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Antioxidant metabolism variation associated with salt tolerance of six maize (*Zea mays* L.) cultivars

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ABSTRACT

Salt stress is a major limiting factor for crop production in many regions. This study examined antioxidant metabolism variation associated with salt stress tolerance of six maize cultivars (Luyu39, Huanong138, Xianyu335, Aoyu3007, Yayu8, Jinping618) under growth chamber environments. The seedlings of six cultivars were subjected to seven NaCl concentrations ranging from 0 to 295 mM for 20 days. The salt stress tolerance of the six cultivars varied largely, with their salt tolerance threshold values ranging from 184.5 to 303.4 mM. Luyu39 had the highest threshold value and was considered as salt tolerant cultivar, and Jinping618 had the lowest threshold (184.5 mM) and was considered as salt sensitive cultivar. Luyu39 had lower MDA content, higher antioxidant enzyme (SOD, CAT, and POD) activity, and lower proline content when compared to Jinping618 at 245 mM and 295 mM NaCl levels. The results suggest that MDA, antioxidant enzyme activity, and proline content can be used as metabolic markers to evaluate relative salt tolerance of different maize cultivars under severe salt stress (245 mM or higher concentration NaCl) conditions.

1. Introduction

Salinity stress has become a serious threat to crop production in arid and semi-arid regions of the world due to the limited rainfall and high evapotranspiration demand, coupled with poor soil and water management practices [1]. Although the general perception is that soil salinization occurs only in the arid and semi-arid regions, no climatic zone is free from this problem [2]. More than 800 million ha of land worldwide is affected by either salinity or sodicity [3].

Salinity stress induces a multitude of responses in plants including morphological, physiological, and molecular changes [4]. High concentration of NaCl in soil reduces water potential, resulting in osmotic stress. Ionic toxicity, osmotic stress as well as nutrient deficiency under salinity may also disrupt plant photosynthetic function and reduce plant growth [5–6]. The disruption of photosynthetic function may make photosystem II unable to transduce or dissipate the exceeded energy absorbed through the light-harvesting complex [7]. The excess energy may be directed to O_2 and result in accumulation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion ($O^{2\bullet-}$), singlet oxygen ($^1O_2$), and hydroxyl radicals (OH•) [8]. ROS may damage proteins, DNA, and lipids [9–10]. Plants have developed antioxidant defense system to scavenge ROS toxicity. Plant enzymatic antioxidant system, which mainly consists of superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT) and ascorbate peroxidases (APX) can effectively suppress ROS and protect plant cells under salt stress [11]. Briefly, SOD constitutes the first line of defense against ROS by dismutating the $O^{2\bullet-}$ to H_2O_2 [12]. H_2O_2 is then regulated by CAT and an array of peroxidases such as POD and APX [11,13]. In the corresponding cell compartments, the multiple forms of these enzymes coordinate to achieve a balance of the formation and removal of ROS, maintaining H_2O_2 at the levels required for cell signaling [14–15].

Osmotic adjustment is the key adaptation of plants at cellular level to minimize effects of salinity-induced osmotic stress [1]. In response to salt stress, plants accumulate organic and inorganic solutes to lower water potential without lessening actual water content [16]. Proline serves as a osmolyte for osmotic adjustment and also ROS scavenger to reduce ROS toxicity. Recently study with switchgrass showed that salt tolerant varieties have lower level of proline than salt sensitive ones under severe salt stress (250 mM·NaCl). This suggests that salt tolerant varieties may complete osmotic adjustment by accumulating

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other simple solutes such as sugar and potassium ions rather than proline which requires more energy to synthesize than sugar.

Maize (*Zea mays* L.) is an economically important cereal crop and relatively sensitive to salt stress. Several studies have shown that wide variation exists between maize cultivars and salt tolerant cultivars may have higher antioxidant enzyme activity under salt stress. In addition, salt sensitive cultivars had higher MDA content relative to salt tolerant ones. However, no study has been reported on screening maize cultivars for salt tolerance using physiological parameters including proline, antioxidant enzyme and MDA content. The objectives of this study were to examine antioxidant metabolism variation associated with salt stress tolerance in six maize cultivars.

2. Materials and methods

2.1. Plant materials and growth conditions

Six cultivars, including Huanong138, Xianyu335, Luyu39, Aoyu3007, Jinping618, and Yayu8, were used in this study. Uniform seeds of each cultivar were selected and soaked in water at room temperature for 12 h before planted in pots (25 cm diameter's, 18 cm deep) filled with the same amount of vermiculite in a growth chamber with photosynthetic active radiation (PAR) at 500 μmol m^{-2} s^{-1}, temperature at 25 ± 2 °C (day)/20 ± 2 °C (night), relative humidity at 60%, and 14 h photoperiod. Five seeds were planted in each pot. The pots were irrigated to 80% container capacity with full strength Hoagland's solution mixed with one of seven NaCl levels (0, 45, 95, 145, 195, 245, and 295 mM). The pots were irrigated with Hoagland's solution only thereafter. The salt treatment lasted for 20 days.

2.2. Sample collection

The maize leaf samples were collected from the youngest fully developed leaf at 20 days after initiation of salt stress treatment. The leaf samples were frozen with liquid N immediately after sampling and stored at −80 °C for analysis of MDA, antioxidant enzyme activity and proline.

