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Full Length Research Paper

Sodium nitroprusside (SNP) alleviates the oxidative stress induced by $NaHCO_3$ and protects chloroplast from damage in cucumber

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Oxidative damage is often induced by abiotic stress, nitric oxide (NO) is considered as a functional molecule in modulating antioxidant metabolism of plants. In the present study, effects of sodium nitroprusside (SNP), a NO donor, on the phenotype, antioxidant capacity and chloroplast ultrastructure of cucumber leaves were studied under $NaHCO_3$ stress. 30 mM $NaHCO_3$ treatment significantly induced accumulation of H_2O_2 and thiobarbituric acid-reactive substances (TBARS) in cucumber leaves, and led to serious electrolyte leakage. Application of 100 µM SNP stimulated reactive oxygen species (ROS)-scavenging enzymes and increased antioxidant capacity, resulting in lower lipid peroxidation and membrane damage induced by $NaHCO_3$ stress. As a main organelle of ROS formation, chloroplast ultrastructure was seriously damaged by $NaHCO_3$ stress and SNP treatment obviously reversed the damage. On the contrary, the above effects of SNP were not observed by application of potassium ferrocyanide which is an analog of SNP that does not release NO. Therefore, it could be concluded that the NO from SNP might account for the alleviating effect of $NaHCO_3$ stress on cucumber plants.

Key words: Cucumber, alkaline stress, nitric oxide, antioxidant, chloroplast ultra structure.

INTRODUCTION

Salinity and alkalinity are important environmental factors limiting crop production in semi-arid and arid regions. There are 831 million hectares of soils affected by excessive salinity and alkalinity in the world. Of this, area under sodic soils (alkaline soils) is 434 million hectares, compared to 397 million hectares of saline soils (Jin et al., 2008). It is well known that salinity soil is mainly due to the accumulation of NaCl and Na_2SO_4, and alkalinity soil is mainly due to the accumulation of $NaHCO_3$ and Na_2CO_3 (Shi and Sheng, 2005; Yang et al., 2009). Higher plant tolerance to salinity stress has previously been extensively studied; unfortunately, the adaption mechanism to alkalinity in plants is short of deep investigation (Jin et al., 2008). Osmotic stress and ion-induced injury are generally considered to be involved in salt stress on plants, while except for the same stress with salt stress, there is the influence of high-pH on plants under alkaline conditions (Liu et al., 2010). The most conspicuous symptom of alkaline salt stress on plants is the induction of leaf chlorosis and stunted growth, which is greatly related with the precipitation of metal ions and phosphorus as well as the disruption of ionic balance and pH homeostasis in tissues caused by high-pH environment surrounded the roots (Yang et al., 2007, 2008).

Like other environmental stress, alkaline stress also induces oxidative stress (Cellini et al., 2011). As the results of oxidative stress, lipid is peroxidised, protein synthesis is inhibited, enzyme is inactivated, and membrane systems are damaged (Bursal and Gülçin, 2011; Gülçin et al., 2011; Tanou et al., 2009). The balance between free radical generation and scavenging determines the survival of

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Abbreviations: EC, Electricity conductivity; **HEPES,** 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid; **SNP,** sodium nitroprusside; **TBARS**, thiobarbituric acid-reactive substances; **TCA**, trichloroacetic acid; **TBA,** 2-thiobarbituric acid.

plants under alkaline stress. To counteract the oxidative stress, plants have evolved an antioxidant system which is composed of ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Jung, et al., 2000). Many studies indicated that plant tolerance to salt stress was closely related with the expression and activities of these antioxidant enzymes (Nawaz and Ashraf, 2010; Noreen et al., 2010; Seckin et al., 2010). Therefore, increasing antioxidant capacity by exogenous substance application could be a practical measure in the detoxification of alkaline stress-induced excessive free radical production.

Nitric oxide (NO) is a small, highly diffusible gas and a ubiquitous bioactive molecule. Its chemical properties make NO a versatile signal molecule that functions through interactions with cellular targets via either redox or additive chemistry (Lamattina et al., 2003). Recent studies provided increasing evidences that NO is involved in many key physiological processes of plants, such as germination (Beligni and Lamattina, 2000), growth and development of plant tissue (Durner and Klessig, 1999; Pagnussat et al., 2003), iron homeostasis (Graziano and Lamattina, 2007), regulation of plant maturation and senescence (Guo and Crawford, 2005; Leshem et al., 1998). In abiotic stress tolerance, it has been obtained that NO plays an important role in resistance to salt, drought, temperature, UV-B and heavy metal stress (Siddiqui et al., 2011). However, there are few investigations about exogenous NO modulating plants tolerance to alkaline stress. Recently, Cellini et al. (2011) observed that nitric oxide content was associated with tolerance to bicarbonate-induced chlorosis in micropropagated *Prunus* explants. In the experiment, Effects of SNP, a nitric oxide donor, on phenotype, antioxidant capacity and choloroplast ultrastructure of cucumber treated with $NaHCO_3$ stress were investigated; the obtained results would provide a basis for further investigation of NO role in cucumber tolerance to alkaline stress.

MATERIALS AND METHODS

Cucumber (*Cucumis sativus* L. cv. Jinchun 5) seeds were germinated on moisture filter paper in the dark at 28°C for 2 days, and germinated seedlings were transferred to the growth chamber filled with vermiculite and grown in greenhouse for 8 days. Then they were transplanted into 5 L black plastic containers containing aerated full nutrient solution: 4 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2.5 mM KH_2PO_4, 2 mM $MgSO_4$, 29.6 µM H_3BO_3, 10 µM $MnSO_4$, 50 µM Fe-EDTA, 1.0 µM $ZnSO_4$, 0.05 µM H_2MoO_4, 0.95 µM $CuSO_4$, with three seedlings per container. After 9 days of pre-culture, the treatments were started. The experimental design consisted of a control (no added SNP and $NaHCO_3$, indicated as CK) and three treatments ($NaHCO_3$: 30 mM $NaHCO_3$ treatment; Na+ SNP: 30 mM $NaHCO_3$ treatment+100 µM SNP treatment; Na + SF, 30 mM $NaHCO_3$ treatment+100 µM potassium ferrocyanide treatment) and was arranged in a randomized, complete block design with three replicates, giving a total of 12 containers. The plants were cultivated under natural conditions in a glass greenhouse after 12 days the leaves were taken for further assay.

