Introduction

According to the two-phase model proposed by Munns (1993), growth reduction in the first phase of salt stress can be ascribed to osmotic stress whereas ion toxicity decreases growth in a second phase. The model originally proposed for wheat was confirmed for maize (Fortmeier and Schubert 1995) but it was also shown that, to a minor extent, ion toxicity may contribute to growth reduction in the first phase (Sümer et al. 2005). Based on the two-phase model, a breeding program was initiated to combine physiological knowledge with classic breeding methods. Since it was demonstrated that ion toxicity in maize is predominantly related to sodium (Na^+) toxicity (Fortmeier and Schubert 1995), as a first step an Na^+-excluding inbred line was established. For this purpose, a relatively efficiently Na^+-excluding maize hybrid (Pioneer 3906) was used to produce variability in the F2 generation by self-crossing. Individuals of the F2 generation were screened for low Na^+ concentration in the 4th leaf after 9 d defined NaCl treatment. Lowest Na^+ concentrations were correlated with low Na^+ uptake at the root surface and low root-to-shoot translocation. The best individuals were selected and after seven recurrent selfings and selections a very efficiently Na^+-excluding maize inbred line (NaExIl) was obtained. This inbred line combined very low Na^+ uptake at the root surface with extremely low Na^+ root-to-shoot translocation (Schubert and Zörb 2005). At the same time, NaExIl did not demonstrate improved salt resistance in the first phase of salt stress.

Establishment of salt-resistant maize hybrids

In a second step to produce salt-resistant maize hybrids, about 200 maize inbred lines (generously provided by Südwestsaat, Germany) were screened for superior leaf growth under osmotic stress. Osmotic stress was exerted by applying 150 mM NaCl to the nutrient solution and growth of the fourth leaf during the first phase of salt stress was compared with leaf growth of untreated plants.

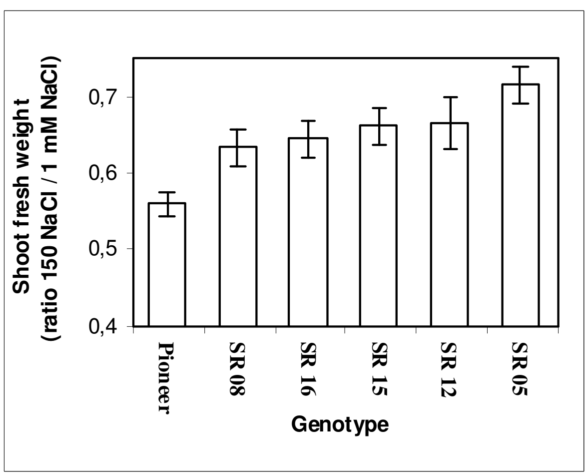

Figure 1: Osmotic resistance of newly established maize hybrids (SR hybrids) relative to maize cv. Pioneer 3906. Resistance is expressed as shoot fresh weight of plants grown for 9 d in nutrient solution + 150 mM NaCl relative to control plants. Values represent means of four replicates ±SE (Schubert, Neubert, and Zörb, unpublished results).

About 20 inbred lines were identified that showed superior osmotic stress resistance relative to the other inbred lines. The resistant inbred lines were crossed with NaExIl and the resulting

SR hybrids were investigated for vegetative growth under 150 mM NaCl relative to control conditions during the first phase of salt stress. A number of SR hybrids showed improved osmotic stress resistance under saline conditions during the first phase of salt stress (Fig. 1). The two-phase model originally proposed that only in the second phase of salt stress genotypic differences occur (Munns 1993). The newly established SR hybrids proved for the first time that genotypic variation does occur in the first phase of salt stress.

Some of the newly developed SR hybrids not only showed better performance in the first phase of salt stress but were also characterized by low Na^+ uptake and Na^+ root-to-shoot translocation (Fig. 2). The Na^+ exclusion strategies were thus successfully inherited.

