Introduction

Soil salinity is one of the major environmental constraints limiting agricultural production worldwide. Especially irrigated land systems in arid and semiarid climates are adversely affected mainly due to non-adapted irrigation practices. Current data indicate that more than 50% of all irrigated areas are already affected by soil salinity. In most saline environments, NaCl is the predominant salt species, whose principle adverse effect in non-resistant plants is growth inhibition due to an inhibition of cell division and cell elongation caused by osmotic effects, ion toxicity, and mineral disturbances in plants. However, the various deleterious effects of salinity on growth are not completely understood yet.

A concept for understanding of salt-induced growth repression has been put forward by Munns (1993) with a biphasic model of growth response. In the first phase, growth is reduced by osmotic stress, while the second phase is mainly characterized by ion toxicity. While there are only small genetic variations in growth due to osmotic effects, sensitive and resistant genotypes can be distinguished within the second phase, with resistant genotypes maintaining a higher growth rate under saline conditions. For the purpose of designing salt-resistant crops, the complete understanding of cellular mechanisms regarding reduced cell growth under salt exposure is essential.

Plant growth under salinity

In order to understand plant growth reduction under salinity, two maize genotypes varying in salt resistance, Pioneer 3906 and a newly developed SR hybrid (Schubert and Zörb 2005), were compared. Salt treatment (100 mM NaCl) for a period of 8 d resulted in a significant decrease of shoot fresh weight of both maize genotypes (Fig. 1). The genotypes differed significantly with the more resistant SR 03 producing the maximum and Pioneer 3906 the minimum shoot fresh weight under saline conditions.

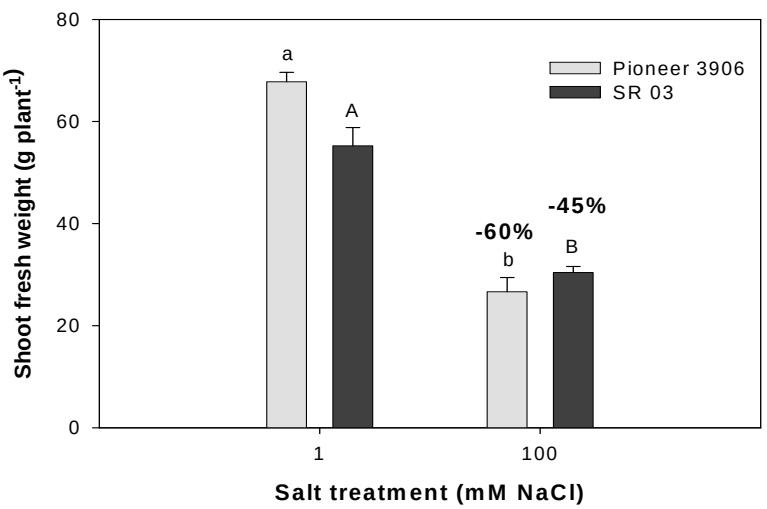

Figure 1: Shoot fresh weights of two maize genotypes as affected by salinity. The values represent means ± SE of four independent experiments. Significant differences ($P \leq 5\%$) are indicated by different letters.

Salt-induced changes of apoplastic pH

According to the acid-growth theory (Hager 2003), acidification of the leaf apoplast mediated by the plasma membrane H^+-ATPase is a major requirement to increase cell wall extensibility. To investigate the effect of apoplastic pH on growth, both maize genotypes were compared using two different *in vivo* techniques: the ratiometric fluorescence microscopy and pH-sensitive microelectrodes.

Both approaches yielded the same result, namely a significant apoplastic alkalization of the salt-sensitive Pioneer 3906, but not of SR 03 (Fig. 2). Although a direct comparison of absolute pH-values is not feasible, this work was not aiming to quantify exact numerical pH-values but to examine the influence of salinity on apoplastic pH-changes. Therefore, the relative variations are crucially. Thus an inversion of the acid-growth argument would permit the conclusion that in growing tissues a pH increase of less than half a unit is sufficient to inhibit growth (Pitann et al. 2009a).

