INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–031/2018/20–6–1454–1462
DOI: 10.17957/IJAB/15.0701
http://www.fspublishers.org

Full Length Article

Methyl Jasmonate Pretreatment Promotes the Growth and Photosynthesis of Maize Seedlings under Saline Conditions by Enhancing the Antioxidant Defense System

Biao Ji[1,2], Zan Li[3], Wanrong Gu[1,2], Jing Li[1,2], Tenglong Xie[1,2] and Shi Wei[1, 2*]
[1]*College of Agriculture, Northeast Agricultural University, Harbin, 150030, China*
[2]*The Observation Experiment Station of the Ministry of Agriculture for Crop Cultivation Science in Northeast Area, Harbin 150030, China*
[3]*Harbin Academy of Agricultural Sciences, Harbin, 150029, China*
[*]For correspondence: weishi5608@163.com

Abstract

Salinity could affected and give the stress for crop growth and development. Methyl jasmonate (MeJA) can mediate diverse developmental processes, but its specific role in plant salt tolerance is poorly understood. In order to identify the influence of MeJA under the salt tolerance in maize (*Zea mays* L.), plant growth， photosynthesis and antioxidant defense system were investigated. MeJA attenuated the crop development and oxidative damage caused by salt stress by generating less superoxide anion, hydrogen peroxide and malondialdehyde. Electrolyte leakage (EL), ascorbic acid (AsA), glutathione (GSH), hydrogen ascorbic acid type (DHA) and oxidized glutathione (GSSG), ratio of AsA/DHA and GSH/GSSG, were significantly reduced during seedling leaves. This is because MeJA maintained high SOD (Superoxide Dismutase), ascorbic acid peroxidase, glutathione reductase, monodehydroascorbate reductase and dehydroascorbate reductase activity under salt water. MeJA pretreatment ameliorated the adverse effect induced by salt stress on photosynthetic pigment content, photosynthetic capacity, and *PSII* photochemistry efficiency. The findings demonstrated that this promoted the development and photosynthesis of MeJA-treated maize seedlings under saline conditions by increasing the efficiency of the antioxidant defense system against oxidative damage.

Keyword: Maize; MeJA; Photosynthesis; Saline conditions; Antioxidant defense system

Introduction

As an environmental stress, salinity severely limits growth and reduces crop yields around the world (Deinlein *et al.*, 2014). Moreover, it exerts a negative impact on nearly 7% of the lands of Earth. The amount of salt-affected land continues to increase (Zhu and Gong, 2014). Hence, improving salt tolerance is essential for sustaining crop production.

Plants adapted to saline conditions through a series of physiological and morphological changes (Gupta and Huang, 2014). To conserve water under saline conditions, stomata are closed to decrease transpiration, inhibit the absorption and fixation of CO_2, reduce photosynthesis, and decrease organic compound synthesis. This results in significant inhibition of plant growth (Xu *et al*., 2014). The main toxic reactive oxygen species generated by chloroplasts and mitochondria are efficiently maintained at sublethal levels by the antioxidant defense systems in unstressed plants (Munns and Tester, 2008; Petrov *et al*., 2015). Under saline conditions, chloroplasts were exposed to excessive excitation energy that led to increased production of ROS (reactive oxygen species) that disrupted the balance between ROS generation and detoxification. This led to oxidative stress that severely disrupted cell normal metabolism (Misra and Gupta, 2006). Salinity-induced oxidative damage such as reduced photosynthetic pigments content, inactivation of photosynthetic enzymes, and damage of membranes structure is considered to represent the main non-stomatal constraint (Gao *et al*., 2008; Gill and Tuteja, 2010; Turan and Tripathy, 2013; Nedjimi, 2014).

The antioxidant defense system always showed the significant effect on detoxification of salt-induced ROS and maintaining growth under stress conditions. The study showed that the salt stress tolerance of crops was positively correlated with the enzymatic [SOD, APX (aseorbate peroxidase), GR (glutathione reductase), MDHAR (monodehydroascorbate reductase), DHAR (Dehydroascorbate reductase)] and non-enzymatic components of the antioxidant defense system (Parida and Das, 2005; Abraham and Dhar, 2010; Noreen *et al*., 2010; Mishra *et al.*, 2013).

To cite this paper: Ji, B., Z. Li, W. Gu, J. Li, T. Xie and S. Wei, 2018. Methyl jasmonate pretreatment promotes the growth and photosynthesis of maize seedlings under saline conditions by enhancing the antioxidant defense system. *Int. J. Agric. Biol*., 20: 1454–1462

Exogenously applied plant growth regulators salicylic acid, brassinolids, and indole acetic acid can dramatically increase plant growth and enhance salt tolerance on maize (He *et al*., 1991; Gunes *et al*., 2007; Kaya *et al*., 2014). Methyl jasmonate (MeJA) was studied to ameliorate the negative stress effects, including drought, chilling, and heavy metals (Rwm *et al*., 2004; Anjum *et al*., 2011), regulate the closure of stomata (Rohwer and Erwin, 2008) and up-regulate defense genes expression in crops (Ding and Smith, 2002). However, its specific role in plants was identified in few crops, and few studies have focused on maize. Moreover, the effects of MeJA for crop salt tolerance are less understood.

Maize (*Zea mays* L.), was identified as a salt-sensitive species, and its growth was greatly influenced by salinity at both the germination and seedling stages (Chaum and Kirdmanee, 2009; Ahmad *et al.*, 2011). Therefore, this study was performed to explore mechanism of MeJA in salt tolerance of maize seedlings by monitoring photosynthesis and the enzymatic and non-enzymatic components of antioxidant defense system. The result will give the foundation to better understand the mechanisms of MeJA involved the improved salt stress tolerance of maize.

Materials and Methods

Experimental Material and Plant Growth Conditions

Seeds of maize (*Zea mays* L. cv. Zhengdan 958) were supplied by the Henan Academy of Agricultural Sciences, were used in this experiment. Methyl jasmonate (MeJA) (95% purity, CAS No. 39924-52-2) was bought from Sigma-Aldrich.

The Petri dish (15 cm diameter) lined with damp filter paper was used for seed germination after high-temperature (120°C) sterilization for 30 min. Maize seeds were grown in glasshouse conditions for 5 days at day/night temperature 28/20°C, 14/10 h light/dark and photoperiod (400 μmol m^{-2} s^{-1}) and 65–70% relative humidity until the emergence of shoots and roots. The moist filter paper was replaced every day. On the fifth day, 40 maize seedlings with same growth state were chose and transferred to one plastic culture box (length × width × height: 30 × 20 × 7 cm) containing modified 1/2 Hoagland solution (pH = 6.5) and fixed in perforated foam board by an opening sponge.