Determination of H_2O_2 content

H_2O_2 content was determined according to Patterson et al. (1984). The assay was based on the absorbance change of the titanium-peroxide complex at 415 nm. Absorbance values were quantified using standard curve generated from known concentrations of H_2O_2.

Determination of Lipid peroxidation

Lipid peroxidation (LPO) was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) using the thiobarbituric acid method described by Heath and Packer (1968). 0.3 g of tissue homogenized in 3 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 10 min and 3 ml of 20% TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA) was added to 1 ml of supernatant. The mixture was heated at 95°C for 30 min and the reaction was stopped by quickly placing in an ice-bath. The cooled mixture was centrifuged at 10,000 g for 10 min, and the absorbance of the supernatant at 532 and 600 nm was read. After substrating the non-specific absorbance at 600 nm, the TBARS concentration was determined by its extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of electrolyte leakage percentage

Electrolyte leakage percentage (ELP) was used to assess membrane permeability. Electrolyte leakage percentage was measured using an electrical conductivity meter according to the method of Lutts et al. (1996). Leaf samples were cut into 1 cm segments and placed in individual stoppered vials containing 10 ml of distilled water after three washes with distilled water to remove surface contamination. These samples were incubated at room temperature on a shaker for 24 h. Electrical conductivity of bathing solution (EC1) was read, and then samples were placed in the thermostatic water bath at 95°C for 15 min and then the electrical conductivity (EC2) was read after cooling the bathing solutions to room temperature. ELP was calculated as EC1/EC2 and expressed as percentage.

Fe^{2+}-chelating activity

For the determination of Fe^{2+}-chelating activity, 0.3 g sample was suspended in 3 ml of serine borate buffer (100 mM Tris-HCl, 10mM borate, 5 mM serine, and 1 mM diethylenetriaminepentacetic acid, pH 7.0). The slurry was centrifuged at 5,000 g for 10 min at 4°C and the supernatants were used for the *in vitro* antioxidant assays. All samples were placed on ice during the experiments.

Fe^{2+}-chelating activity was measured according to the method of Gülçin (2011), Köksal et al. (2011) and Manda et al. (2010). The reaction mixture (2.0 ml) contained 100 µl of cucumber radicles extract, 100 µl $FeCl_2$ (0.6 mM), and 1.7 ml deionised water. The mixture was shaken vigorously and left at room temperature for 5 min; 100 µl of ferrozine (5 mM in methanol) were then added, mixed, and left for another 5 min to complex and was measured at 562 nm against a blank. Disodium ethylenediamineteracetic acid (EDTA-Na_2) was used as the control. The chelating activity of the extract for Fe^{2+} was calculated as:

$$\text{Chelating effect} = [1-(A_1-A_2)/A_0] \times 100\%.$$

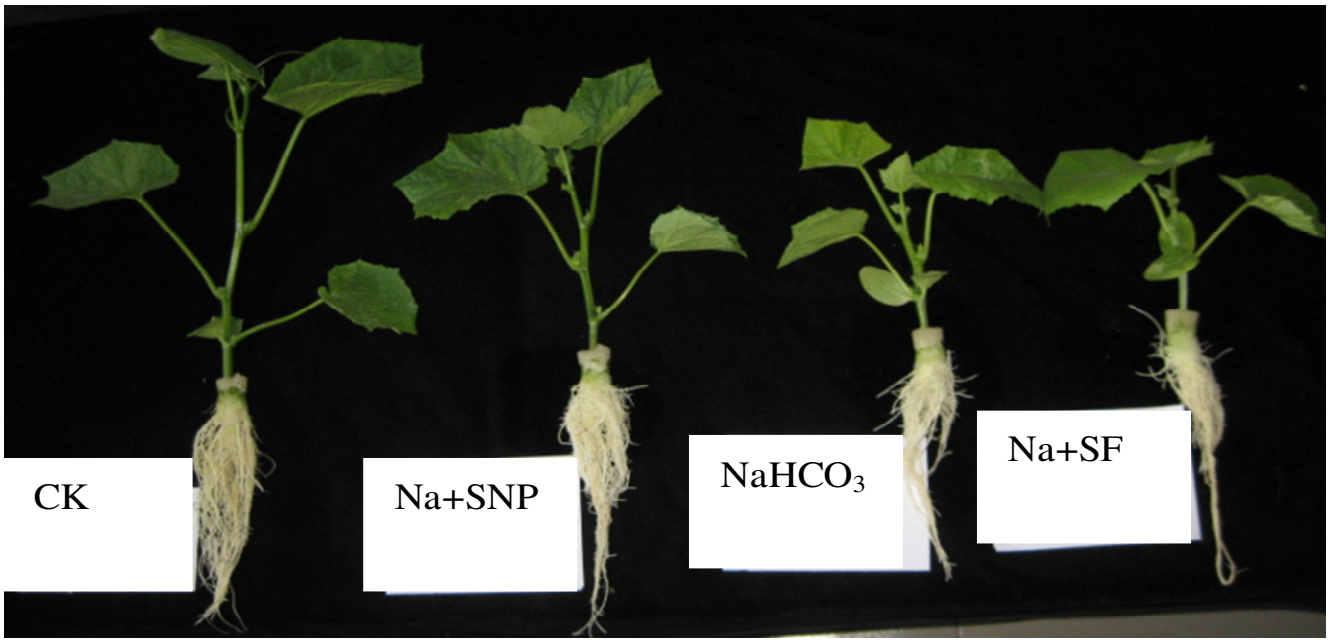

Figure 1. Effects of SNP (sodium nitroprusside, a nitric oxide donor) on the phenotype of $NaHCO_3$-stressed cucumber plants. CK, Control; $NaHCO_3$, 30 mM $NaHCO_3$ treatment; Na+SNP, 30 mM $NaHCO_3$ + 100 µM SNP treatment; Na + SF, 30 mM $NaHCO_3$ + 100 µM potassium ferrocyanide treatment (potassium ferrocyanide, an analog of SNP that does not release NO).

Where, A_0 is the absorbance of the control (without extract); A_1 is the absorbance in the presence of the extract and A_2 is the absorbance without ferrozine.

Enzyme extraction

For enzyme assays, 0.3 g leaves were ground with 3 ml ice-cold 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate and 2% PVP. The homogenates were centrifuged at 4°C for 20 min at 12,000 g and the resulting supernatants were used for determination of enzymatic activities (Zhu et al., 2000). All spectrophotometric analyses were conducted on a SHIMADZU UV-2450PC spectrophotometer.