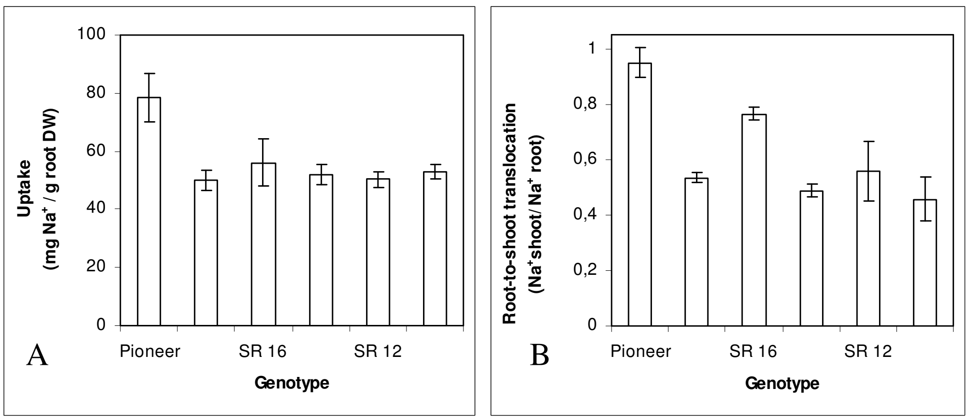

Figure 2: Sodium uptake (A) and Na^+ root-to-shoot translocation (B) of newly developed maize hybrids (SR hybrids) in comparison to cv. Pioneer 3906 (Schubert, Neubert, and Zörb, unpublished results)

Yield performance under saline conditions as an integrative parameter of salt resistance was improved in SR hybrids relative to the parent hybrid Pioneer 3906 (Tab. 1).

Table 1: Grain yield of newly developed SR hybrids and cv. Pioneer 3906 grown in saline soil (10 dS m^{-1}) in comparison with plants grown under non-saline conditions (Schubert, Neubert, and Zörb, unpublished results)

Treatment	**Maize genotypes**					
	cv. Pioneer 3906	**SR 05**	**SR 08**	**SR 12**	**SR 15**	**SR 16**
Control	146 ±3	151 ±6	123 ±6	133 ±4	143 ±3	119 ±2
Salt stress (10 dS m^{-1})	87 ±17	120 ±6	112 ±4	109 ±3	110 ±7	84 ±3
Ratio (salt stress / control)	0.59	0.79	0.91	0.82	0.76	0.71

Whereas Na^+ exclusion, as an important strategy to avoid Na^+ toxicity and to realize salt resistance in the second phase of salt stress, was sufficiently increased, osmotic resistance in the first phase of salt stress was improved only to a small extent. The aim of our ongoing investigations is, therefore, to understand the problems associated with the first phase of salt stress.

Water availability and growth during the first phase of salt stress

It is generally assumed that plant growth in saline environments may be reduced because water availability is restricted by the low osmotic potential. However, despite growth reduction of cv. Pioneer 3906 and SR 05 in salinized nutrient solution (not shown), the transpiration rates were unaffected (Fig. 3) indicating that plant water uptake did not limit leaf extension growth.

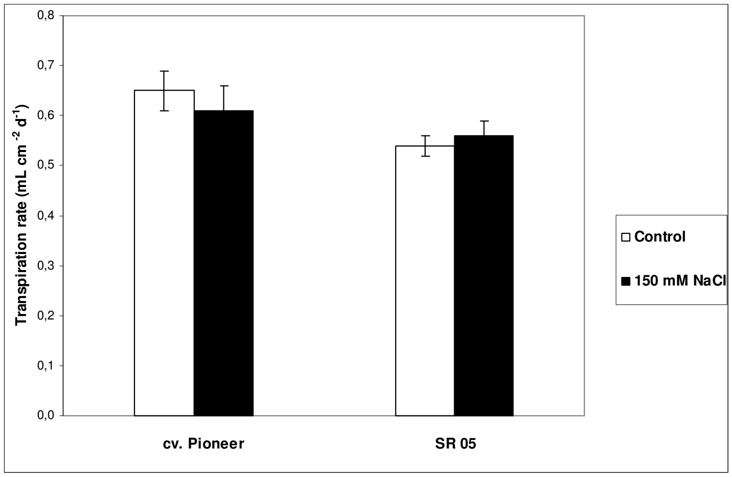

Figure 3: Effect of 150 mM NaCl salt stress on the transpiration rates of two maize hybrids in nutrient solution (Ingold, unpublished results)