Treatments

At the three-leaf stage, maize seedlings growing in the plastic box were subjected to the following treatments by root drenching: (1) 0 mM NaCl+0 mM MeJA (CK), (2) 100 mM NaCl + 0 mM MeJA (N), (3) 0 mM NaCl+0.1 mM MeJA (M) (4) 100 mM NaCl+0.1 mM MeJA (NM). Maize seedlings of M and NM treatments were pretreated with 0.1 mM MeJA nutrient solution for 24 h before stress. Each treatment was repeated five times, and forty maize seedlings from one plastic box were considered a repeat of each treatment. All plastic boxes were arranged completely randomly. The MeJA concentration was selected after using a range of MeJA concentrations (0.05−0.15 mM) in a preliminary growth experiment (data are not given). The best MeJA concentration was chosen based on the improved growth of the plants in nutrient solution with 200 mM NaCl. The second leaf numbered basipetally were assessed after 0d, 1d, 3d of exposure to salt stress with existence or none of MeJA for physiological characters.

Growth Parameters

The fresh weight of shoot and root was scored after sampling and the root and shoot part was dried under the condition of 105°C for 30 min, and 80°C for 24 h to determining the dry weight of root and shoot. We calculated the RLWC in leaf tissue based on the work of Munné-Bosch *et al.* (2003). We measured the fresh weight immediately after weighing, and the leaves of maize were immersed in the deionized water for 24 h, and remove water from surface with bibulous paper before measure the saturated fresh weight. Then, samples were dried in drying oven with 80°C for 72 h. The weight was measured using electronic analytical balance (BSA224S, Sartorius, Taiwan). We measured the area of the whole leaf by Li-3000 leaf area meter (Li-Cor, Inc., Lincoln, NE, USA). The mean values of the 10 plants that from one same dish were deemed as one replication.

Photosynthetic Pigment Contents

The pigment content was calculated by spectrophotometer (Arnon, 1949). The frozen leaf sample approximately 0.1 g was mashed and then mixed with 10 mL of miscible liquids which composed of acetone and 95.5% ethyl alcohol in the ratio of 1:1 in a centrifuge tube (15 mL). The tubes were placed in the dark until sample white. Then, 1 mL filtered extract mixed with 6 mL of absolute ethanol , and the contents of Chl a, Chl b, and Chl a+b were measured separately through the absorbance 645 nm, 652 nm, 663 nm.

Observation of Chloroplast by Transmission Electron Microscope

The approximately 1 mm^2 fragments of leaf samples which were cut were fixed overnight by glutaraldehyde in 0.1 M PBS (pH 7.4). Each fixed sample was washed 3 times for 10 min with the same solution. The following step were the sample post-fixed in 1% osmium tetroxide with the cacodylate buffer for 2 h then washed 3 times with 0.1 M PBS (pH 7.4). Then, the samples were dehydrated in gradient concentration ethanol (50, 70, 90, and 100%) and absolute acetone for 15 min. The samples were inserted in Spurr's resin, and then cut ultrathin sections were stained by uranium acetate and then lead citrate in series. The ultrathin

sections were accumulated on copper grids and then examined under a HITACHI HT7700 transmission electron microscope.

Gas Exchange Parameters

We measured the stomatal conductance (gs), transpiration rate (Tr), net photosynthetic rate (Pn) and intercellular CO_2 concentration (Ci) by a calibrated portable LI-6400 gas exchange system (Li-6400, Li-Cor Inc., USA) from 9:00 to 11:00 a.m. The analyses were performed under the condition of an air flow rate of 200 μmol s^{-1} at 25°C, 65% humidity, 350 μmol (photon)·m^{-2}·s^{-1} light intensity, and 350 μmol·mol^{-1} ambient CO_2 concentration. The measurement was done once for apiece leaf and for five different leaves each treatment.

Chlorophyll Fluorescence

We measured the Chlorophyll fluorescence parameters by a pulse-amplitude-modulated (PAM-2500) fluorometer (Walz, German). The leaves of maize were kept in adaptation last 20 min; after exposing to low modulated light for 0.8 s, the *Fo* and *Fm* values were measured. After the second saturation pulse, the maximum fluorescence was recorded in the light-adapted state (*Fm'*). The actinic light (7,000 μmol m^{-2} s^{-1}) was then turned off, and the far-red light was turned on in a light-adapted state (*F'o*) to measure the minimal fluorescence. The *Fv/Fm*, *ΦPSII*, and *NPQ* values were counted as follows: (*Fm–Fo*)/*Fm*, (*Fm'–F')/Fm'* and *Fm/Fm'*–1, respectively.

Production Rate of $O_2^{\cdot-}$, H_2O_2, MDA Content and Electrolyte Leakage (EL)

The measured of production rate of $O2^{\cdot-}$ was based on the method of Bian and Jiang (2009). H_2O_2 content was measured according to Bian and Jiang (2009). Lipid peroxidation was estimated through measuring MDA content according to the thiobarbituric acid (TBA) test according to Heath and Packer (1968). The MDA content was determined based on Heath and Packer (1968) by the thiobarbituric acid (TBA) reaction as previously described. Electrolyte leakage (EL) was measured by electrical conductivity meter according to Lutts *et al.* (1995).

Antioxidant Enzymes Assay

Leaf tissue (0.5 g) were extracted with 2 mL of 50 mM phosphate buffer (pH 7.8), containing 0.2 mM EDTA, 2% (w/v) polyvinylpyrrolidone (PVP), and 2 mM reduced ascorbate (AsA) and then centrifuged at 12000 *g* for 20 min at 4°C. The supernatant was used as enzyme extract.

SOD (EC 1.15.1.1) activity was assayed according to Giannopolitis and Ries (1977). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed based on the method of Nakano and Asada (1981). Glutathione reductase (GR, EC 1.6.4.2) activity was determined according to the method of Grace and Logan (1996). Monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activity was estimated according to Miyake and Asada (1992). Dehydroascorbate reductase (DHAR, EC 1.8.5.1) activity was assayed according to the method of Miyake and Asada (1992).