Determination of enzymatic activities

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium following the method of Stewart and Bewley (1980). CAT activity was measured as the decline in absorbance at 240 nm due to the decrease of extinction of H_2O_2 using the method of Patra et al. (1978). GPX activity was measured as the increase in absorbance at 470 nm due to guaiacol oxidation (Nickel and Cunningham, 1969). APX activity was measured by the decrease in absorbance at 290 nm as ascorbate was oxidized (Nakano and Asada, 1981). DHAR activity was assayed by measuring the increase in absorbance at 265 nm due to reduced ascorbate formation (Nakano and Asada, 1981). GR activity was measured according to Foyer and Halliwell (1976), which depended on the rate of decrease in the absorbance of NADPH at 340 nm.

Determination of chloroplast ultrastructure

Chloroplast ultrastructure was determined by TEM according to the method of Xu et al. (2008). The cucumber leaves were fixed in 3.5% glutaraldehyde for 24 h, washed with 0.1 M phosphate buffer (pH 7.2) and then postfixed with 1% osmic acid at 4°C for 4 h. Cells were dehydrated with ascending concentrations (from 30 to 100%) of ethanol and embedded in spur resin at 60°C for 24 h. After thin sections have been cut with an LKB Ultratome Nova (Bromma, Sweden) and picked on 250-mesh grids, leaf cells were postained with uranyl acetate and lead citrate and then were observed using a transmission electron microscope (JEM-1200EX; JEOL Ltd., Tokyo, Japan) at 80 kV.

The statistics method

Values presented were means ± standard deviation (SD) of three replicates. Statistical analyses were carried out by analysis of variance (ANOVA) using SPSS10.0 software. Differences between treatments were analyzed by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Effects of SNP on symptom of cucumber plants under $NaHCO_3$ stress

Figure 1 shows that $NaHCO_3$ stress significantly induced cucumber leaf chlorosis and inhibited the plant growth, it is well known that chlorosis is often caused by deficiency of iron (Fernández et al., 2008) which easily precipitates under alkaline conditions (Riadh et al., 2006). Nitric oxide, as a gas signal molecular, can readily form complexes with transition metal ions in aqueous solutions or those present in diverse nucleophylic compounds such as metalloproteins (Stamler et al., 1992). The Fe(III)NO

complex appears to undergo a charge transfer reaction to form $Fe(II)NO^+$ (Olson, 1981) and Graziano et al. (2002) observed that NO could increase the availability of iron in plants. In the present experiment, application of SNP reversed the chlorosis of cucumber leaves induced by $NaHCO_3$ stress; the mechanism might be partly attributed to its function in modulating iron metabolism. Because the NO donor SNP contains iron in its molecule, it is important to exclude the effect of iron in SNP for the elucidation of NO function. Therefore, potassium ferrocyanide, an analog of SNP that does not release NO was chosen to treat cucumber under $NaHCO_3$ stress, the results showed that it had no effect on reversing $NaHCO_3$-induced cucumber leaf chlorosis, based on this phenomenon it could be concluded that the function of SNP was due to the NO from it.

Effects of SNP on H_2O_2, lipid peroxidation accumulation and electrolyte leakage percentage of cucumber leaves under $NaHCO_3$ stress

When plants subject to environmental stresses like salinity, drought and extreme temperatures, oxidative damage is caused either directly or indirectly by triggering increased production of reactive oxygen species (ROS) (Suzuki et al., 2011). H_2O_2 is one important member of ROS which are produced as products of membrane linked electron transport activities as well as by a number of metabolic pathways (Blokhina and Fagerstedt, 2010). Increased H_2O_2 accumulation has been obtained in many plants such as cucumber (Zhu et al., 2004), and tomato (Mittova et al., 2003) under neutral salt NaCl stress, however, there is short of investigations about the effects of alkaline salt on H_2O_2 production. In the experiment, $NaHCO_3$ treatment, as a simulation of alkaline environment, dramatically induced accumulation of H_2O_2 in cucumber leaves (Figure 1A). The results indicate that the ROS balance in cucumber leaves was destroyed under $NaHCO_3$.

Estimation of cell membrane integrity in plants based on measurements of electrolyte leakage has become a widely accepted method of estimating cell viability (Ingram and Buchanan, 1981), and enhanced electrolyte from cells can occur as a result of changes in membrane permeability caused by oxidative damage to the plasma membrane. The present experiment showed that $NaHCO_3$ treatment significantly induced electrolyte leakage of cucumber leaves (Figure 2C); similar results have been obtained in NaCl-treated cucumber (Zhu et al., 2004). TBARS were often used to indicate lipid peroxidation, in the experiment, in accordance with the change of electrolyte leakage; their concentrations were much higher in $NaHCO_3$ treatment than in the control (Figure 2B). TBARS formation was considered to be caused by oxidative degradation of polyunsaturated fatty acids, in particular linolenic acid, since most of the linolenic acid in leaves is localized in the thylakoid glycolipids, TBARS formation in leaves is likely a good measure for peroxidative damage to chloroplast membrane (Van-Hasselt et al., 1996).

David et al. (2011) observed that NaCl treatment showed a transient positive effect on expression of NO signal in root tips and the process might be involve in the regulation of specific transporter proteins. However, the antioxidant capacity modulation by NO was still widely thought as one of important pathways in regulating abiotic stress (Hao et al., 2009; Qiao and Fan, 2008). There were a number of investigations indicating the role of NO in hydrogen peroxide-dependent induction of abiotic stress tolerance by BRs (Cui et al., 2011), SA (Zottini et al., 2007; Gémes et al., 2011), and other signal molecule (Acharya et al., 2011). In the present study, SNP treatment significantly decreased H_2O_2 accumulation, electrolyte leakage and lipid peroxidation, however, potassium ferrocyanide did not obviously ameliorate them (Figure 2A, B, C), therefore, it could be concluded that NO from SNP should be responsible for the lower oxidative stress induced by $NaHCO_3$ stress and exogenous NO could protect cucumber membrane system from damage.

