3 ml of assay solution, consisting of 3 ml of reaction mixture containing 13 mM guaiacol, 5 mM H2O2 and 50 mM Na-phosphate (pH 6.5) (Chance and Maehly, 1955). An increase of the optical density at 470 nm for 1 min at 25°C was recorded using a spectrophotometer. POD activity was expressed as change in absorbance min-1 mg-1 protein. The increase in A470 was measured for 3 min and activity expressed as ΔA470/mg protein/min.

Polyphenol oxidase activity (PPO) activity was assayed with 4-methylcatechol as a substrate according to the method of Zaubermann et al. (1991). Half gram of fresh leaf was ground with 10 ml of 0.1 mol/l sodium phosphate buffer (pH 6.8) and 0.2 g of polyvinylpyrrolidone (PVP, insoluble). After centrifugation at 19 000g for 20 min, the supernatant was collected as the crude enzyme extract. The assay of the enzyme activity was performed using 1 ml of 0.1 mol/l sodium phosphate buffer (pH 6.8), 0.5 ml of 100 mmol/l 4- methylcatechol, and 0.5 ml enzyme solution. The increase in absorbance at 410 nm at 25 °C was recorded automatically for 5 min. One unit of enzyme activity was defined as an increase of 0.01 in absorbance per min per mg protein.

Dry Weight

Three randomly selected plants per replicate were divided into shoots and roots, and dried in a forced air oven at 70ºC for two days to determine dry weights.

Statistical analysis

The experiment was performed twice under the same environmental conditions. Statistical analysis (ANOVA) indicated that there were no significant differences in measurements between the

two runs; data presented here are the averages of the two experiments. A two way analysis of variance was performed on all datas and the LSD was calculated at P≤0.05.

RESULTS

Plant Growth

In this experiment dry and fresh weight of both shoot and root was significantly inhibited by salt and water stress (Table 1). Reduction in total plant dry weight in high salinity (**C+S_H**: plant grown nutrient solution plus 10 ds/m NaCl) treatment was 52 % compared to the control (plant grown nutrient solution), while it was 56 % in high salinity and PEG 6000 (**S_H+PEG**:plant grown nutrient solution plus 10 ds/m NaCl plus PEG 6000). Inhibition on plant growth was not significantly affected by PEG 6000 in addition to NaCl treatment, but water stress had been effective on root and shoot dry weight. The high concentrations of NaCl were more harmful than PEG 6000 in maize plants. Total dry weight decreased with increasing concentration of the osmotic agents, with a drastic effect at the highest NaCl concentration.

Table 1. Effects of NaCl and PEG 6000 treatments on the fresh and dry weight of shoot and root of maize (*Zea mays* L.) plants.

	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
C	250a	80a	20a	7,5a
C+PEG	203b	75a	19a	6,1b
C+S_L	195b	75a	14b	5,3c
S_L+PEG	134c	59b	11c	4,2d
C+S_H	94d	54b	9d	4,1d
S_H+PEG	90d	50b	9d	3,2e

Values followed by different superscript letters in each column differ significantly (LSD test, P ≤ 0.05).
C: Control**, C+PEG:** PEG 6000 treatment**, C+S_L:** 5 ds/m NaCl treatment**, S_L+PEG:** 5 ds/m NaCl treatment plus PEG 6000 treatment**, C+S_H:** 10 ds/m NaCl treatment**, S_H+PEG:** 10 ds/m NaCl plus PEG 6000 treatment.

Enzyme Activities

We report in Fig 1, 2, and 3 the effects of various salt concentrations and PEG 6000 on soluble protein content and antioxidant enzyme activities such as SOD, POX and PPO. The activity of SOD, which converts superoxide radical to H_2O_2, was higher in all leaf of maize plants under stress conditions compared to the control plants. SOD activity was observed to be significantly (p≤0.05) higher in high salt and PEG 6000 treated plants than in the only high salt treated plants, and activity was more pronounced in plants under NaCl stress than in the PEG 6000 treated plants. However, high salinity and PEG 6000 treated plants maintained higher (p≤0.05) SOD activity than all other treatments.