Ascorbic Acid and Glutathione

The contents of AsA and DHA were determined according to the modified method of Hodges *et al.* (1996). DHA was estimated from the difference between tAsA and AsA. The contents of GSH and GSSG were determined by the modified method of Griffith (1980). GSH was estimated from the difference between tGSH and GSSG.

Statistical Analysis

The experiment utilized a stochastic complete block design. SPSS 17.0 were for data analysis. All values were shown as the mean±SE. The least significant difference (LSD) test at the 5% probability level was used for means separating. The utilization of difference between treatments were considered significant difference ($P < 0.05$), and no difference were considered as no significant difference.

Results

Growth and RLWC of Maize Seedlings under Saline Conditions by MeJA Treatment

Under non-saline conditions, MeJA pretreatment significantly increased the shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total leaf area by 8.72, 8.69, 7.23, 9.02 and 7.30%, respectively. The result showed no significant effects on the RLWC of maize seedlings (Table 1). Salt stress inhibited seedling growth significantly, and the magnitudes of this decrease were smaller in MeJA-treated seedlings exposed to saline conditions than they were in untreated ones. The shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, total leaf area and RLWC were decreased by 42.52, 35.60, 30.45, 30.98, 42.21 and 36.93% in the salt-stressed seedlings, respectively. They also decreased by 26.78, 28.45, 22.37, 13.73, 24.40 and 18.20%, respectively, in the salt-stressed seedlings pretreated with MeJA.

Photosynthetic Pigment Content of Maize Seedlings under Saline Conditions by MeJA Treatment

Under non-saline conditions, MeJA pretreatment significantly increased chlorophyll (Chl) a content by 7.51%, but the result showed no significant effect on Chl b, total Chl content, or the ratio of Chl a /Chl b (Table 2).

Table 1: Fresh and dry weights of the shoots and roots and the leaf area of maize seedlings exposed to saline conditions and treated or not with MeJA

Treatment	Fresh weight (g·plant^{-1})		Dry weight (g·plant^{-1})		Leaf area (m^2·plant^{-1})	RWC (%)
	Shoot	Root	Shoot	Root		
Control	2.083 ±0.068 b	0.681± 0.017 b	0.1680±0.0081b	0.051± 0.003 a	38.880± 1.171 b	97.320±1.501a
MeJA	2.265 ±0.101 a	0.735± 0.021 b	0.1826±0.0078a	0.051± 0.003 a	41.718± 0.720 a	97.520±1.543a
NaCl	1.20± 0.082 d	0.474 ±0.028 c	0.1082±0.0072d	0.035± 0.003 c	22.470± 2.049 d	61.378±3.298c
NaCl+MeJA	1.53 ±0.148 c	0.529 ±0.028 d	0.1202±0.0082c	0.044± 0.005 b	29.394± 2.459 c	85.006±3.214b

The values represent the mean±SE (n=5). Values with the same letters in the columns are not significantly different at P<0.05 (LSD test)

The chlorophyll content was significantly decreased in salt stress, and the magnitude of decrease was smaller in MeJA-treated seedlings under saline conditions than it was in untreated ones. The contents of Chl a, Chl b, and total Chl was reduced of 55.17, 66.02 and 61.56%, respectively, and the ratio of Chl a/Chl b increased by 32.06% in the salt-stressed maize, respectively. The Chl a, Chl b, and total Chl contents reduced by 21.58, 32.04 and 27.75%, respectively, in the salt-stressed seedlings pretreated with MeJA, the ratio of Chl a/Chl b was increased by 15.21%.

Table 2: Photosynthetic pigment content (mg/g FW) of maize seedlings exposed to saline conditions and treated or not with MeJA

Treatment	Chl a	Chl b	Chl a+b	Chl a/b
Control	3.97± 0.16 b	5.69± 0.24 a	9.65± 0.15 a	0.70± 0.05 c
MeJA	4.26± 0.19 a	5.68± 0.22 a	9.95± 0.34 a	0.75± 0.03 bc
NaCl	1.78± 0.11 d	1.93± 0.15 c	3.71± 0.22 c	0.92± 0.07 a
NaCl+MeJA	3.11± 0.21 c	3.86± 0.18 b	6.97± 0.33 b	0.81± 0.05 b

The values represent the mean±SE (n=5). Values with the same letters in the columns are not significantly different at P<0.05 (LSD test)

Gas Exchange Parameters of Maize Seedling under Saline Conditions by MeJA Treatment

Under non-saline conditions, MeJA-treated seedlings showed a higher *Pn*, *Gs*, *Tr* and *Ci* by 7.63, 7.19, 8.69, 6.82 and 8.20%, respectively, than control at the 3rd day (Fig. 1 A−D). *Pn*, *Gs* and *Tr* were drastically decreased in both non-MeJA and MeJA-treated seedlings exposed to saline conditions. Interestingly, the magnitudes of this decrease were smaller in leaves of MeJA-pretreated seedlings than they were in non-MeJA-pretreated seedlings. Compared to controls, the values of *Pn*, *Gs* and *Tr* decreased by 51.62, 50.26 and 54.69%, respectively, and *Ci* increased by 18.65% in the salt-stressed seedlings. These parameters decreased by 25.34, 23.17, 32.97 and 10.83% in the salt-stressed seedlings pretreated with MeJA, respectively.

Compared to controls, *Ci* decreased first and increased afterwards. It increased by 18.65% at the 3rd day in the salt-stressed seedlings. *Ci* decreased first, remained stable, and then decreased by 10.83% at the 3rd day in the salt-stressed seedlings that were pretreated with MeJA.

