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

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Abstract

Salinity could affective and give the stress for crop growth and development. MeJA (Methyl Jasmonate) 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 seedlings leaves. This is because MeJA maintained high SOD (Superoxide Dismutase), ascorbic acid peroxidase, glutathione reductase, mono dehydroascorbate 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. © 2018 Friends Science Publishers

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 CO₂, 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 (Munn 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 reaction (SOD, APX (ascorbateperoxidase), 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).

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Exogenously applied plant growth regulators salicylic acid, brassinoloids, 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 (Chau 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 were 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 washed 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 mm² 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 CO₂ 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 CO₂ 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 Fo/Fm, ΦPSII, and NPQ values were counted as follows: (Fm–Fo)/Fm, (Fm'–F'/Fm' and Fm/Fm'–1, respectively.

**Production Rate of O₂⁻, H₂O₂, MDA Content and Electrolyte Leakage (EL)**

The measured production rate of O₂⁻ was based on the method of Bian and Jiang (2009). H₂O₂ 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).
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 were reduced by 21.58, 32.04 and 27.75%, respectively, under saline-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%.

### 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.

### 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 Fv/Fm, \( \Phi_{PSII} \) 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 (F0) significantly increased by 71.58% in non-MeJA treatment and by 31.07% compared to control at the 3rd day.

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**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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g plant(^{-1}))</th>
<th>Dry weight (g plant(^{-1}))</th>
<th>Leaf area (m(^2) plant(^{-1}))</th>
<th>RWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>2.083 ± 0.068 b</td>
<td>0.681 ± 0.017 b</td>
<td>0.1680 ± 0.0081 b</td>
<td>0.051 ± 0.003 a</td>
</tr>
<tr>
<td>MeJA</td>
<td>2.265 ± 0.101 a</td>
<td>0.735 ± 0.021 b</td>
<td>0.1826 ± 0.0078a</td>
<td>0.051 ± 0.003 a</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.20 ± 0.082 d</td>
<td>0.474 ± 0.028 c</td>
<td>0.1082 ± 0.0072d</td>
<td>0.035 ± 0.003 c</td>
</tr>
<tr>
<td>NaCl+MeJA</td>
<td>1.53 ± 0.148 c</td>
<td>0.529 ± 0.028 d</td>
<td>0.1202 ± 0.0082c</td>
<td>0.044 ± 0.005 b</td>
</tr>
</tbody>
</table>

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).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl a+b</th>
<th>Chl b/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.97 ± 0.16 b</td>
<td>5.69 ± 0.24 a</td>
<td>9.65 ± 0.15 a</td>
<td>0.70 ± 0.05 c</td>
</tr>
<tr>
<td>MeJA</td>
<td>4.26 ± 0.19 a</td>
<td>5.68 ± 0.22 a</td>
<td>9.95 ± 0.34 a</td>
<td>0.75 ± 0.03 bc</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.78 ± 0.11 d</td>
<td>1.93 ± 0.45 c</td>
<td>3.71 ± 0.22 c</td>
<td>0.92 ± 0.07 a</td>
</tr>
<tr>
<td>NaCl+MeJA</td>
<td>3.11 ± 0.21 c</td>
<td>3.86 ± 0.18 b</td>
<td>7.01 ± 0.33 b</td>
<td>0.81 ± 0.05 b</td>
</tr>
</tbody>
</table>

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).

**Fig. 1**: Effects of MeJA on the Gs, Tr, Ci and Pn of the fully developed second leaf (numbered basipetally) of maize seedlings under salinization 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.

**Generation of O\(_2\)\(^-\) Content of H\(_2\)O\(_2\) and MDA, and EL Levels under Salinity and MeJA Treatment**

No significant effects on the content of H\(_2\)O\(_2\) and MDA, and EL were observed in plants treated with MeJA alone, but MeJA pretreatment significantly increased the generation rate of O\(_2\)\(^-\) in leaves under non-saline conditions compared with the control (Fig. 3A-D).
Fig. 2: Effects of MeJA on the Fo, Fv/Fm, 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−, H2O2 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 O2−, content of H2O2, 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 O2−, content of H2O2, 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 49.97, 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, 37.69, 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 treatment. 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 by 12.25 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).
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. 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

**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 (Rwmi 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 (Mustard 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 lower $Ci$ 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 assimilates 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 overproduction 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$_2^-$ and H$_2$O$_2$ under salt stress (Fig. 3A-D). This phenomenon is consistent with the disordered grana thylakoid lamellae and increased osmophilic 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$_2^-$ and H$_2$O$_2$ 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$_2^-$, as expressed by increased generation rate of O$_2^-$. However, MeJA pretreatment further enhanced the SOD activity in maize seedlings under saline conditions, which may accelerate the reduction from O$_2^-$ to H$_2$O$_2$. (Sun and Tao, 2011). APX is a key component of the AsA-GSH cycle in plants, and it is predominantly responsible for converting H$_2$O$_2$ into H$_2$O 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$_2$O$_2$, this increased APX activity induced by MeJA under saline conditions could be responsible for efficient control of H$_2$O$_2$ content and preventing H$_2$O$_2$-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$_2$O$_2$ 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$_2^-$ and H$_2$O$_2$ generation, attenuated the oxidative damage affected by salt stress, reduced salt-induced damage for the photosynthetic system and improved maize seedling growth.

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