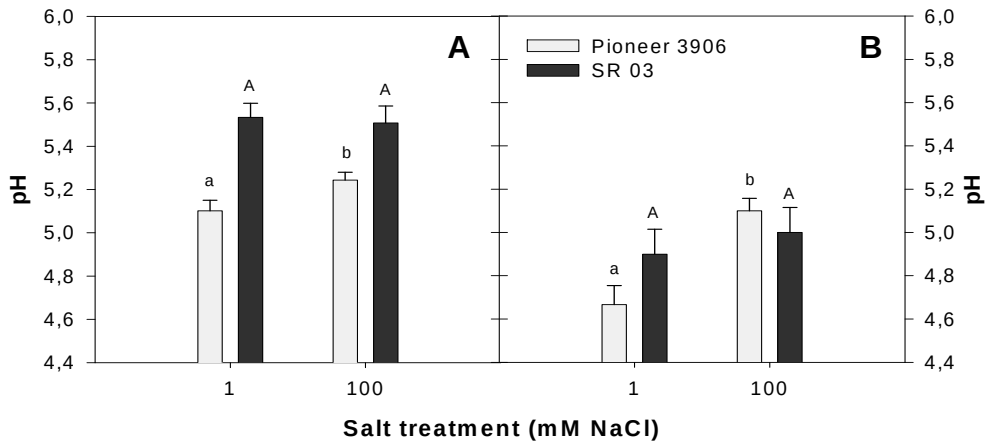

Figure 2: Effect of salt stress on apoplastic pH of intact maize leaves: (A) vacuum infiltration with fluorescein tetramethylrhodamine-dextran (FTMR, 20 µM) and ratiometric fluorescent microscopy at excitation wavelengths of 490 and 440 nm; (B) pH-sensitive microelectrodes.

The role of plasma membrane H^+-ATPase in salt resistance

It is assumed that salt stress-induced apoplastic alkalization and leaf growth reduction are caused by a reduced plasma membrane pump activity. In *inside-out* vesicles isolated from leaf tissue it could be shown that hydrolytic activity of plasma membrane H^+-ATPase remained unaffected under saline conditions (Pitann et al. 2009b). In contrast, H^+ pumping activity was reduced by 47% in Pioneer 3906, but was unchanged in SR 03 (Tab. 1).

Since total concentration of plasma membrane H^+-ATPase did not alter in both genotypes there are indications that transcription of specific isoforms of the *MHA* family underlies modification in Pioneer 3906 under salt stress conditions (Zörb et al. 2005). Currently, subsequent studies aim to show that in SR 03 expression of ineffective isoforms such as *MHA4* is avoided, resulting in maintenance of proton pumping and apoplastic acidification necessary for plant growth.

Table 1: Hydrolytic activity of plasma membrane ATPase and H^+ transport in vesicles isolated from leaves of two 3 week-old maize genotypes grown under control and salt-stress conditions. The values represent means ± SE of three independent experiments. Significant differences ($P \leq 5\%$) are indicated by different letters.

Salt treatment	Hydrolytic Activity ($\mu mol\ P_i\ mg^{-1}\ min^{-1}$)	Active H^+ Transport	
		Initial Rate $\Delta A_{492}\ min^{-1}$	pH Gradient ΔA_{492}
Pioneer 3906			
Control	0.31 ± 0.12 a	0.017 ± 0.001 a	0.07 ± 0.00 a
100 mM NaCl	0.35 ± 0.10 a	0.009 ± 0.003 b	0.05 ± 0.00 b
SR 03			
Control	0.41 ± 0.20 a	0.016 ± 0.001 a	0.05 ± 0.01 a
100 mM NaCl	0.54 ± 0.10 a	0.016 ± 0.001 a	0.05 ± 0.01 a

Conclusion

We have shown that salinity reduces apoplastic acidification in leaves of salt-sensitive maize due to the reduction of H^+-pumping of the plasma membrane ATPase. There is evidence that not only the increase of apoplastic pH itself causes shoot growth reduction but that other factors such as a reduced activation of cell wall loosening proteins, e.g. expansins, may be responsible for growth inhibition of resistant maize genotypes in the first phase of salt stress (Pitann et al. 2009c).

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