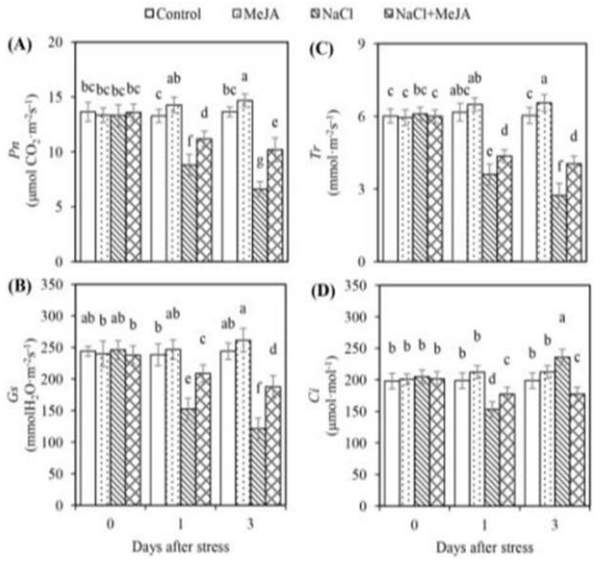

Fig. 1: Effects of MeJA on the *Gs*, *Tr*, *Ci* and *Pn* of the fully developed second leaf (numbered basipetally) of maize seedlings under saline conditions. The data represent the means of independent measurements with five replicates, and the standard deviations are indicated by the vertical error bars. Values with the same letters on the bars are not significantly different at P<0.05

Chlorophyll Fluorescence Parameters of Maize Seedlings under Saline Conditions by MeJA Treatment

The result showed no significant differences in chlorophyll fluorescence parameters of the non-treated and MeJA-treated seedlings under non-saline conditions (Fig. 2A-D). Under saline conditions, the values of the *F*v/*F*m, *ΦPSII* and *NPQ* were decreased, by 43.83, 57.89 and 48.83%, respectively, in non-MeJA pretreatment, and by 21.25, 29.36 and 32.78%, in MeJA pretreatment at the 3rd day with CK (Fig. 2). The values of the minimal fluorescence (*F*o) significantly increased by 71.58% in non-MeJA treatment and by 31.07% compared to control at the 3rd day.

Generation of O_2^- Content of H_2O_2 and MDA, and EL Levels under Salinity and MeJA Treatment

No significant effects on the content of H_2O_2 and MDA, and EL were observed in plants treated with MeJA alone, but MeJA pretreatment significantly increased the generation rate of $O_2\cdot^-$ in leaves under non-saline conditions compared with the control (Fig. 3A−D).

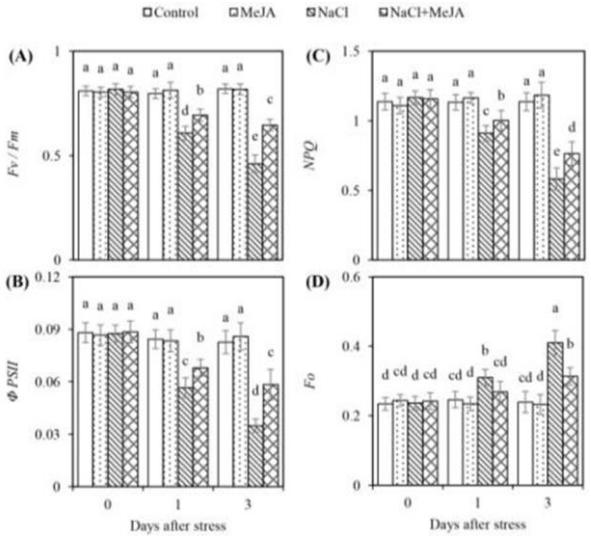

Fig. 2: Effects of MeJA on the *F*o, *F*v/*F*m, *NPQ* and *ΦPSII* of the fully developed second leaf (numbered basipetally) of maize seedlings under saline conditions. The data represent the means of independent measurements with five replicates, and the standard deviations are indicated by the vertical error bars. Values with the same letters on the bars are not significantly different at P<0.05

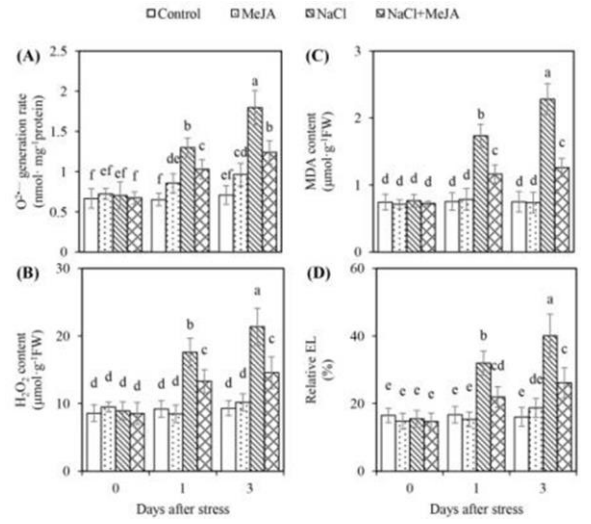

Fig. 3: Effects of MeJA on the production rate of O2·⁻, H_2O_2 content, MDA content and EL of the fully developed second leaf (numbered basipetally) of maize seedlings under saline conditions. The data represent the means of independent measurements with five replicates, and the standard deviations are indicated by the vertical error bars. Values with the same letters on the bars are not significantly different at P<0.05

Under saline conditions, maize seedlings showed a marked increase in the generation rate of $O_2^{\cdot-}$, content of H_2O_2, MDA, and EL levels, and by 153.53, 129.17, 204.25 and 149.55%, respectively, compared to the control plants at the 3rd day. However, MeJA pretreatment significantly decreased the generation rate of $O_2^{\cdot-}$, content of H_2O_2, MDA, and EL, and by 30.97, 31.82, 44.71 and 34.80% at the 3rd day, respectively, compared to the single NaCl treatment. However, the values in these treatments were still much higher than that in control.

Activities of Antioxidative Enzymes, MDHAR, and DHAR under Saline conditions by MeJA Treatment

In response to saline conditions and MeJA pretreatment, the SOD activity was increased by 108.81 and 23.83% on the 3rd day, respectively (Fig. 4A). Moreover, SOD activities in MeJA-pretreated seedlings were significantly higher (24.67% over untreated plants at the 3rd day) than those in untreated ones under saline conditions. Under non-saline conditions, MeJA has not significantly effect on the activities of APX, GR, DHAR and MDAR (Fig. 4B−E). After exposure to saline conditions, the activities of APX, GR, DHAR and MDAR were decreased by 39.97, 41.77, 48.15 and 42.07% significantly, respectively, on the 3rd day compared to control. Under saline conditions, MeJA pretreatment enhanced these activities by 33.02, 37.68, 34.09, and 28.58%, respectively, compared to salt stress alone on the 3rd day.

