

Effect of Salinity Stress on Degradation of Polyamines and Amine Oxidase Activity in Maize Seedlings

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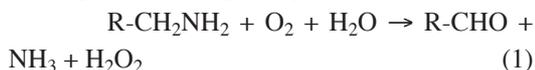
ABSTRACT

The objective of this study was to obtain information on the interrelation between polyamine (PA) degradation and diamine oxidase (DAO) activity under salt stress. To identify a quantitative correlation between PA degradation and DAO activity in maize under salt stress, the changes in endogenous free PA levels and DAO activity were analyzed in maize seedling roots, using treatments with different concentrations of NaCl and aminoguanidine (AG, a specific inhibitor of DAO). The results showed that the levels of putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm) decreased significantly with increasing salt concentrations. This occurred because salt stress strongly promoted DAO activity to stimulate PA degradation. AG treatment increased the accumulation of endogenous free PAs because of a strong retardation of DAO activity. A close correlation was observed between the changes in DAO activity and the PA contents among various treatments. The results indicated that salt stress could enhance PA degradation, suggesting that DAOs might increase their functions under salt stress.

Key words: amine oxidase, maize, sodium chloride

INTRODUCTION

Diamine oxidases (DAO, EC 1.4.3.6) catalyze the oxidative deamination of primary amine groups of several biogenic amines in the presence of molecular oxygen, by accepting two electrons from the substrate and transferring them to oxygen, according to Equation 1:



Diamine oxidases are frequently referred as semicarbazidesensitive amine oxidases (SSAO), due to their characteristic sensitivity to inhibition by this compound. Diamine oxidases include the subclass of diamine oxidases, which oxidize

preferentially putrescine and cadaverine.

Recently, many reports have demonstrated that the involvement of DAO enzymes in polyamine (PA) catabolism and the products derived from their degradation are involved in a variety of important physiological processes. These include: cell wall maturation and lignification during cell development (Cona *et al.*, 2003), wound-healing and cell wall reinforcement during pathogen invasion (Yoda *et al.*, 2003) and abiotic stresses, such as osmotic stress, phytohormones and salinity (Bouchereau *et al.*, 1999). In response to salt stress, diamine oxidase and polyamine oxidase (PAO, EC 1.5.3.11) activities were elevated in oat seedlings (Smith,

1985), and a response to salt stress has been found to be associated with a strong increase in 1,3-diaminopropane in tomato leaf explants. This reflects a salt-stress promoted PA oxidation (Aziz *et al.*, 1998). In the present study, the changes in endogenous free PA levels and DAO activity were analyzed in maize seedling roots using treatments with different concentrations of NaCl and aminoguanidine (AG, a specific inhibitor of DAO). A recovery test for the effects of salinity on these physiological indexes was also monitored. The main objective of the study was to further reveal a quantitative correlation between PA degradation and DAO activity in maize under salt stress.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of maize (*Zea mays* L.) were sterilized with 0.5% HgCl₂ for 5 min, soaked for 6 h in distilled water after being washed five times, then germinated at 25°C for 24 h on moist filter paper in plastic boxes.

Chemical treatments

Fourteen-day-old seedlings were subjected to solutions containing: 1) 0, 25, 50, 75, 100, 125, 150, 175 and 200 mM NaCl; 2) 0, 1.0 mM AG, 1.0 mM AG + 150 mM NaCl; 3) 150 mM NaCl for 14 days. All solutions were adjusted to pH 7.0 and renewed every 2 d to reduce fluctuation in the components in the culture solutions.

Determination of plant height and root dry weight

The plant height of at least 20 maize seedlings was directly measured using a centimeter ruler and expressed as a mean \pm standard error (SE). For the determination of dry weight, root samples were harvested after treatment, heat killed at 105°C for 10 min, then kept at 80°C until the

dry weight remained constant. After cooling at room temperature, dried samples were obtained using electronic scales.

Determination of DAO activity

Plant root tissues (0.5 g) were ground with a pestle and mortar at 4°C in 1.6 ml 0.1 M sodium phosphate buffer (pH 6.5). Homogenates were centrifuged at 10,000 \times g for 20 min at 4°C. The supernatants were used to determine DAO activity (Su *et al.*, 2005). Reaction mixtures (3.0 ml) contained 2.5 ml 0.1 M sodium phosphate buffer (pH 6.5), 0.1 ml crude enzyme extracts, 0.1 ml peroxidase (250 U ml⁻¹) and 0.2 ml 4-aminoantipyrine/N,N-dimethylaniline reaction solutions. The reaction was initiated by the addition of 0.1 ml 20 mM Put. A 0.01 value of the changes in absorbance at 555 nm was regarded as one activity unit of the enzyme.

