



RESEARCH ARTICLE

Enhancing maize grain yield under salt-affected field conditions using salt-resistant maize hybrids and higher planting density

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Abstract

Background: In arid and semiarid countries, grain yield of maize is increasingly impaired by soil salinity. Beside soil amelioration, the development of salt-resistant cultivars is a possibility to enhance crop yield on salt-affected soils.

Aims: This study aimed at testing yield performance in the field of salt-resistant maize hybrids on a salt-affected soil. In addition, planting density was optimized under the saline conditions.

Methods: Four salt-resistant maize hybrids (*Zea mays* L. SR-05, SR-12, SR-15, and SR-16) were grown under control ($EC = 2.0\text{--}2.5\text{ dS m}^{-1}$) and saline ($EC = 10.0\text{--}12.0\text{ dS m}^{-1}$) field conditions and compared to the salt-sensitive maize cv. Pioneer-3906. Planting density (5, 8, or 11 plants m^{-2}) was optimized for saline soil conditions for SR-12 and the local hybrid EV-78.

Results: Yield of Pioneer-3906 was significantly reduced under salinity because of inhibited kernel setting, whereas the SR hybrids showed no decrease in grain yield. Based on grain yield, the optimum planting density was 8 plants m^{-2} with no further increase with 11 plants m^{-2} . In contrast to SR-12, for cv. EV-78 no increase of harvest index with 8 relative to 5 plants m^{-2} was observed.

Conclusions: Vegetative growth of Pioneer-3906 and the SR hybrids was decreased due to Phase-I effects but neither due to water deficiency nor ion toxicity. The experiment corroborated the salt resistance of the SR hybrids under field conditions. Under saline conditions, optimum planting density of salt-resistant cultivars may be higher than under nonsaline conditions when sufficient water supply by artificial irrigation is guaranteed.

KEYWORDS

EC, harvest index, soil salinity, three-phase model, *Zea mays*

1 | INTRODUCTION

Soil salinity is an important problem threatening the future of agriculture around the world, particularly in the arid and semiarid regions. Salt-affected soils occur in at least 75 countries and cover about

62 Mha irrigated land and over 800 Mha total land (Qadir et al., 2007, 2014). Soil amelioration is expensive, and the availability of high-quality water for the leaching of salts in areas of salt-affected soils is restricted. Therefore, as early as 1941 Lyon suggested to breed for salt-resistant crop plants (cited by Epstein, 1985). However, because of an incomplete understanding of the physiological basis and the multigenic

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nature of salt resistance (Yamaguchi & Blumwald, 2005), breeding success since then has been limited.

Understanding the action of soil salinity on plant growth was significantly promoted with the two-phase model first suggested by Munns (1993). In this conceptual model, the growth reaction to salt is separated into a Phase I and a Phase II. In Phase I, the decrease of water potential in the root medium after addition of salt decreases the water availability in the root medium. However, due to acclimation processes plants avoid water deficiency and turgor of leaf cells is maintained (De Costa et al., 2007; Munns, 1988). With prolonged plant growth and high external ion concentrations, plants enter Phase II, which is characterized by the accumulation of toxic ion concentrations, particularly in leaves (Munns & Tester, 2008). Munns (1993) already mentioned a turgor decrease when plants are salt-treated in nutrient solution experiments. This transient decrease in turgor, membrane potential, and proteome was subsequently characterized as Phase 0, extending the two-phase model to a three-phase model of salt stress (Schubert, 2011).

Differences in salt resistance of various genotypes were demonstrated for Phase I and Phase II. Although both phases overlap (Sümer et al., 2004) the model enabled a breakthrough in developing salt-resistant maize hybrids (SR hybrids; Schubert et al. 2009). Plants can exclude Na^+ from cytoplasm into the vacuoles with the help of H^+/Na^+ antiporters at the tonoplast (Apse et al., 1999; Saqib et al., 2005), which also leads to tissue tolerance of Na^+ (Munns & Tester, 2008; Neubert et al., 2005) hence contributing to osmoregulation. At root level, this process leads to a reduced Na^+ translocation from root to shoot (Neubert et al., 2005). A restricted sodium entry into the roots (Amtmann et al., 2005; Schubert & Läuchli, 1990) and the reabsorption of sodium from xylem by xylem parenchyma cells (Davenport et al., 2005; Yeo et al., 1977) provide other opportunities of sodium exclusion and helped to increase the salt resistance of wheat (Davenport et al., 2005).

Like wheat (Munns, 1993), maize growth is also affected by salinity following the three-phase growth model with an osmotic stress in Phase I and ion toxicity (particularly Na^+ toxicity) in Phase II (Fortmeier & Schubert, 1995). Therefore, a salt-resistant maize genotype needs to combine traits of resistance to osmotic stress (Phase I) as well as ion toxicity (Phase II). The exclusion of Na^+ at the surface of the root, restriction of Na^+ translocation from root to shoot, and plant resistance to osmotic stress were essential targets to develop salt-resistant maize genotypes (Schubert et al., 2009).

Keeping this in mind, salt-resistant maize hybrids (SR hybrids) were developed which show better growth and yield performance compared to the salt-sensitive maize cv. Pioneer-3906 when exposed to salinity under controlled conditions (Schubert et al., 2009). However, for agricultural purposes, salt resistance must be tested in the “real world,” that is, under field conditions.