AsA, DHA, GSH and GSSG and Ratios of AsA/DHA and GSH/GSSG under Saline Conditions by MeJA Treatment

The contents of AsA, GSH, DHA and GSSG, and the ratio of AsA/GSH and GSH/GSSG did not showed the significantly difference for non-treated and MeJA-treated seedlings under non-saline tratement. Salt stress can reduce the content of AsA and GSH (by 24.58 and 30.94% compared with control on the 3rd day), and increase the content of DHA and GSSG (by 61.07 and 26.35% compared to control on the 3rd day) (Fig. 5A−F). This resulted in significant decreases in the ratios of AsA/DHA and GSH/GSSG (by 53.44 and 45.33% compared to control at the 3rd day). Moreover, seedlings pretreated with MeJA had higher AsA and GSH contents at salt stress condition (increased by12.25 and 18.08% at the 3rd day) and lower DHA and GSSG contents (decreased by 24.55 and 11.22% on the 3rd day) than did stressed untreated plants, which significantly increased the ratios of GSH/GSSG and AsA/DHA (increased by 48.99 and 33.26% compared to untreated plants on the 3rd day).

Chloroplast Ultrastructure of Maize Seedlings under Saline Conditions by MeJA Treatment

The chloroplasts in maize seedlings pretreated with or without MeJA under unstressed conditions were related with the membrane tidily, and they contained smooth thylakoid membranes and well-arranged grana (Fig. 6).

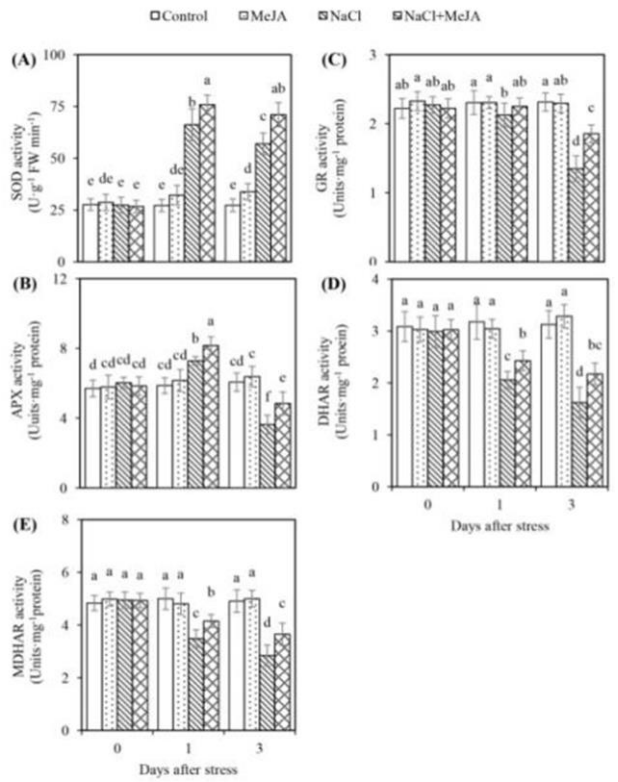

Fig. 4: Effects of MeJA on the activity of SOD, APX, GR, DHAR and MEHAR of the fully developed second leaf (numbered basipetally) of maize seedlings under saline conditions. The data represent the means of independent measurements with five replicates, and the standard deviations are indicated by the vertical error bars. Values with the same letters on the bars are not significantly different at P<0.05

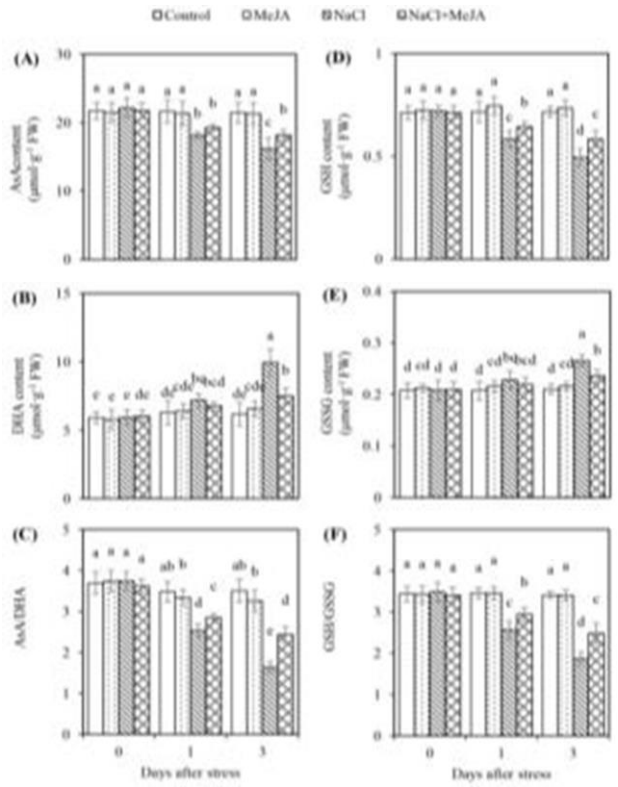

Fig. 5: Effects of MeJA on the content of AsA, DHA, GSH, GSSG, and ratio of AsA/DHA and GSH/GSSG of the fully developed second leaf (numbered basipetally) of maize seedlings under saline conditions. The data represent the means of independent measurements with five replicates, and the standard deviations are indicated by the vertical error bars. Values with the same letters on the bars are not significantly different at P<0.05

However, the chloroplasts under salt stress were divided from the plasma membrane, and their shape changed smoothly, from the ellipse to the near circle. The chloroplasts showed disarranged thylakoids with an increased number and size of osmiophilic plastoglobuli. Additionally, the inner lamellar of the stroma thylakoid (SL) had been damaged by the disintegration of the stacked granal thylakoid (GL) system. But the MeJA pretreatment alleviated the changes of chloroplasts structure. Pretreated plants contained fewer osmiophilic plastoglobuli induced by salt stress.

Discussion

The growth inhibition induced by salt stress has been obtained in previous reports in many crops (Agong *et al*., 2004; Yamaguchi and Blumwald, 2005; Hajer *et al*., 2006; Ferreira-Silva *et al.,* 2012). For maize, the growth and physiological parameters in the seedling stage are frequently used for salt tolerance evaluation (Giaveno *et al*., 2007).