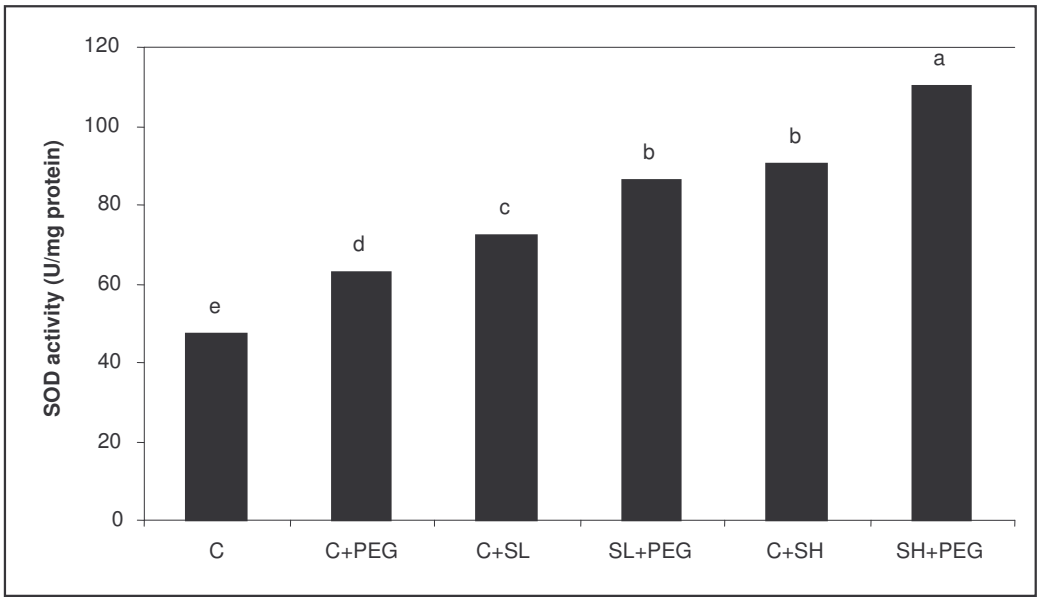

Figure 1. Effects of NaCl and PEG 6000 treatments on SOD activity of maize plants.

Values followed by different superscript letters in each column differ significantly (LSD test, $P \leq 0.05$); SOD, superoxide dismutase.

C: Control, C+PEG: PEG 6000 treatment, C+S_L: 5 ds/m NaCl treatment, S_L+PEG: 5 ds/m NaCl treatment plus PEG 6000 treatment, C+S_H: 10 ds/m NaCl treatment, S_H+PEG: 10 ds/m NaCl plus PEG 6000 treatment.

POX activity, which decomposes the H_2O_2 produced by SOD, also changes with respect to salinity and water stres. The activity of POX increased with increasing salinity and water stress. But POX activity was observed not to be significantly ($p \leq 0.05$) higher plants under water stress than the salinity treated plants. However maize plants exhibited an increase in POX activity with increasing magnitude of salinity and water stress conditions.