Metal chelating capacity could be used to express antioxidant capacity of plant tissues. Compared to the control, $NaHCO_3$ stress dramatically decreased Fe^{2+}-chelating activity, and application of SNP significantly enhanced Fe^{2+}-chelating activity in $NaHCO_3$-treated cucumber leaves (Figure 2D). Increase of antioxidant capacity in plants usually depends on antioxidant metabolites such as ascorbic acid, glutathione, polyphenols and flavonoids (Ksouri et al., 2007), and there were reports indicating that exogenous NO could induce synthesis of these antioxidant metabolites (Ksouri et al., 2007; Ferreira et al., 2010; Wu et al., 2007). In the experiment, it was SNP rather than potassium ferrocyanide which enhanced Fe^{2+}-chelating activity in $NaHCO_3$-treated cucumber leaves, therefore, the function of SNP increasing antioxidant capacity depended on NO from it.

Effects of SNP on activities of antioxidant enzyme in cucumber leaves under $NaHCO_3$ stress

As key elements in the plant defense mechanisms, varying reactions of ROS-scavenging enzymes in plants have been observed under salt stress. For example, under neutral salt NaCl stress, it has been reported that ROS-scavenging enzymes increased under saline conditions in the case of salt-tolerant cotton (Meloni et al., 2003), shoot cultures of rice (Fadzilla et al., 1997), cucumber (Lechno et al., 1997), but decreased in wheat roots (Meneguzzo et al., 1999). In the aspect of alkaline stress, Cellini et al. (2011) found that SOD activity was not affected, and CAT and GPX displayed reduced trend in *Prunus* explants under bicarbonate stress. In the present study, alkaline salt $NaHCO_3$ stress inhibited activities of all antioxidant

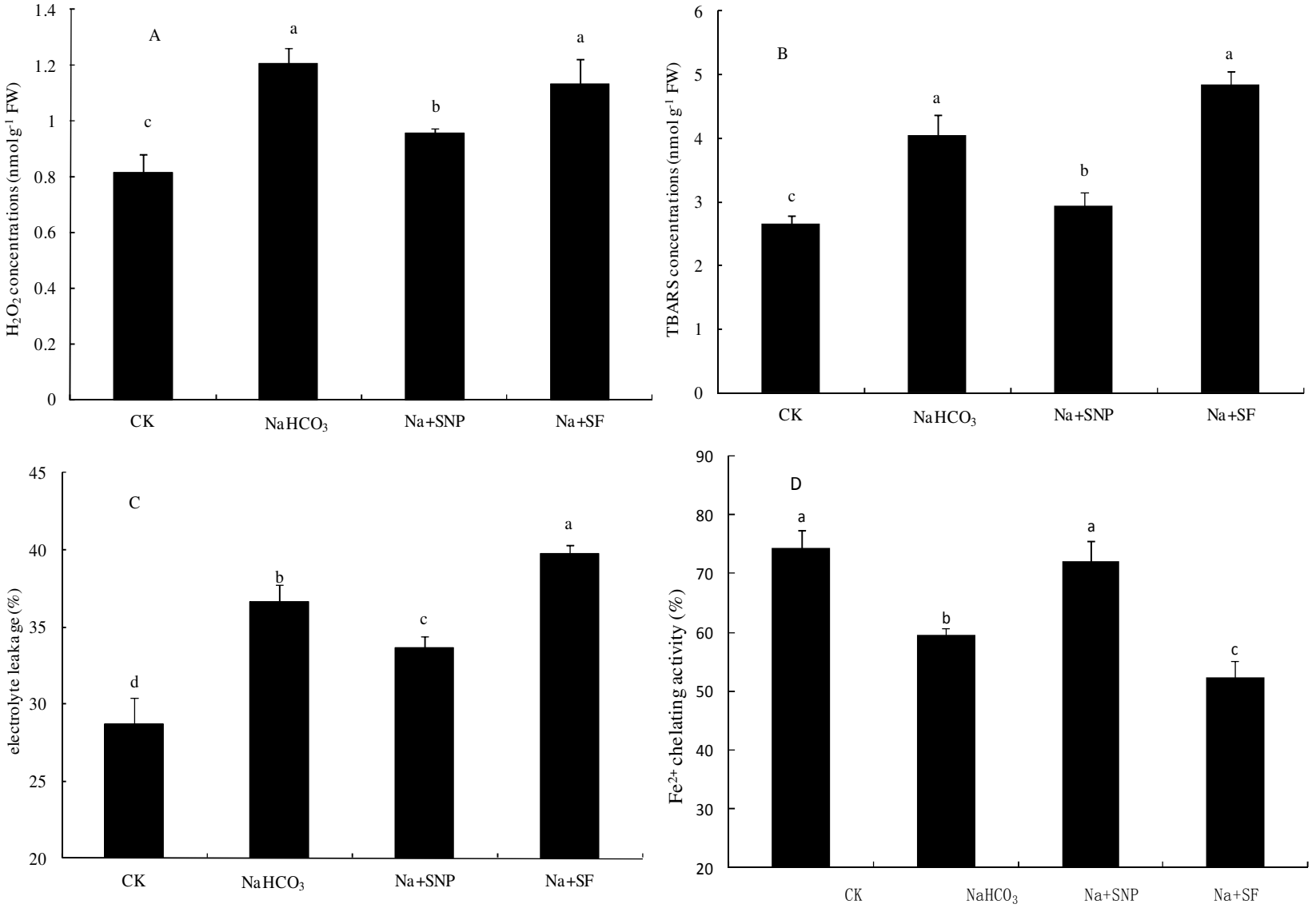

Figure 2. Effects of SNP (sodium nitroprusside, a nitric oxide donor) on the concentrations of H_2O_2 (A) and TBARS (B), electrolyte leakage (C) and Fe^{2+}-chelating activity (D) of cucumber leaves under $NaHCO_3$ stress. Data are means ± SD of three replicates. Mean values followed by different letters (a to c) are significantly different ($P < 0.05$). CK, Control; $NaHCO_3$, 30 mM $NaHCO_3$ treatment; Na+SNP, 30 mM $NaHCO_3$ + 100 µM SNP treatment; Na + SF, 30 mM $NaHCO_3$ + 100 µM potassium ferrocyanide treatment (potassium ferrocyanide, an analog of SNP that does not release NO).