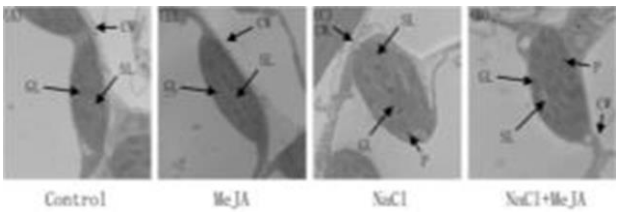

Fig. 6: Effects of MeJA on the ultrastructure of the photosynthetic apparatus of the leaves of maize seedlings grown in nutrient solutions with or without NaCl. The second leaves (numbered basipetally) were sampled for ultramicroscopic observations on day 3 after the drought treatment (15% PEG-60000). SL, stroma lamella; GL, grana lamellae; CW, cell wall; and P, plastoglobule. The scale bars for the photosynthetic apparatus represent 2000 nm. (A) Control, (B) MeJA, (C) NaCl, (D) NaCl+MeJA

Here, we found that all investigated growth parameters decreased significantly under saline conditions (Table 1). Photosynthesis is the primary process of biomass production

and maintenance that is affected by salinity. The growth reduction of maize seedlings might be due to lower photosynthetic capacity and low water status based on the reduction of *Pn* and *RWC* induced by salt stress (Munns *et al*., 2006) (Fig. 1A). Notably, the salt-induced growth inhibition of maize seedlings was partly reversed by MeJA treatment. This was manifested by increased growth parameters. Similar to previous reports showing that exogenous MeJA application could increase plant tolerance to environmental stresses, including drought, chilling, heavy metal (Rwm *et al*., 2004; Anjum *et al*., 2011), the results suggested that MeJA treatment could enhance the salinity resistance of maize seedlings.

As previously seen with many other crops, stomatal closure decreases the transpiration rate. This may be an important adaptation mechanism for salt tolerance. It also limits CO_2 diffusion to the intercellular spaces within leaves under stress, leading to a reduction in photosynthesis (Mustárdy *et al*., 2010; Barbieri *et al*., 2012). Other reports ascribed the reduction to non-stomatal limitation (Dunn and Neales, 1993; Zhang *et al*., 2015). We also observed that the decrease in *Ci* was not accompanied by a significant corresponding change in *Ci* under saline conditions. Seedlings exposed to saline conditions have a relatively higher *Ci* and lower *Gs*. These results show that the reduction of *Pn* in maize seedlings exposed to saline conditions associated with both stomatal and non-stomatal limitations and their combination (Fig. 1A, B and D). The seedlings pretreated with MeJA maintained higher *RWC*, *Gs* and *Tr* compared to untreated seedlings under saline conditions. One possible reason is that MeJA enhanced root water uptake and tissue water maintenance (Tanentzap *et al*., 2015). Under saline conditions, MeJA pretreatment reversed salt-induced stomatal closure to produce more CO_2 in the pore space of the cell, protect the photochemical mechanism, and maintain significantly higher CO_2 assimilation rates. This is suggested by the higher *gs* and lowers *Ci* under saline conditions. Compared to untreated seedlings, MeJA pretreatment maintained appropriate leaf water status, large leaf area and high photosynthetic rate in salt-stressed maize seedlings, enabling a much greater supply of assimilate to growing tissue and promoting seedling growth.

For photosynthesis, it always begins with the suck up light by pigment molecules located in the thylakoid membrane. Chlorophyll content is positively correlated with photosynthetic rate, and is widely used as an indicator of plant abiotic tolerance (Melkozernov and Blankenship, 2006). The decrease in *Chl* content of maize under saline conditions agrees with previous reports in sunflowers (Akram and Akram, 2009) and wheat (Shahbaz *et al*., 2008). This can be attributed to chlorophyll enzymes degradation quickly or damage of chloroplast structure and associated proteins (Singh and Dubey, 1995) induced by the over-production of ROS and account for the decrease of *Pn* (Table 2 and Fig. 1A, Fig. 3A and B). Compared with non-MeJA treated ones, MeJA pretreatment mitigated the adverse effects induced by saline conditions on Chl content, which contributes to the enhanced capacity for light-absorbing efficiency and leaf photosynthetic rate of MeJA-pretreated maize seedlings, increasing salt tolerance.

There are many physiological factors involved in salt stress, including MDA and EL. Determination of the MDA level as an indicator of lipid peroxidation-induced ROS formation is a widely used methods (Miao *et al*., 2010). EL from leaf tissues has been reported as an important indicator to measure the permeability of cell membrane (Cui *et al*., 2015). Here, in response to saline conditions, membrane lipid peroxidation and loss of cell membrane integrity (as expressed by MDA and EL, respectively) were also observed in maize seedling leaves. This is presumably due to increased ROS like $O_2^{\cdot-}$ and H_2O_2 under salt stress (Fig. 3A−D). This phenomenon is consistent with the disordered grana thylakoid lamellae and increased osmiophilic particles (Fig. 6C). However, the magnitudes of increase of MDA and EL were smaller in MeJA-pretreated seedlings than the non-pretreated ones (Fig. 3C and D). There was similarity between this result of Wang (1999), Anjum *et al*. (2011), which demonstrated that exogenous MeJA treatment can reduce lipid peroxidation of strawberries and soybeans exposed to water stress. Moreover, MeJA pretreatment significantly decreased the separation of the plasma membrane from plasmolysis, and it alleviates these indications of salt stress damage by making its shape and structure look normal (Fig. 6D). These results demonstrated that MeJA pretreatment minimizes the damage to the photosynthetic apparatus and helps maintain photosynthetic pigments under environmental may contribute to effectively protect photosynthetic apparatus from oxidative damage under saline conditions. This effect could be ascribed to MeJA pretreated seedlings with more efficient $O_2^{\cdot-}$ and H_2O_2 scavenging.

Aremu *et al*. (2014) reported that primary reactions of chlorophyll fluorescence photosynthesis are always functioned by subtle reflection. It has been widely used in describing the photosynthetic physiology and photosynthesis mechanism under environmental stress (Daymond and Hadley, 2015). The *Fv*/*Fm* ratio is a way to evaluate the maximal quantum yield of *PSII* photochemistry (Sharma *et al*., 2015) and indicated the utilization of light energy for photosynthesis. Compared to the non-MeJA pretreated samples, *Fv/Fm* was maintained at higher values in MeJA treated seedlings (Fig. 2A). Moreover, MeJA pretreated plants maintained higher *ΦPSII* and *ETR* and lower *NPQ* (Fig. 2B−D). This indicated that MeJA could protect the salt stress induced damage for the *PSII* activity and *PSII* reaction centers were damaged. A possible reason is that MeJA maintained the efficiency of photochemical and activity of electron transportation, which associated with the structural integrity and orderliness of chloroplast (Fig. 6D) (Mittal *et al*., 2012).