Polyamine extraction and high performance liquid chromatography (HPLC) analysis

Root tissues (0.5 g) were ground in a mortar with 4 ml 5% (v/v) HClO₄. After extraction for 1 h on an ice bath, the homogenate was centrifuged (15,000 \times g for 20 min) at 4°C. Five hundred microliters of the supernatant containing the free diamines was mixed with 1 ml 2 M NaOH. After the addition of 10 μ l benzoylchloride, vortexing for 20 s, and incubation for 20 min at 37°C, 2 ml of ether was added. After centrifugation (1500 \times g for 5 min), 1 ml of the ether phase was collected. The sample was evaporated to a dry state and redissolved in 100 ml of methanol (HPLC grade). Standards were treated in a similar way, with up to 50 nmol of Put, Cad, Spd and Spm, respectively. Benzoyl-polyamines (10 ml) were analyzed using a Waters HPLC System (USA), consisting of an isocratic pump equipped with a reverse phase C18 column (Nova-pak, 150 \times 3.9 mm, particle size 4 μ m). Methanol/acetonitrile/H₂O (48:2:50) (v/v/v) was used as an isocratic eluting solvent at a flow rate of 1 ml min⁻¹. The

polyamine content was expressed as nmol g^{-1} fresh weight (mean \pm SE).

RESULTS

Effect of salt stress on maize vegetative growth

When fourteen-day-old maize seedlings were subjected to different salt concentrations for 14 d, inhibition of vegetative growth was observed. The plant height and total dry weight of roots gradually decreased as salt concentrations increased in the nutrient solution. There were different responses between plant height and root dry weight to salt stress. Plant height exhibited a significant decrease when NaCl concentrations exceeded levels of 100 mM, while even at 50 mM NaCl the root growth was significantly inhibited. These different responses could be related to the distinctive environmental growth conditions (Figures 1 and 2).

PA levels in maize roots under salt stress

The level of Cad in maize seedling roots was the highest, while the Spm level was very low

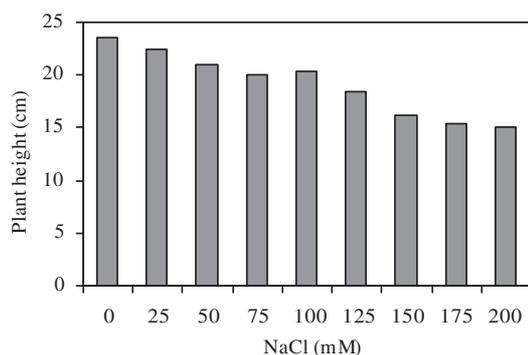


Figure 1 Maize plant height under different NaCl concentrations. Seeds of maize (*Zea mays* L.) with a germination yield >90% were utilized. The seed germination was carried out in the dark. Fourteen-day-old maize seedlings were subjected to 0-200 mM NaCl for 14 d at room temperature.

(Figures 3 and 6). The contents of four common polyamines decreased in varying degrees with increasing salt concentrations in the growth medium, reflecting their different responses to salinity (Figures 4-6).

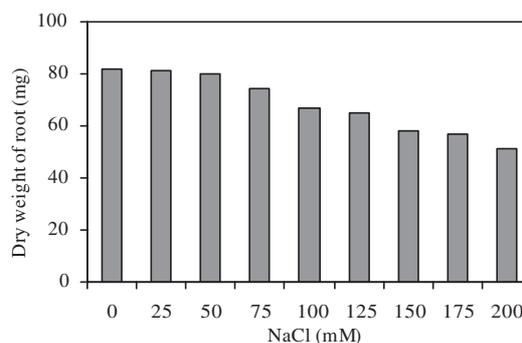


Figure 2 Dry weight of maize roots under salt stress. Fourteen-day-old maize seedlings were subjected to 0-200 mM NaCl for 14 days at room temperature. Root dry weight was measured after being heat-killed at 105°C for 10 min, then kept at 80°C for 6 h.