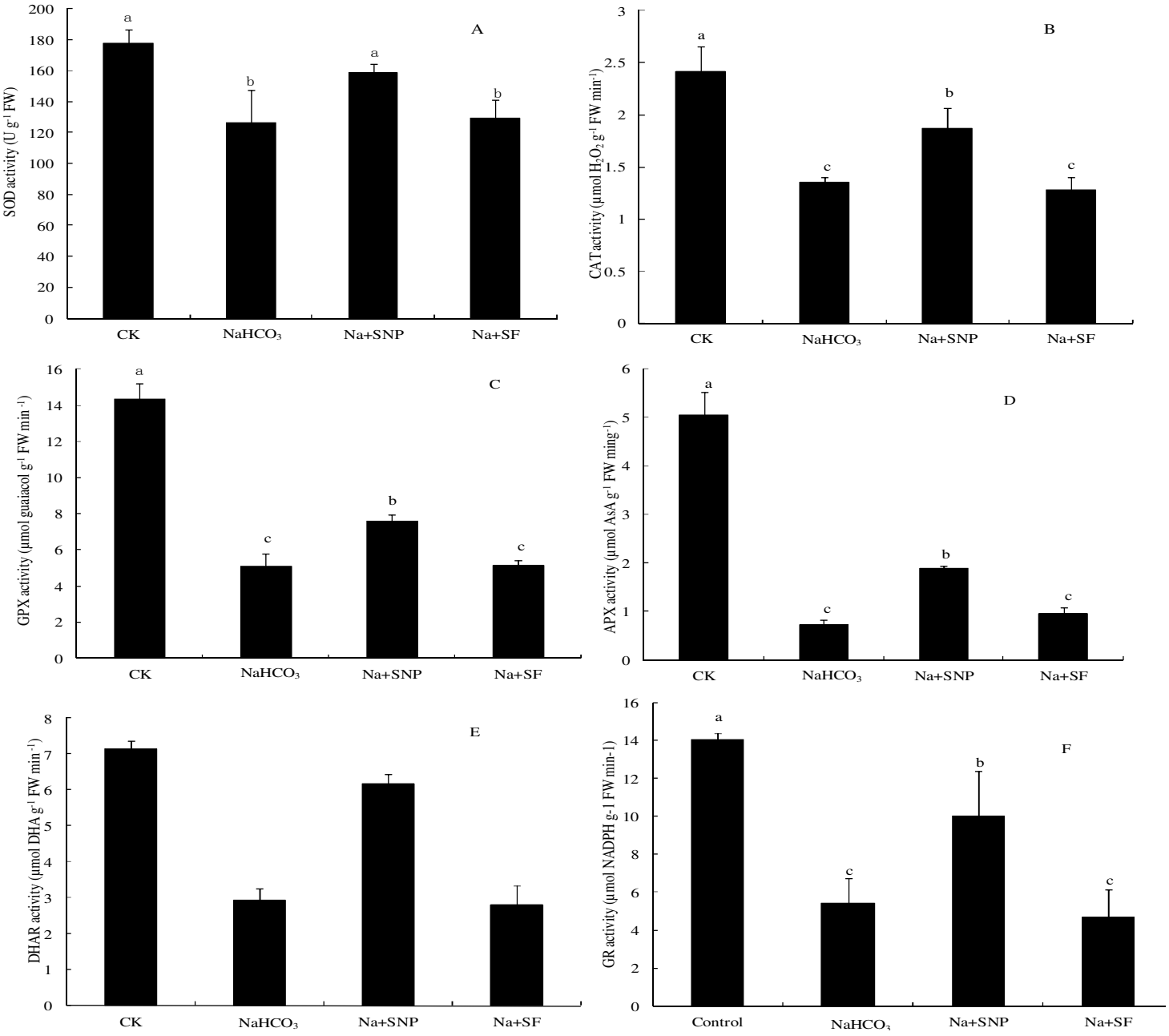

Figure 3. Effects of SNP (sodium nitroprusside, a nitric oxide donor) on the activities of SOD (A), CAT (B), GPX (C) APX (D) DHAR (E) and GR (F) of cucumber leaves under $NaHCO_3$ stress. Data are means ± SD of three replicates. Mean values followed by different letters (a to c) are significantly different (P<0.05). CK, Control; $NaHCO_3$, 30 mM $NaHCO_3$ treatment; Na+SNP, 30 mM $NaHCO_3$ + 100 μM SNP treatment; Na + SF, 30 mM $NaHCO_3$ + 100 μM potassium ferrocyanide treatment (potassium ferrocyanide, an analog of SNP that does not release NO).

enzymes including SOD, CAT, GPX, APX, DHAR and GR (Figure 3). The different reactions showed that the influence of abiotic stress on the antioxidant enzymes were very complex and related to plant treatment period, plant tissues, plant species and genotypes. However, higher activities of antioxidant enzymes were always thought to play important role in alleviating oxidative stress induced by environmental stress.

In many investigations, it was observed that the func-tion of NO alleviation of oxidative stress was due to induction of various ROS-scavenging enzyme activity (Laspina et al., 2005; Sun et al., 2007; Yi et al., 2008). Cheng et al. (2002) reported that the inhibition of poly-ethylene glycol (PEG)- and dehydration (DH)-enhanced senescence of rice leaves by NO was most likely modulated through increasing SOD activity which resulted in lower lipid peroxidation. In the present study, applica-tion of SNP significantly alleviated the inhibited level of