Antioxidative enzymes are the key component to provide the protection of the cell structures to stressful

conditions (Reddy *et al*., 2004). After exposure to salt stress, maize seedlings showed a comparatively high SOD activity. This is similar to observations in many crop species, and it may be a plant adaptation to stress (Fig. 4A). It seemed to be insufficient to scavenge all $O_2^{\cdot -}$, as expressed by increased generation rate of $O_2^{\cdot -}$; However, MeJA pretreatment further enhanced the SOD activity in maize seedlings under saline conditions, which may accelerate the reduction from $O_2^{\cdot -}$ to H_2O_2 (Sun and Tao, 2011). APX is a key component of the AsA-GSH cycle in plants, and it is predominantly responsible for converting H_2O_2 into H_2O, with AsA as the electron donor. Some researchers reported the similar result (Moradi and Ismail, 2007). Due to the high affinity of APX for H_2O_2, this increased APX activity induced by MeJA under saline conditions could be responsible for efficient control of H_2O_2 content and preventing H_2O_2-mediated cell damage. Compared to the samples exposed to salt stress only, maintaining higher AsA in a high redox state in MeJA-pretreated seedlings might be due to enhanced activities of MDHAR and DHAR. In that case, APX scavenges H_2O_2 efficiently (Fig. 4B, D and E, Fig. 5A, B and C). GR, the rate-limiting enzyme, plays a key function at keeping the GSH/GSSG ratio advantageous to ascorbate reduction (Gossett *et al*., 1994). Under saline conditions, the MeJA-treated seedlings exhibited a higher GR activity compared to the untreated ones. The increasing of GR activity with MeJA pretreatment may affect high GSH content and a high GSH/GSSG ratio in the leaves of MeJA-treated seedlings that are advantageous to ascorbate reduction (Gossett *et al*., 1994; Fig. 4C, Fig. 5D, E and F).

Conclusion

MeJA pretreatment enhanced the activities of antioxidative enzyme in maize leaves and maintained redox homeostasis under saline conditions. This led to a lower rate of $O_2^{\cdot -}$ and H_2O_2 generation, attenuated the oxidative damage affected by salt stress, reduced salt-induced damage for the photosynthetic system and improved maize seedling growth.

Acknowledgements

This work was funded and supported by the National Key Research and Development Program of China (2016YFD0300103), the National Key Research and Development Program of China (2017YFD0300506), and "Academic Backbone" Project of Northeast Agricultural University (17XG23).

References

Abraham, G. and D.W. Dhar, 2010. Induction of salt tolerance in azolla microphylla, kaulf through modulation of antioxidant enzymes and ion transport. *Protoplasma*, 245: 105–111

Agong, S.G., Y. Yoshida, S. Yazawa and M. Masuda, 2004. Tomato response to salt stress. *Acta Hortic*., 637: 93–97

Ahmad, S.Q., S. Khan, M. Ghaffar and F. Ahmad, 2011. Genetic diversity analysis for yield and other parameters in maize (*Zea mays* L.) genotypes. *Asian J. Agric. Sci*., 3: 385–388

Akram, M.M. and N. Akram, 2009. Effectiveness of potassium sulfate in mitigating salt-induced adverse effects on different physio-biochemical attributes in sunflower (*Helianthus annuus* L.). *Flora*, 204: 471–483

Anjum, S.A., L. Wang, M. Farooq, I. Khan and L. Xue, 2011. Methyl jasmonate-induced alteration in lipid peroxidation, antioxidative defence system and yield in soybean under drought. *J. Agron. Crop Sci.*, 197: 296–301

Aremu, A.O., N.A. Masondo, T.O. Sunmonu, M.G. Kulkarni, M. Zatloukal, L. Spichal and V.S. Johannes, 2014. A novel inhibitor of cytokinin degradation (incyde) influences the biochemical parameters and photosynthetic apparatus in NaCl-stressed tomato plants. *Planta*, 240: 877–889

Arnon, D.T., 1949. Copper enzyme in isolated chloroplasts polyphe-noloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1–15

Barbieri, G., S. Vallone, F. Orsini, R. Paradiso, S.D. Pascale, and F. Negre-Zakharov, 2012. Stomatal density and metabolic determinants mediate salt stress adaptation and water use efficiency in basil (*Ocimum basilicum* L.). *J. Plant Physiol*., 169: 1737–1746

Bian, S.M. and Y.W. Jiang, 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Sci. *Hortic.*, 120: 264–270

Chaum, S. and C. Kirdmanee, 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.*, 41: 87–98

Cui, D., D. Wu, J. Liu, D. Li, C. Xu and S. Li, 2015. Proteomic analysis of seedling roots of two maize inbred lines that differ significantly in the salt stress response. *PloS One*, 10: e0116697

Daymond, A.J. and P. Hadley, 2015. The effects of temperature and light integral on early vegetative growth and chlorophyll fluorescence of four contrasting genotypes of cacao (*Theobroma cacao*). *Ann. Appl. Biol.*, 145: 257–262

Deinlein, U., A.B. Stephan, H. Tomoaki, L. Wei, X. Guohua and S. Zhulian, 2014. Plant salt-tolerance mechanisms. *Trends Plant Sci.*, 19: 371

Ding, C.K. and D.L. Smith, 2002. Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta*, 214: 895–901

Dunn, G.M. and T.F. Neales, 1993. Are the effects of salinity on growth and leaf gas exchange related *Photosynthetica*, 29: 33–42

Ferreira-Silva, S.L., E.L. Voigt, E.N. Silva, J.M. Maia, T.C.R. Aragão and J.A.G. Silveira, 2012. Partial oxidative protection by enzymatic and non-enzymatic components in cashew leaves under high salinity. *Biol. Plant.*, 56: 172–176

Gao, S., C. Ouyang, S. Wang, Y. Xu, L. Tang and F. Chen, 2008. Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. *Plant Soil Environ*, 54: 374–381

Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutases i. occurrence in higher plants. *Plant Physiol*., 59: 309–314

Giaveno, C.D., R.V. Ribeiro, G.M. Souza and R.F.D. Oliveira, 2007. Screening of tropical maize for salt stress tolerance. *Crop Breed. Appl. Biotechnol*., 7: 304–313

Gill, S.S. and N. Tuteja, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, 48: 909

Gossett, D.R., E.P. Millhollon and M.C. Lucas, 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Sci.*, 34: 1057–1075

Grace, S.C. and B.A. Logan, 1996. Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. *Plant Physiol.*, 112: 1631–1640