Also, under comparable conditions cv. Pioneer 3906 and newly established SR hybrids demonstrated solute accumulation in the shoot sap (Fig. 4a) and turgor maintenance (not shown). These data indicate that it is not turgor but cell wall extensibility that restricts cell extension growth. According to a model proposed by Davies and Zhang (1991) root tips sense a decrease in water availability and release signals to the shoot, which may modify shoot metabolism. One of these signals is abscisic acid (ABA) that is accumulated under salt stress (Fig. 4b). A comparison of cv. Pioneer 3906 with salt-resistant SR hybrids revealed that under salt stress relative to control conditions ABA accumulated more in the resistant hybrids (Fig. 4b) indicating that it may contribute to salt resistance in the first phase of salt stress.

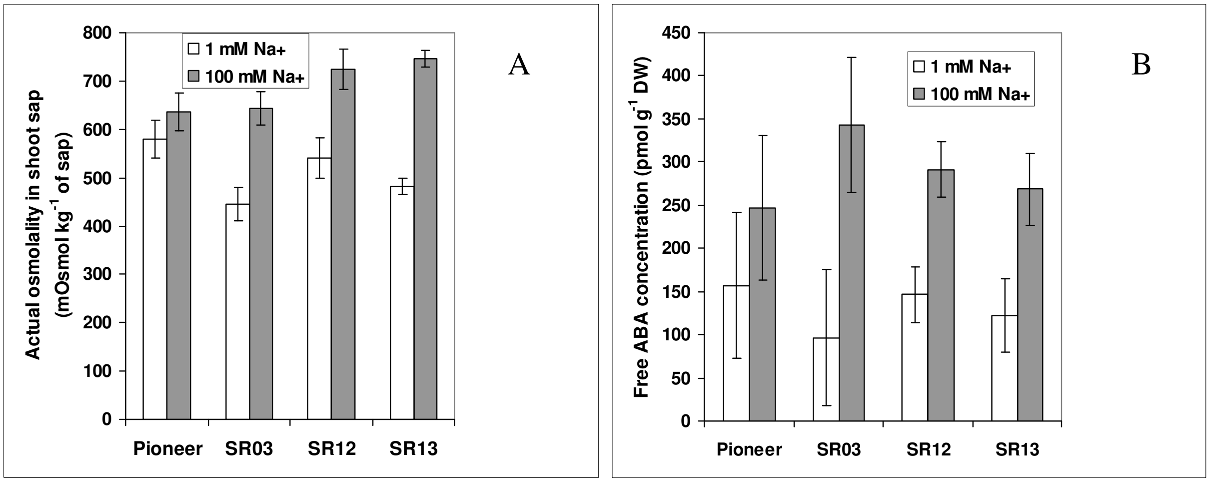

Figure 4: Effect of salt stress on the (A) actual osmolality of shoot sap and (B) abscisic acid (ABA) concentration of the growing region of the fourth leaf of various SR hybrids. Values are means of four replicates. Error bars indicate the 95% confidence interval for the comparison of salt treatment with control (De Costa et al. 2007).

Cell wall acidification and resistance in the first phase of salt stress

According to the acid-growth theory, H^+-ATPase-mediated cell-wall acidification is an essential requirement for leaf extension growth (Hager 2003). It was shown that *in vitro* H^+-pumping activity of plasma membrane H^+-ATPase in vesicles isolated from leaf tissue of cv. Pioneer 3906 was decreased in comparison to vesicles isolated from control plants (Fig. 5).