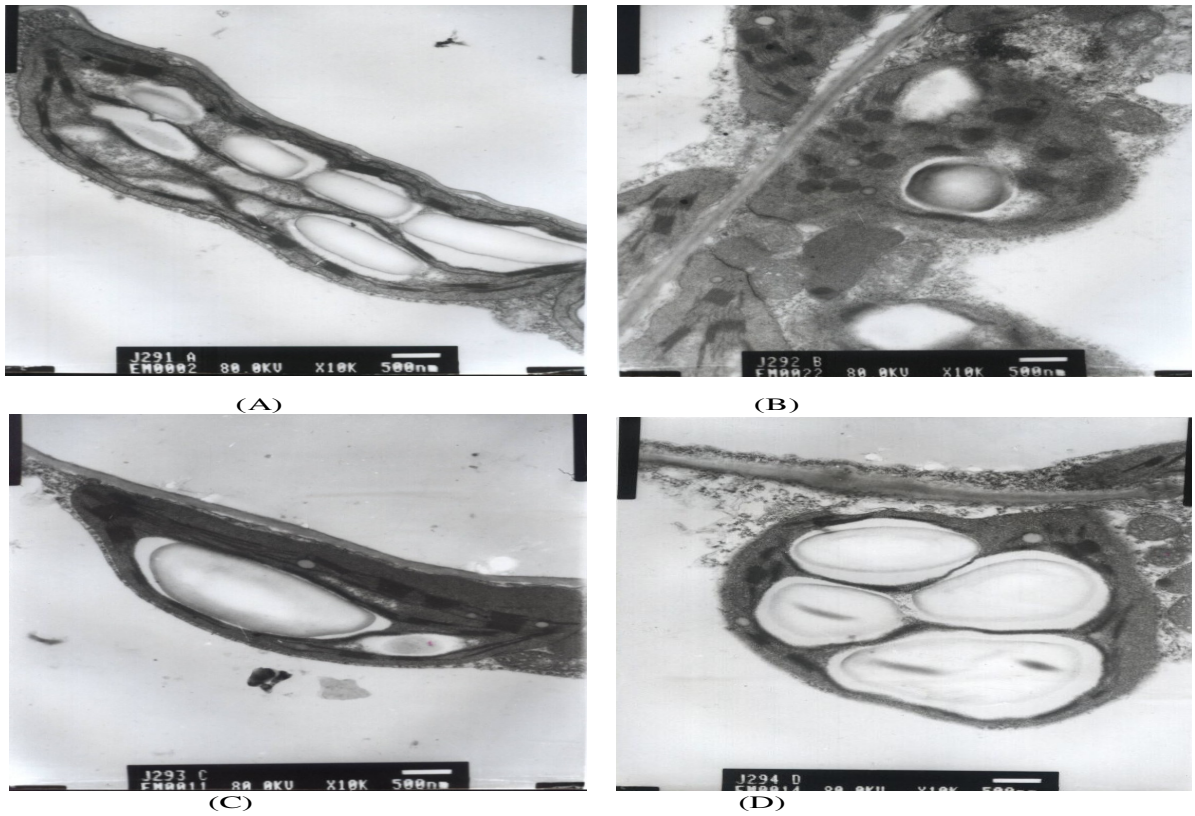

Figure 4. Effects of SNP (sodium nitroprusside, a nitric oxide donor) on chloroplast of cucumber leaves under $NaHCO_3$ stress. (A), Control; (B) 30 mM $NaHCO_3$ treatment; (C) 30 mM $NaHCO_3$ + 100 µM SNP treatment; (D) 30 mM $NaHCO_3$ + 100 µM potassium ferrocyanide treatment (potassium ferrocyanide, an analog of SNP that does not release NO).

SOD activity by $NaHCO_3$ stress (Figure 3A), which suggested that application of NO could promote the conversion from $O_2^{\cdot-}$ into H_2O_2 and O_2. It is well known that SOD includes Mn-SOD, Cu,Zn-SOD and Fe-SOD, and previous study indicated that NO could increase the Fe utilization efficiency in higher plants (Graziano and Lamattina, 2007), based on the facts, it might be able to conclude that the increasing SOD activity by SNP might be partly attributed to higher Fe efficiency. H_2O_2, as the products of $O_2^{\cdot-}$ descomposition, can rapidly diffuse across the membrane and is toxic because it acts both as an oxidant as well as reductant (Foyer et al., 1997), under such situation, the highly efficiency of H_2O_2 scavenging is very vital. In the experiment, SNP significantly increased CAT and GPX activity, which can directly scavenge H_2O_2 and play important role in reducing oxidative stress induced by abiotic stress (Figure 3B, C), similar results were obtained in wheat seed germination treated with NaCl (Zheng et al., 2009). Besides SOD, CAT and APX, there exists another important ROS-scavenging system which is ascorbate-glutathione cycle in chloroplast (Murshed et al., 2008). The cycle is mainly composed of enzymes such as APX, DHAR, GR and antioxidants such as ascorbate and glutathione. In the experiment, SNP significantly induced activities of APX, DHAR and GR (Figure 3D, E, F), which are very important in making chloroplast avoid oxidative damage. On the contrary, the effects of SNP on antioxidant enzymes were not observed by potassium ferrocyanide treatment under $NaHCO_3$ stress (Figure 3), which indicated that the increase of antioxidant enzymes induced by exogenous NO was greatly responsible for decreasing oxidative stress induced by $NaHCO_3$.

Effects of SNP on ultrastructure of cucumber chloroplast under $NaHCO_3$

Chloroplast is the organelle of photosynthesis and the center ROS formation in plant leaves, especially under stress conditions. It has been demonstrated that chloroplast membrane ruptured under saline or osmotic stress (Yamane et al., 2003) and that the thylakoidal structure of the chloroplast is disrupted by salt stress (Hernández et al., 1995). In this study, compared to elliptical shape and thylakoid integrity of chloroplast in the control, $NHCO_3$ treatment led chloroplast become completely swollen, membrane disappear and thylakoid in a disorganized form (Figure 4B). The results could account for a notable reduction of net photosynthesis rate in $NaHCO_3$-treated

cucumber plants in our earlier investigation (Lin et al., 2010). SNP treatment reversed the damage of chloroplast ultrastructure and membrane system induced by $NaHCO_3$ stress, on the contrary, potassium ferrocyanide did not obviously inhibit the chloroplast disturbance caused by $NaHCO_3$ (Figure 4C, D). This indicated that exogenous NO could protect the chloroplast ultrastructure of cucumber under $NaHCO_3$ stress. The protective effect of NO on chloroplast has been observed in Fe-deficiency maize (Magdalena et al., 2002).

Conclusion

Exogenous NO application had significant beneficial effects on $NaHCO_3$-exposed cucumber plants, effectively revered the chlorosis and alleviating growth inhibition of cucumber induced by $NaHCO_3$. The preliminary investigation indicated that the protective effects of exogenous NO were partly attributed to its role in modulating ROS metabolism and holding the integrity of chloroplast. The results suggest that a practical potential method in alleviating alkaline stress by NO metabolism. Because NO is also an important endogenous signal biomolecule which was verified to be effective in gene expression regulation (Besson-Bard et al., 2009), therefore, the genetic method of NO should also be considered to be a direction to increase plants tolerance to abiotic stress including alkaline stress.