Griffith, O.W., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.*, 106: 207–212

Gunes, A., A. Inal, M. Alpaslan, F. Eraslan, E.G. Bagci and N. Cicek, 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol.*, 164: 728–36

Gupta, B. and B. Huang, 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genom.*, 2014: 701596

Hajer, A.S., A.A. Malibari, H.S. Alzahrani and O.A. Almaghrabi, 2006. Responses of three tomato cultivars to sea water salinity 1. effect of salinity on the seedling growth. *Pak. J. Biol. Sci*., 3: 855–861

He, R.Y., G.J. Wang and X.S. Wang, 1991. Effects of brassinolide on growth and chilling resistance of maize seedlings. *Acs Symposium Series Amer. Chem. Soc*., 1932

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. i. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*., 125: 189–198

Hodges, D.M., C.J. Andrews, D.A. Johnson and R.I. Hamilton, 1996. Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines. *Physiol. Plant.*, 98: 1105–1113

Kaya, C., A.L. Tuna and A.M. Okant, 2014. Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. *Turk. J. Agric. For*., 34: 529–538

Lutts, S., J.M. Kinet and J. Bouharmont, 1995. Changes in plant response to nacl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J. Exp. Bot.*, 46: 1843–1852

Melkozernov, A.N. and R.E. Blankenship, 2006. Photosynthetic functions of chlorophylls. *Adv. Photosynt. Respir*., 25: 397–412

Miao, B.H., X.G. Han and W.H. Zhang, 2010. The ameliorative effect of silicon on soybean seedlings grown in potassium-deficient medium. *Ann. Bot.*, 105: 967–973

Mishra, P., K. Bhoomika and R.S. Dubey, 2013. Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive indica rice (*Oryza sativa* L.) seedlings. *Protoplasma*, 250: 3–19

Misra, N. and A.K. Gupta, 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in catharanthus roseus seedlings. *J. Plant Physiol.*, 163: 11–18

Mittal, S., N. Kumari and V. Sharma, 2012. Differential response of salt stress on brassica juncea: photosynthetic performance, pigment, proline, d1 and antioxidant enzymes. *Plant Physiol. Biochem.*, 54: 17–26

Miyake, C. and K. Asada, 1992. Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. *Plant Cell Physiol*., 33: 433–439

Moradi, F. and A.M. Ismail, 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive, stages in rice. *Ann. Bot.*, 99: 1161–1173

Munné-Bosch, S., T. Jubany-Marí and L. Alegre, 2003. Enhanced photo- and antioxidative protection, and hydrogen peroxide accumulation in drought-stressed cistus clusii and cistus albidus plants. *Tree Physiol*., 23: 1–12

Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59: 651–681

Munns, R., R.A. James and A. Läuchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57: 1025–1043

Mustárdy, L.A., T.T. Vu and Á. Faludi-Dániel, 2010. Stomatal response and photosynthetic capacity of maize leaves at low temperature. a study on varietal differences in chilling sensitivity. *Physiol. Plant.*, 55: 31–34

Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*., 22: 867–880

Nedjimi, B., 2014. Effects of salinity on growth, membrane permeability and root hydraulic conductivity in three saltbush species. *Biochem. Syst. Ecol*., 52: 4–13

Noreen, Z., M. Ashraf and N.A. Akram, 2010. Salt-induced regulation of some key antioxidant enzymes and physio-biochemical phenomena in five diverse cultivars of turnip (brassica rapa l.). *J. Agron. Crop Sci.*, 196: 273–285

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Saf.*, 60: 324–349

Petrov, V., J. Hille, B. Mueller-Roeber and T.S. Gechev, 2015. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front. Plant Sci.*, 6: 69

Reddy, A.R., K.V. Chaitanya, P.P. Jutur and K. Sumithra, 2004. Differential antioxidative responses to water stress among five mulberry (morus alba, l.) cultivars. *Environ. Exp. Bot.*, 52: 33–42

Rohwer, C.L. and J.E. Erwin, 2008. Horticultural applications of jasmonates. *J. Hortic. Sci. Biotechnol*., 83: 283–304

Rwm, F., C.Y. Wang, D.L. Smith, K.C. Gross and M. Tian, 2004. MeSA and MeJA increase steady-state transcript levels of alternative oxidase and resistance against chilling injury in sweet peppers (*Capsicum annuum* L.). *Plant Sci.*, 166: 711–719

Shahbaz, M., M. Ashraf and H.U.R. Athar, 2008. Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.). *Plant Growth Regul*., 55: 51–64

Sharma, D.K., S.B. Andersen, C.O. Ottosen and E. Rosenqvist, 2015. Wheat cultivars selected for high fv/fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiol. Plant.*, 153: 284–98

Singh, A.K. and R.S. Dubey, 1995. Changes in chlorophyll a and b contents and activities of photosystems i and ii in rice seedlings induced by nacl. *Photosynthetica*, 31: 489–499

Sun, X. and W. Tao, 2011. Physiological roles of plastid terminal oxidase in plant stress responses. *J. Biosci.*, 36: 951

Tanentzap, F.M., A. Stempel and P. Ryser, 2015. Reliability of leaf relative water content (RWC) measurements after stress. *Botany*, 93: 535–541

Turan, S. and B.C. Tripathy, 2013. Salt and genotype impact on antioxidative enzymes and lipid peroxidation in two rice cultivars during de-etiolation. *Protoplasma*, 250: 209–222

Wang, S.Y., 1999. Methyl jasmonate reduces water stress in strawberry. *J. Plant Growth Regul*., 18: 127–134

Xu, W.Z., X.P. Deng, B.C. Xu, Z.J. Gao and W.L. Ding, 2014. Photosynthetic activity and efficiency of bothriochloa ischaemum, and lespedeza davurica, in mixtures across growth periods under water stress. *Acta Physiol. Plant.*, 36: 1033–1044

Yamaguchi, T. and E. Blumwald, 2005. Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci.*, 10: 615–620

Zhang, R.H., X.H. Zhang, J.J. Camberato and J.Q. Xue, 2015. Photosynthetic performance of maize hybrids to drought stress. *Russ. J. Plant Physiol.*, 62: 788–796

Zhu, Y. and H. Gong, 2014. Beneficial effects of silicon on salt and drought tolerance in plants. *Agron. Sustain. Dev.*, 34: 455–472

(Received 01 March 2018; Accepted 23 March 2018)