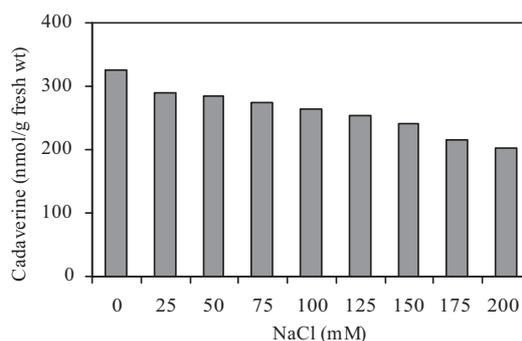


Figure 3 Effect of salt stress on cadaverine content in maize seedling roots. Fourteen-day-old maize seedlings were treated and cultivated in 0-200 mM NaCl for 14 d at room temperature. Root tissues were collected, treated and the endogenous levels of cadaverine were determined of HPLC.

DAO activity in salt stressed maize roots

DAO activity in maize roots was determined. The activity was markedly stimulated by salt stress with about a 2.5-90.5% increase (Figure 7). The increase in DAO activity by 1.03- to 1.91-fold compared with the control (Figure 7),

was consistent with the decrease in the endogenous PA levels in salt-stressed roots (Figures 3-6). In terms of the precursor product correlation among PAs, it was thus reasonable to suppose that the salt in maize roots involved in PA oxidation was catalyzed by DAO.

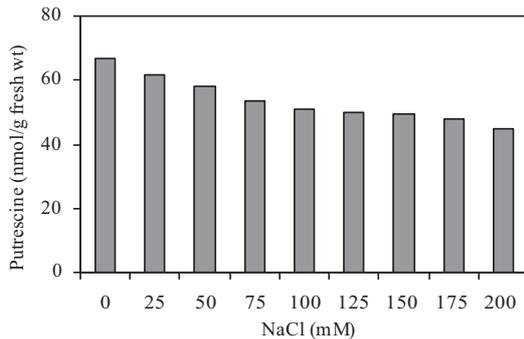


Figure 4 Putrescine salt stress at different concentrations. Fourteen-day-old maize seedlings were treated and cultivated in 0-200 mM NaCl for 14 d at room temperature. Root tissues were collected to determine the endogenous level of putrescine by HPLC.

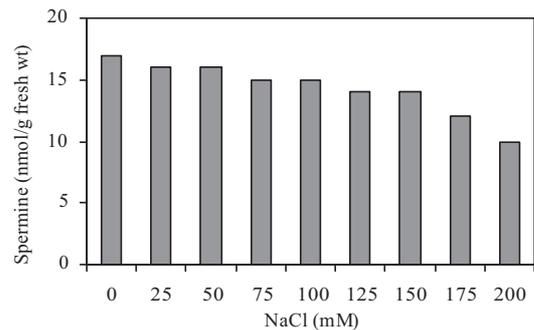


Figure 6 Endogenous spermine salt stress at different concentrations. Fourteen-day-old maize seedlings were treated and cultivated in 0-200 mM NaCl for 14 d at room temperature. Root tissues were collected to determine the endogenous level of spermine by HPLC.

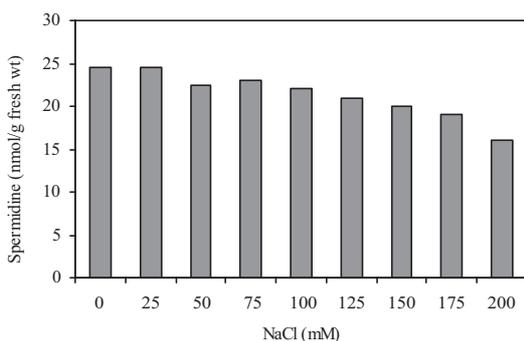


Figure 5 Endogenous spermidine salt stress at different concentrations. Fourteen-day-old maize seedlings were treated and cultivated in 0-200 mM NaCl for 14 d at room temperature. Root tissues were collected to determine the endogenous level of spermidine by HPLC.

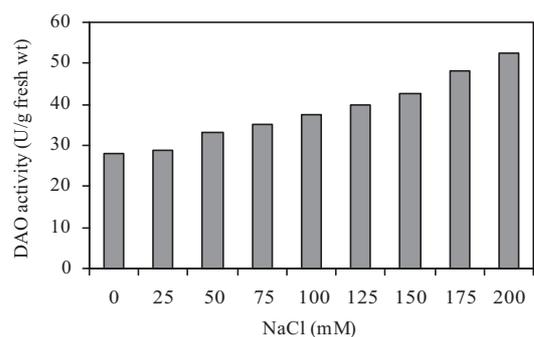


Figure 7 Effect of salt stress at different concentrations on DAO activity in maize roots. Fourteen-day-old maize seedlings were subjected to 0-200 mM NaCl for 14 d and DAO activity in maize roots was measured.