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REFERENCES

Acharya K, Chakraborty N, Dutta AK, Sarkar S, Acharya R (2011). Signaling role of nitric oxide in the induction of plant defense by exogenous application of abiotic induces. Arch. Phytopathol. Plant Protect. 44: 1501-1511.

Beligni MV, Lamattina L (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyls elongation, three light-inducible responses in plants. Planta, 210: 215-221.

Besson-Bard A, Astier J, Rasul S, Wawer I, Dubreuil-Maurizi C, Jeandroz S, Wendehenne D (2009). Current view of nitric oxide-responsive genes in plants. Plant Sci. 177: 02-309.

Blokhina O, Fagerstedt KV (2010). Oxidative metabolism, ROS and NO under oxygen deprivation. Plant Physiol. Biochem. 48: 359-337.

Bursal E, Gülçin I (2011). Polyphenol contents and in vitro antioxidant activities of lyophilised aqueous extract of kiwifruit (*Actinidia deliciosa*). Food Res. Int. 44: 1482-1489.

Cellini A, Corpas FJ, Barroso JB, Masia A (2011). Nitric oxide content is associated with tolerance to bicarbonate-induced chlorosis in micropropagated *Prunus* explants. J. Plant Physiol. 168: 1543-1549.

Cheng FY, Hsu SY, Kao CH (2002). Nitric oxide counteracts the senescence of detached rice leaves induced by dehydration and polyethylene glycol but not by sorbitol. Plant Growth Regul. 38: 265-272.

Cui J, Zhou Y, Ding J, Xiao X, Shi K, Chen S, Asami T, Chen Z, Yu J (2011). Role of nitric oxide in hydrogen peroxide-dependent induction of abiotic stress tolerance by brassinosteroids in cucumber. Plant Cell Environ. 34: 347-358.

David A, Yadav S, Bhatla SC (2010). Sodium chloride stress induces nitric oxide accumulation in root tips and oil body surface accompanying slower oleosin degradation in sunflower seedlings. Physiol. Planta. 140: 342-354.

Durner J, Klessig DF (1999). Nitric oxide as a signal in plants. Curr. Opin. Plant Biol. 2: 369-374.

Fadzilla NM, Finch RP, Burdon RH (1997). Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J Exp. Bot. 48: 325-331.

Fernández V, Eichert T, del Río V, López-Casado G, Heredia-Guerrero JA, Abadía A, Heredia A, Abadía J (2008). Leaf structural changes associated with iron deficiency chlorosis in field-grown pear and peach: physiological implications. Plant Soil, 311: 161-172.

Ferreira LC, Cataneo AC, Remaeh LMR, Corniani N, Fátima Fumis Tde, Souza YAde, Scavroni J, Soares BJA (2010). Nitric oxide reduces oxidative stress generated by lactofen in soybean plants. Pestic. Biochem. Phys. 97: 47-54.

Foyer CH, Halliwell B (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 133: 21-25.

Gémes K, Poór P, Horváth E, Kolbert Z, SzopkóD, Szepesi Á, Tari I (2011). Cross-talk between salicylic acid and NaCl-generated reactive oxygen species and nitric oxide in tomato during acclimation to high salinity. Physiol. Planta. 142:179-192.

Graziano M, Beligni MV, Lamattina L (2002). Nitric oxide improves internal iron availability in plants. Plant Physiol. 130: 1852-1859.

Graziano M, Lamattina L (2007). Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots. Plant J. 52: 949-960.

Guo FQ, Crawford NM (2005). Arabidopsis nitric oxide synthase 1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. Plant Cell, 17: 3436-3450.

Gülçin I (2011) Antioxidant activity of eugenol: a structure-activity relationship study. J. Med. Food 14: 975-985.

Gülçin I, Topal F, Çakmakçı R, Gören AC, Bilsel M, Erdogan U (2011). Pomological features, nutritional quality, polyphenol content analysis, and antioxidant properties of domesticated and 3 wild ecotype forms of raspberries (*Rubus idaeus* L.). J. Food Sci. 76: 585-593.

Hao G, Du X, Zho F, Shi R, Wang J (2009). Role of nitric oxide in UV-B-induced activation of PAL and stimulation of flavonoid biosynthesis in Ginkgo biloba callus. Plant Cell Tiss. Org. Cul. 97: 175-185.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189-198.

Hernández JA, Olmos E, Corpas FJ, Sevilla F, Del Río LA (1995). Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci. 105: 151-167.

Ingram DL, Buchanan D (1981). Measurement of direct heat injury of roots of three woody plants. HortScience, 16: 769-771.

Jin H, Kim HR, Plaha P, Liu SK, Park JY, Piao YZ, Yang ZH, Jiang GB, Kwark SS, An G, Son M, Jin YH, Sohn JH, Lim YP (2008). Expression profiling of the genes induced by Na_2CO_3 and NaCl stresses in leaves and roots of *Leymus chinensis*. Plant Sci. 175: 784-792.

Jung S, Kim JS, Cho KY, Tae GS, Kang BG (2000). Antioxidant responses of cucumber (*Cucumis sativus*) to photoinhibition and oxidative stress induced by norflurazon under high and low PPFDs. Plant Sci .153: 145-154.

Koksal E, Bursal E, Dikici E, Tozoğlu F, Gülçin İ (2011). Antioxidant activity of *melissa officinalis* leaves. J. Med. Plants Res. 5: 217-222.

Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C,. Abdelly C (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiol. Biochem. 45: 244-249.

Lamattina L, García-Mata C, Graziano M, Pagnussat G (2003). Nitric oxide: the versatility of an extensive signal molecule. Annu. Rev. Plant Biol. 54: 109-136.

Laspina NV, Groppa MD, Tomaro ML, Benavides MP (2005). Nitric oxide protects sunflower leaves against Cd-induced oxidative stress.