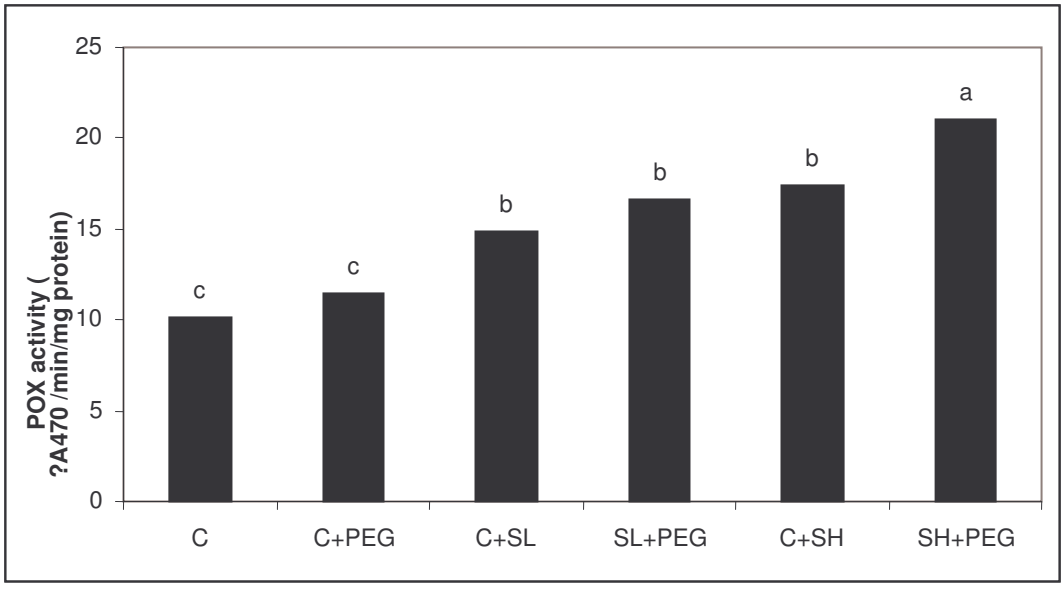

Figure 2. Effects of NaCl and PEG 6000 treatments on POX activity of maize plants.

Values followed by different superscript letters in each column differ significantly (LSD test, P ≤ 0.05); P0X, peroxidase.

C: Control**, C+PEG:** PEG 6000 treatment**, C+S_L:** 5 ds/m NaCl treatment**, S_L+PEG:** 5 ds/m NaCl treatment plus PEG 6000 treatment**, C+S_H:** 10 ds/m NaCl treatment**, S_H+PEG:** 10 ds/m NaCl plus PEG 6000 treatment.

The effect of increasing magnitude of salinity and water stress on PPO activity in the leaves of maize plants is shown in Fig 3. PPO activity showed an increasing trend with the increase in the NaCl concentrations. The highest PPO activity was found in high salinity and PEG 6000 treatment, whereas the lowest was determined in control plants. As POX activity, salinity was more effective than water stress in increase of PPO activity. However, the interactive effects of salt and water stress was found very significant on PPO activity.

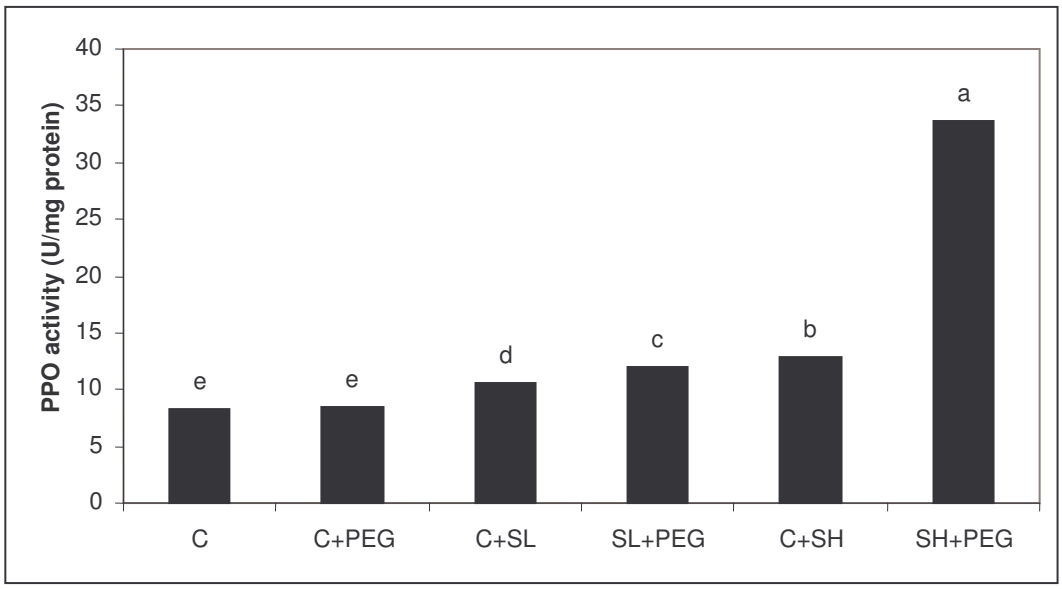

Figure 3. Effects of NaCl and PEG 6000 treatments on PPO activity of maize plants.