Effects of aminoguanidine on the contents of polyamine and DAO activity

Salt was partly resulted in PA degradation due to the promotion of DAO activity, so treatment with AG, a specific inhibitor of DAO, could lead to a PA accumulation. An exposure to 1.0 mM of AG markedly increased the accumulation of endogenous free PAs, especially Put and Cad in both control and salt-stressed roots (Table 1).

DISCUSSION

When 14-day-old maize seedlings were subjected to different salt concentrations, a significant decrease in endogenous PA levels (Put, Cad, Spd and Spm) in maize roots was observed with increasing salt concentrations (Figures 3-6). It has been reported that DAO or PAO activity was stimulated by salinity in oat seedlings or *Brassica campestris* (Smith, 1985; Das *et al.*, 1995). In tomato leaf explants, salt-stress promoted PA oxidation (Aziz *et al.*, 1998). The current study suggested that decreases in endogenous PA levels in maize seedlings under salt stress could be related to an increase in DAO or PAO activity. The measurement of DAO activity in maize roots showed that its activity increased greatly when salinity increased (Figure 7), indicating the possibility that the decrease in PA levels under salt stress was associated with an increase in DAO

activity. As described using rice (Maiale *et al.*, 2004) and tomatoes (Aziz *et al.*, 1998), PA levels were also associated with PA biosynthetic enzymes, such as arginine decarboxylase, spermidine synthase and S-adenosyl-L-methionine decarboxylase. These enzymes were affected by salt stress. Some reports demonstrated that the endogenous contents of Put and Spd decreased either with the concentrations of saline medium or with time, in studies on salt-sensitive and salt-tolerant tomato plants (Santa-Cruz *et al.*, 1997), rice (Maiale *et al.*, 2004) and *Lotus glaber* (Sanchez *et al.*, 2005). However, Basu and Ghosh (1991) and Kativar and Dubey (1990) reported that salinity resulted in the accumulation of PA compounds in rice seedlings. Krishnamurthy and Bhagwat (1989) also reported that salt-tolerant rice cultivars rapidly accumulated high levels of Spd and Spm resulting in an enhanced level of total PAs, with a relative decrease in Put content. The most significant aspect of salt-sensitivity in rice cultivars was their excessive accumulation of Put and their inability to maintain high levels of Spd and Spm in their root system when exposed to a saline environment. The differences in endogenous PA levels in response to salt stress were generally assigned to plant species, development stage, nature of the stress and the duration of the treatment (Botella *et al.*, 2000).

Table 1 Effect of aminoguanidine treatment on free polyamine content and diamine oxidases activity in salt-stressed maize roots.

Treatment	Amine content (nmol/g fresh wt)				DAO activity (U/g fresh wt)
	Cadaverine	Putrescine	Spermidine	Spermine	
Control	325.3 ± 21.4	65.2 ± 3.2	24.6 ± 2.4	17.5 ± 3.2	31.4 ± 5.6
AG ^a	537.8 ± 36.2	92.3 ± 7.4	37.9 ± 4.3	26.7 ± 4.6	4.2 ± 3.2
NaCl ^b	241.2 ± 17.4	51.6 ± 4.6	20.4 ± 2.7	14.8 ± 2.3	42.6 ± 2.1
AG ^a + NaCl ^b	464.7 ± 23.6	76.4 ± 3.2	32.6 ± 6.4	22.6 ± 1.7	32.2 ± 6.4

^a concentration of AG was 1 mM

^b concentration of NaCl was 150 mM

Fourteen-day-old maize seedlings were selected and subjected to several treatments and cultivated for 14 d at room temperature. The values in this table are the means ± SE of triplicates.

CONCLUSIONS

This study investigated the effects of short-term salinity stress using a salinized nutrient solution on the polyamine metabolism in the roots of maize seedlings. DAO activity in the roots was much higher in seedlings in nutrient solution salinized with 200 mM NaCl than in the control, while a marked decrease in PA (putrescine, cadavarine, spermidine and spermine) content was evident in the roots after exposure to salinity. The data supported the hypothesis that salt-induced PA oxidation is related to Na⁺ uptake and subsequent accumulation in maize roots. Such accumulation would help osmotic adjustment of the plant. The decrease in the PA content could be important in adjusting the cation content of the tissues. The present study indicated that salt stress could increase DAO activity and result in a decreased PA content in maize roots.

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