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**The Proceedings of the International Plant Nutrition  
Colloquium XVI**

**Title**

Salt stress affects polyamine concentrations and plasma membrane H<sup>+</sup>-ATPase proton pumping in maize

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<https://escholarship.org/uc/item/0dx1c1qb>

**Authors**

Ingold, Mariko  
Hanstein, Stefan  
Schubert, Sven

**Publication Date**

2009-04-13

Peer reviewed

## Introduction

High salt concentrations in soils lead to distinct growth reductions in all crop plants. The reasons for the immediate growth reduction, which is due to an inhibited cell elongation, are not clear yet. The cell-wall extensibility is reduced under salt stress, due to the inhibition of pumping activity of the plasmalemma  $H^+$ -ATPase (Zörb et al. 2005). It is well known that the plasmalemma- $H^+$ -ATPase acidifies the apoplast by pumping protons out of the cell and promotes cell elongation (Hager 2003). The lowered pH activates cell wall loosening enzymes that break bonds in the cell wall and enables turgor to drive cell elongation (Cosgrove 2005). Why the activity of the plasmalemma- $H^+$ -ATPase is reduced under salt stress is not clear but it is considered that hormonal signals may trigger this reaction.

A group of small aliphatic molecules, the polyamines, may have an important function in plant growth and may play a role in stress reactions and resistance (Smith 1990). The major polyamines in plants are spermidine and spermine and their precursor putrescine. At physiological pH they are cations, which enables them to interact with anionic compounds of nucleic acids, membranes, proteins and the cell wall (Edreva 1996). Under salt stress diverse changes of polyamine levels were observed depending on species, varieties and plant tissue (Bouchereau et al. 1999). There are indications that polyamines are modulators of salt-stress resistance (Krishnamurthy and Baghwat 1989) and influence the activity of the plasmalemma  $H^+$ -ATPase and thus plant growth (Liu et al. 2005).

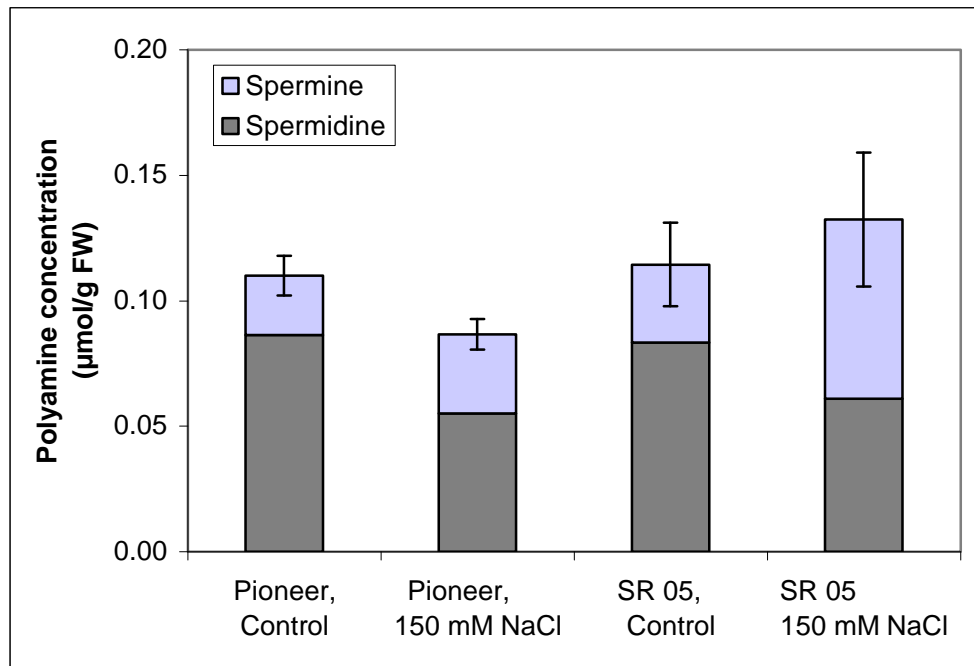
The aim of the present work was to examine how salt stress influences polyamine concentrations and if polyamines have an effect on the activity of the plasmalemma  $H^+$ -ATPase.