Values followed by different superscript letters in each column differ significantly (LSD test, P ≤ 0.05); PPO, polyphenol oxidase.

C: Control**, C+PEG:** PEG 6000 treatment**, C+S_L:** 5 ds/m NaCl treatment**, S_L+PEG:** 5 ds/m NaCl treatment plus PEG 6000 treatment**, C+S_H:** 10 ds/m NaCl treatment**, S_H+PEG:** 10 ds/m NaCl plus PEG 6000 treatment.

In the present study, in general salinity, was more effective on SOD, POX, and PPO than water stres in maize plants. However, it was found that the interective effects of salinity and water stress induced antioxidant enzymes such as SOD, POX, and PPO. Maize plants under salt and water stress were significantly increased the antioxidant enzymes compared to the control and only salt or PEG 6000 treated plants.

DISCUSSION

In this study, reduction in maize plants growth was more significant, because of water stress treatment in addition to NaCl. Similar results were reported in durum wheat plants by Almansouri et al. (2001). But it seems difficult to determine differences between salt and water stress detrimental effects on plant growth, because PEG 6000 treatment had also been decreased maize plants growth by reduce plant dry and fresh weight.

Antioxidant enzymes play important roles in adaptation to stress conditions (Misra and Gupta, 2006). Therefore, we hypothesized that increased activity of antioxidant enzymes; SOD, POX and PPO contributes to the protection of maize plants from salt and water stress. As a result of this study, it was found that maize plants under salt and water stress were increased antioxidant enzymes activity such as SOD, POX and PPO.

The activity of SOD enzyme, which converts superoxide radical to H_2O_2, was reported to increase under saline conditions in the maize and sunflower seedlings (Rios-Gonzalez et al., 2002), and in cotton (Meloni et al., 2003). Many workers found positive correlation between water stress and the abundance of SOD in plants (McKersie et al., 1996; Burke et al., 1985; Badawi et al., 2004). Our results are in conformity with these results.

It is know that high NaCl (Meloni et al., 2003) and PEG treatment (Li and Staden, 1998) induces POX activity in plants. The results of this study are similar to those reported results. High salinity and PEG 6000 treatment was more effective in increasing POX activity.

Demir and Kocaçalışkan (2001) was found that in bean plants treated with NaCl PPO activity gradually increased as NaCl concentrations increased. Some previous studies have also shown that PPO activity is induced during water stres (Shivishankar, 1988; English-Loeb et al., 1997). Similar results were obtained in this study.

In conclusion, the aim of this work was to evaluate the effects of salt and water stress on growth, the activity of antioxidative enzymes such as SOD, POX and PPO, in order to better understand stress condition's interactive effects on plant growth. The results obtained from this experiment show that high salinity and water stress enhanced antioxidant enzymes (SOD, POX and PPO) activity and salt stress was more effective than water stress on the stress parameters. However, interactive effects of salinity and water stress on growth, and antioxidative enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POX) and polyphenol oxidase (PPO) in hydroponically grown plants of maize, *Zea mays* L.cv DKC647 were more significant than only salt or water stress treatment. Certainly, the present study in *Zea mays* L. plants about its suffering from PEG 6000 and NaCl stresses is probably not sufficient. Nevertheless, these results suggest that maize plants may be increased the activity of antioxidant enzymes to have a better protection against reactive oxygen species (ROS) under salt and water stress.

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