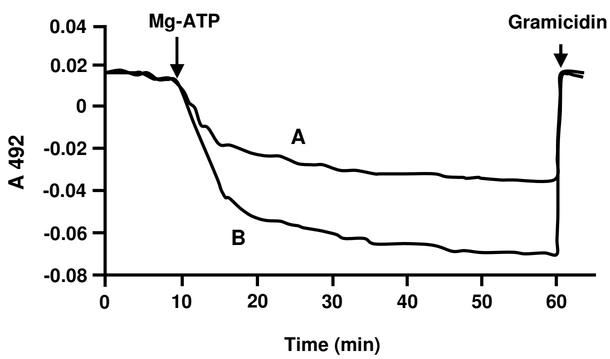

Figure 5: Effect of salt treatment (A: 125 mM) relative to control plants (B) of maize cv. Pioneer 3906 on active H^+ transport into isolated membrane vesicles. The pH gradient formation across membrane vesicle membranes was monitored by absorbance quenching (A492) of acridine orange (Zörb et al. 2005).

This suggested that the requirement for cell-wall acidification was not met and that a lack of active H^+-pumping may be the cause for growth reduction in the first phase of salt stress. Transcription studies indicated that a change in gene expression under salt stress may be responsible because the transcription of specific isoforms showed a modification under salt stress (Fig. 6).

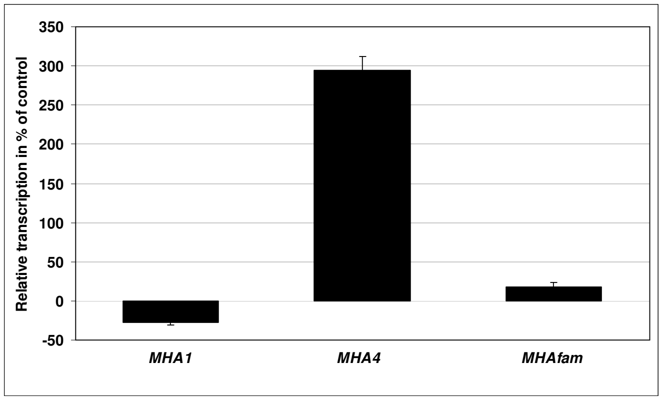

Figure 6: Transcription analysis of plasmalemma H^+ ATPases (MHA_{fam}) and specific isoforms (*MHA1, MHA4*) from maize (cv. Pioneer 3906) plants treated with 125 mM NaCl for 3 d relative to control plants (Zörb et al. 2005)

Decreased *in vitro* plasma membrane ATPase proton pumping of cv. Pioneer 3906 was corroborated in subsequent studies by Pitann et al. (2009) who additionally showed that the *in vivo* apoplastic acidification was significantly decreased (Tab. 2). However, the resistant hybrid SR 03 not only maintained *in vitro* H^+ pumping (not shown) but also *in vivo* cell-wall acidification (Tab. 2). The fact that leaf growth was also decreased in SR 03, although to a smaller degree than in cv. Pioneer 3906, suggests that there must be additional limitations for extension growth than cell wall acidification.

Table 2: Effect of salt stress on apoplastic pH in the growing zone of the youngest, expanding leaf of cv. Pioneer 3906 and SR 03 after 8 d of salt treatment (100 mM NaCl). pH values were measured by means of ratiometric fluorescent microscopy after infiltration with FITC-dextran (50 µM). The values represent means ±SE of four independent experiments. Significant differences ($P \leq 5\%$) between treatments are indicated by different letters (Pitann et al. 2009).

Salt treatment	**Apoplastic pH**	
	cv. Pioneer 3906	**SR 03**
Control	5.5 ± 0.01 a	5.8 ± 0.04 a
100 mM NaCl	5.7 ± 0.02 b	5.8 ± 0.03 a

Conclusions

Due to efficient Na^+ exclusion, newly developed maize hybrids show strongly improved salt resistance in the second phase of salt stress. Osmotic stress resistance in the first phase is partly improved by maintaining proton pumping and cell wall acidification under salt stress probably due to selective expression of H^+ ATPase isoforms. However, additional limitations in cell-extension growth independent of cell-wall acidification still limit the growth of resistant SR hybrids and may be a key to further improve the salt resistance of maize.

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