## Materials and methods

For this purpose plants of two maize hybrids (*Zea mays* L. cv. Pioneer 3906 and SR 05) were grown in nutrient solution in a climate chamber for 15 days. For measuring the polyamine concentrations with HPLC 8 day old plants were adapted gradually to salt stress in a range between 0 and 150 mM NaCl. Roots and shoots of the 15 day old plants were harvested separately and extracted with 3.5%  $HClO_4$  for 1 hour on ice. Extracted polyamines were derivatized with Fmoc according to Manderscheid et al. (1991) and detected using a UV detector. For measuring the activity of the plasmalemma  $H^+$ -ATPase, plants of the cultivar Pioneer 3906 were grown in nutrient solution in a climate chamber for 21 days without salt treatment. Plasmalemma vesicles of roots (*Zea mays* L. cv. Pioneer 3906) were obtained by two-phase partitioning (Yan et al. 1998). The hydrolytic activity was measured according to Yan et al. (1998); the pumping activity was measured with Acridine Orange according to Zörb et al. (2005).

## Effect of salt stress on polyamine concentrations

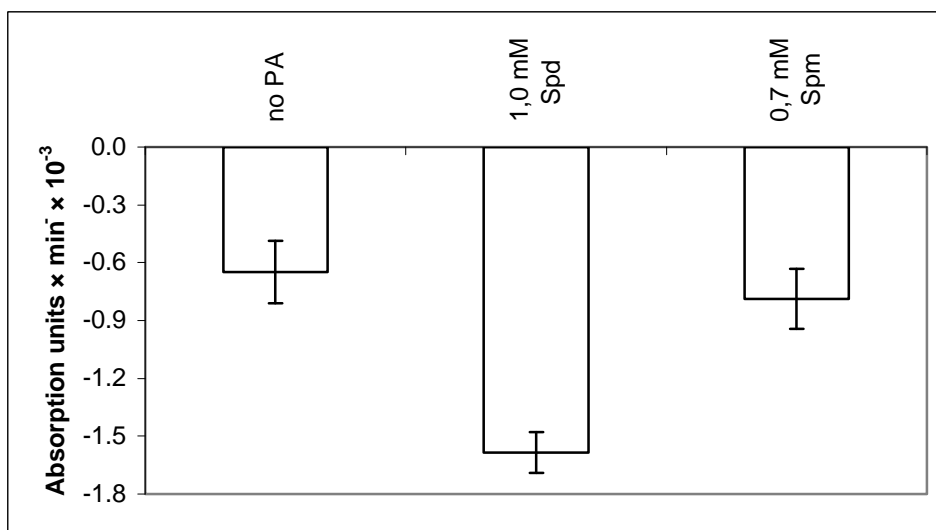
In shoots, the measurement of the polyamine concentrations were in the range of 0,13-0,16  $\mu\text{mol/g}$  FW for spermidine (Spd) and 0,04-0,06  $\mu\text{mol/g}$  FW for spermine (Spm) and showed no significant differences (not shown). The Spd concentrations in the roots of both cultivars were lower under salt treatment (Fig. 1). The Spm concentration was significantly increased by salt stress only in roots of SR 05 whereas cv. Pioneer 3906 showed a decrease in the cumulative polyamine concentration. It seems that there are genotypic differences in the adaptation of Spm concentrations during salt stress which may be related to salt resistance. This is consistent with other findings (Krishnamurthy and Bhagwat 1989).



**Figure 1:** Effect of 150 mM NaCl on concentrations of the polyamines spermidine and spermine in roots of two maize hybrids (cv. Pioneer 3906 and SR 05). Error bars indicate standard errors and refer to the cumulative polyamine concentrations. Values represent means of four replicates.

### Effect of polyamines on the activity of the plasmalemma- $H^+$ -ATPase

Plasmalemma vesicles from roots were treated with 1 mM Spd or 0.7 mM Spm. The pumping activity was significantly increased by incubation with Spd while the treatment with Spm showed no significant effect (Fig. 2). The hydrolytic activity of plasmalemma  $H^+$ -ATPase and passive  $H^+$  transport were not affected by Spd (data not shown). It seems that spermidine enhances the pumping activity of the plasmalemma- $H^+$ -ATPase while ATP consumption remains the same.



**Figure 2:** Effect of 1 mM Spd and 0,7 mM Spm on the pumping activity of the plasmalemma  $H^+$ -ATPase from maize (Pioneer 3906) root vesicles. The pumping activity is expressed as

initial rate and was measured with acridine orange in *inside-out* vesicles with a photometer at 492 nm.

## Conclusions

The polyamine metabolism is altered by salt stress. The concentration of Spd, which has an enhancing effect on the pumping activity of the plasmalemma H<sup>+</sup>-ATPase in roots, is lowered by salt treatment. The synthesis of polyamines under salt stress may contribute to different salt resistance of maize genotypes.

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