Plant Sci. 169:323-330.
Lechno S, Zamski E, Tel-Or E (1997). Salt stress-induced responses in cucumber plants. J. Plant Physiol. 150: 206-211.
Leshem YY, Wills RBH, Ku VVV (1998). Evidence for the function of the free radical gas-nitric oxide (NO) – as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol. Biochem. 36: 825-833.
Lin Y, Hong YY, Shi QH, Wang XF, Wei M, Yang FJ (2010). Effects of SNP on the growth and nitrogen metabolism enzymes activities of cucumber seedlings under $NaHCO_3$ stress. Plant Nutr. Fert. Sci. 16: 1294-1298.
Liu J, Guo WQ, Shi DC (2010). Seed germination, seedling survival, and physiological response of sunflowers under saline and alkaline conditions. Photosynthetica, 48: 278-286.
Lutts S, Kinet JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivar differing in salinity resistance. Ann. Bot. 78: 389-398.
Manda K, Adams DC, and Ercal N (2010). Biologically important thiols in aqueous extracts of spices and evaluation of their in vitro antioxidant properties. Food Chem. 118:589-593.
Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and ability of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 49: 69-76.
Meneguzzo S, Navari-Izzo F, Izzo R (1999). Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. J. Plant Physiol. 155: 274-280.
Mittova V, Tal M, Volokita M, Guy M (2003). Up-regulation of the leaf mitochondrial and peroxisomal antioxidative system in response to salt-induced oxidative stress in the wild salt tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ. 26: 845-856.
Murshed R, Lopez-Lauri F, Sallanon H (2008). Microplate quantification of enzymes of the plant ascorbate-glutathione cycle. Anal. Biochem. 383: 320-322.
Nakano Y, Asada K (1981). Hydrogen peroxide scanvenged by ascorbate-specific peroxidase in spinach chloroplast. Plant cell Physiol. 22: 867-880.
Nawaz K, Ashraf M (2010). Exogenous application of glycinebetaine modulates activities of antioxidants in maize plants subjected to salt stress. J. Agron. Crop Sci. 196: 28-37.
Nickel RS, Cunningham BA (1969). Improved peroxidase assay method using Ieuco 2,3,6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis. Ann. Biochem. 27: 292-299.
Noreen Z, Ashraf M, Akram NA (2010). Salt-induced regulation of some key antioxidant enzymes and physio-biochemistry phenomena in five diverse cultivars of turnip(*Brassica rapa* L.). J. Agron. Crop Sci. 196: 23-285.
Pagnussat GC, Lanteri ML, Lamattina L (2003). Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. Plant Physiol. 132: 1241-1248.
Patra HL, Kar M, Mishre D (1978). Catalase activity in leaves and cotyledons during plant development and senescence. Biochem. Physiol. 172: 385-390.
Patterson BD, Macrae EA, Ferguson IB (1984). Estimation of hydrogen peroxide in plants extracts using titanium (IV). Ann. Biochem .134: 487-492.
Qiao W, Fan L (2008). Nitric oxide signaling in plant responses to abiotic stresses. J. Integr. Plant Biol. 50: 1238-1246.
Riadh R, Sabah M, Mohamed G, Mokhtar L (2006). Biochemical responses to true and bicarbonate-induced iron deficiency in grapevine genotypes. J. Plant Nutr. 29: 305-315.
Seckin B, Turkan I, Sekmen AH, Ozfidan C (2010). The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environ. Exp. Bot. 69: 76-85.
Shi D, Sheng Y (2005). Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. Environ. Exp. Bot. 54: 8-21.
Siddiqui MH, Al-Whaibi MH, Basalah MO (2011). Role of nitric oxide in tolerance of plants to abiotic stress. Protoplasma, 248: 447-455.
Stamler JS, Singel DJ, Loscalzo J (1992). Biochemistry of nitric oxide and its redox-activated forms. Science, 258: 1898-1902.
Stewart RC, Bewley JD (1980). Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiol. 65: 245-248.
Sun B, Jing Y, Chen K, Song L, Chen F, Zhang L (2007). Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*). J. Plant Physiol. 164: 536-543.
Suzuki N, Koussevitzky S, Mittler R, Miller G (2011). ROS and redox signaling in the response of plants to abiotic stress. Plant Cell. Environ (in Press, doi:10.1111/j.1365-3040.2011.02336.x)
Tanou G, Molassiotis A, Diamantidis G (2009). Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. Environ. Exp. Bot. 65: 270-281.
Van-Hasselt PR, Chow WS, Anderson JM (1996). Short-term treatment of pea leaves with supplementary UV-B at different oxygen concentrations: impacts on chloroplast and plasma membrane bound processes. Plant Sci. 120: 1-9.
Wu CH, Tewari RK, Hahn EJ, Paek KY (2007). Nitric oxide elicitation induces the accumulation of secondary metabolites and antioxidant defense in adventitious roots of *Echinacea purprea*. J. Plant Biol. 50: 636-643.
Xu PL, Guo YK, Bai JG, Shang L, Wang XJ (2008). Effects of long-term chilling on ultrastructure and antioxidant activity heaves of two cucumber cultivars under low light. Physiol. Planta. 132: 467-478.
Yamane K, Kawasaki M, Taniguchi M, Miyake H (2003). Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings. J. Plant Physiol. 160: 573-575.
Yang CW, Chong JN, Li CY, Kim CM, Shi DC, Wang DL (2007). Osmotic adjustment and ion balance traits of an alkali resistant halophyte Kochia sieversiana during adaptation to salt and alkali conditions. Plant Soil, 294: 263-276.
Yang CW, Shi DC, Wang DL (2008). Comparative effects of salt and alkali stresses on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *suaeda glauca* (Bge.). Plant Growth Regul. 56: 179-190.
Yang CW, Xu HH, Wang LL, Liu J, Shi D, Wang DL (2009). Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. Photosynthetica, 47: 79-86.
Yi QY, Niu HB, Wang MB, Shao HB, Deng DZ, Chen XX, Ren JP, Li YC (2008). Protective role of exogenous nitric oxide against oxidative-stress induced by salt stress in barley (*Hordeum vulgare*). Collid Surfaces B: Biointerfaces, 65: 220-225.
Zheng C, Jiang D, Liu F, Dai T, Liu W, Jing Q, Cao W (2009) Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. Environ. Exp. Bot. 67: 222-227.
Zhu Z, Wei G, Li J, Qian Q, Yu J (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt stressed cucumber (*Cucumis sativus* L.) Plant Sci. 167: 527-533.
Zhu ZJ, Gerendas J, Bendixen R, Schinner K, Tabrizi K, Sattelmacher B, Hansen UP (2000). Different tolerance to light stress in NO_3^- and NH_4^+ grown *Phaseolus vulgaris* L. Plant Biol. 2: 558-570.
Zottini M, Costa A, De Michele R, Ruzzene M, Carimi F, Lo Schiavo F (2007). Salicylic acid activates nitric oxide synthesis in *Arabidopsis*. J. Exp. Bot. 58: 1397-1405.