2.3. Measurements

At the end of the experiment, plants above ground were harvested and rinsed with deionized water. The biomass was determined after the plant tissues were dried in an oven at 80 °C to a constant weight. The threshold values of salt tolerance were determined based on the regression analysis with salt level as independent variable (X) and biomass as dependent variable (Y). The threshold values at 25% and 50% biomass reduction were determined based on the regression equations.

The content of MDA was measured according to Heath and Packer [17] Leaves tissue (0.05 g) (W) of was homogenized in 10 mL (V) of 0.1% (w/v) thiobarbituric acid (TCA) solution. 1 mL of extract was added to a tube containing 4 mL of 20% (v/v) TCA and 0.5% (v/v) of thiobarbituric acid. The homogenate was then incubated in boiling water for 30 min and cooled to room temperature, and centrifuged at 10,000 × *g* for 10 min. The absorbance of the supernatant was read at 532 nm (A_{532}) and 600 nm (A_{600}). The absorbance for nonspecific absorption at 600 nm was subtracted from the value at 532 nm. The MDA content is calculated based on the following formula: C_{MDA} (nmol g^{-1}) = (A_{532}–A_{600}) V 10^6/(155,000 W).

2.4. Antioxidant activities

For determination of antioxidant activities, 200 mg leaf tissue was ground with liquid nitrogen and extracted with 1 mL 50 mM phosphate buffer (pH 7.8, including 10 g L^{-1} polyvinylpyrrolidone). The supernatant after centrifuged at 15,000 × *g* for 20 min at 4 °C, was used for SOD activity determination [18]. The amount of extract that gave 50% inhibition of p-nitro blue tetrazolium chloride reduction was used as one SOD unit.

The CAT activity was measured according to Chance, B. and Maehly, A. C. [19]. The reaction mixer containing 50 mM of phosphate buffer (pH 7.0), 15 mM of H_2O_2, and 100 μL of enzyme extract were mixed in 3 mL tube. Reaction was started after the addition of the enzyme extract. Within 1 min of the linear decline of absorbance at 240 nm was recorded on spectrophotometer (Thermo Electron Corporation, USA) and the absorbance change (0.01 unit min^{-1}) was used to define the CAT activity.

POD activity was determined according to Hammerschmidt et al. [20]. Leaf tissue (0.2 g) was homogenized in an ice mortar and extracted with 1 mL 200 mM phosphate buffer (pH 6.0). The POD activity was determined by supernatant after centrifugation (5000 × *g*, 4 °C,15 min) using guaiacol as a substrate.

2.5. Experimental design and statistical analyses

A completely randomized block design was used with five replications. The data were analyzed using one-way analysis of variance with SPSS-19 statistical software (SPSS Inc., Chicago, IL, USA). The data were also analyzed using linear regression model. Mean separations were performed using Duncan's multiple range test (DMRT) at $p \leq 0.05$% level.

3. Results

3.1. Threshold value of salt tolerance of different cultivars

The threshold values were determined based on the regression analysis with salt level as independent variable (X) and biomass as dependent variable (Y) (Tables 1 and 2). The NaCl concentrations were found when the biomass was reduced 25% and 50%, respectively, in response to salt treatment. Threshold values differed largely among the six cultivars. Luyu39 and Huanong138 had a higher threshold value relative to other four cultivars. In contrast, Jinping618 had lower threshold value than other cultivars.

3.2. Malondialdehyde (MDA) content

The MDA is an indicator of lipid peroxidation under salt stress. The higher MDA content, the more severe cell membrane damage due to salt stress. The MDA content increased in response to salt stress in all six cultivars (Fig. 1). A significant increase in MDA was observed at 195 mM NaCl in all six cultivars. At 145 and higher NaCl concentrations, MDA content was higher in Jinping618 relative to Luyu39 and Huanong138. At 295 mM NaCl, MDA content increased by 171% in Luyu39, 162% in Huanong138, and 256% in Jinping618 relative the control (no NaCl). No MDA was detected in Jinping618 at 245 and 295 mM NaCl because of leaf senescence.

3.3. Superoxide dismutase (SOD) activity

Leaf SOD activity increased as NaCl concentration increased from 0 to 195 mM in all cultivars except for Jinping618 whose SOD activity

Table 1
Threshold values of salt concentrations determined 25% ($C_{25\%}$) and 50% ($C_{50\%}$) plant growth reduction of six maize cultivars.

Cultivar	Regression equation	R^2	$C_{25\%}$/mmol L^{-1}	$C_{50\%}$/mmol L^{-1}
Luyu39	y = 0.294 − 0.000507x	96.20%	161.9	301.2
Huanong138	y = 0.219 − 0.000366x	97.40%	155.7	303.2
Xianyu335	y = 0.173 − 0.000307x	90.70%	110.3	261.4
Aoyu3007	y = 0.136 − 0.000206x	96.10%	123.1	268.8
Yayu8	y = 0.178 − 0.000341x	95.30%	131.4	261.6
Jinpin618	y = 0.225 − 0.000586x	98.20%	101.1	195.4