Therefore, two field experiments were conducted in Pakistan to evaluate the yield performance of the salt-resistant maize hybrids (SR hybrids) under salt-affected field conditions. It was hypothesized that the superior sodium exclusion (Phase II) and improved extension growth and kernel setting (Phase I) were responsible for the breeding success. Because of growth reduction in Phase I it was hypothe-

sized that yield performance of these salt-resistant maize hybrids is improved by increasing the planting density. It should be noted that the SR hybrids were not originally developed for the environmental and field conditions in Pakistan but that the aim was a proof of concept. To compare the performance of SR-12 with a local cultivar from Pakistan under varying plant density, we included the hybrid EV-78 in the second experiment.

2 | MATERIALS AND METHODS

2.1 | Experiment I

2.1.1 | Plant and soil characteristics

A field experiment was conducted to compare the performance of salt-resistant maize hybrids (*Zea mays* L. SR-05, SR-12, SR-15, and SR-16) to the maize cv. Pioneer-3906 (syn. cv. Ornella) under nonsaline and salt-affected soil conditions. The study was conducted at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. The experiment was laid out in a randomized complete block design with four replications using a plot size of 50 m² (5×10^{-3} ha). After the experiment had been laid out, representative soil samples were collected from 0 to 15 and 15 to 30 cm depths from each plot. The soil samples were taken and analyzed following Ryan et al. (2001). The soil was a sandy clay loam in texture with pH: 8.1–8.4, EC (dS m⁻¹): 2.0–2.5, and SAR (mmol L⁻¹)^{1/2}: 6.0–7.5 under the nonsaline soil conditions, and with pH: 8.4–8.7, EC (dS m⁻¹): 10.0–12.0, and SAR (mmol L⁻¹)^{1/2}: 14–16 under the salt-affected soil conditions.

2.1.2 | Agronomic measures

The soil was prepared for the plantation by plowing and planking followed by making the ridges. The recommended levels of fertilizers (N:P:K in elemental forms at the rate of 120:75:75 kg ha⁻¹) were applied in all the plots. Urea, single super phosphate, and potassium sulfate were used as fertilizers for N, P, and K, respectively. The whole P and K, and one third N fertilizer were added at the time of sowing, whereas one third N fertilizer was added 15 days after sowing and the remaining one third was added at tasseling stage. The seeds of cv. Pioneer-3906 and the salt-resistant maize hybrids were placed in small cotton bags and soaked in running tap water (EC: 0.45 dS m⁻¹) for 12 h. The soaked seeds were then sown on the ridges by dibbling keeping a row-to-row distance of 90 cm and a plant-to-plant distance of 30 cm. At the seedling stage, the number of plants in each plot was reduced to 150 (3 plants m⁻²). The whole crop was irrigated uniformly at 7–10 days intervals during the whole growth season (electrical conductivity of the irrigation water was 3.98 dS m⁻¹). The fields were hoed twice during the growth period to remove the weeds. At maturity, all the plants in each plot were harvested and air-dried for 7 days under the shade. The cobs were removed from the plants and the grains were separated from the cobs. Grain yield, kernel number

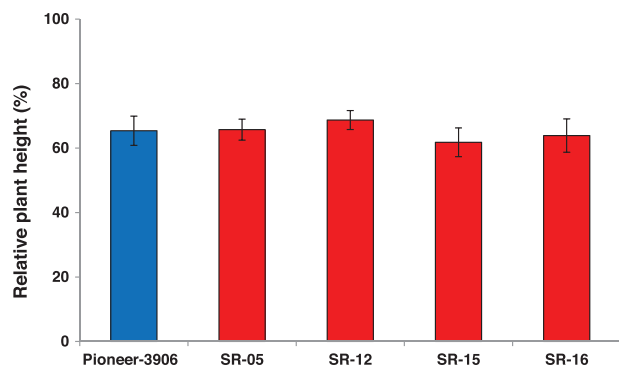


FIGURE 1 Relative plant height of maize cv. Pioneer-3906 and salt-resistant maize hybrids (SR hybrids) at flowering. Relative plant height was calculated by dividing height under saline by height under nonsaline conditions multiplied with 100. Data show means of four replicates \pm standard errors of plants grown under saline conditions relative to nonsaline conditions. Significant decrease ($p \leq 0.05$) under saline conditions; no significant differences among genotypes.

per plant, kernel weight, plant height, and straw yield were recorded. The harvest index was calculated as the ratio of the grain yield to the biological aboveground yield.

2.1.3 | Climatic conditions

The average minimum and maximum temperatures during the growth period were 18.2 and 32.9°C, respectively, and the average minimum and maximum relative humidity was 31% and 63%, respectively. There was 8.7 h average daylight and a total of 8.3 mm rain fall during the growth period.

2.1.4 | Statistics

The data were analyzed for analysis of variance (ANOVA) following Gomez and Gomez (1984), and the significance of the differences among the genotypes was analyzed using the least significant difference test. All data are reported as arithmetic means \pm standard errors of four replicates.

2.2 | Experiment II

This experiment was carried out under salt-affected field conditions following the procedures and methods described for Experiment I except that there were two hybrids and three planting densities.

TABLE 1 Effect of soil salinity on grain yield ($t\ ha^{-1}$) of maize cv. Pioneer-3906 and the SR hybrids

	Pioneer-3906	SR-05	SR-12	SR-15	SR-16
Nonsaline	4.43 \pm 0.10 A	4.29 \pm 0.08 A	4.14 \pm 0.20 A	4.32 \pm 0.16 A	4.08 \pm 0.19 A
Saline	2.36 \pm 0.05 B	3.03 \pm 0.11 A	2.95 \pm 0.10 A	2.88 \pm 0.10 A	2.86 \pm 0.08 A

Note: The values are means of four replicates \pm standard error. Values sharing different letters in a row differ significantly ($p \leq 0.05$).

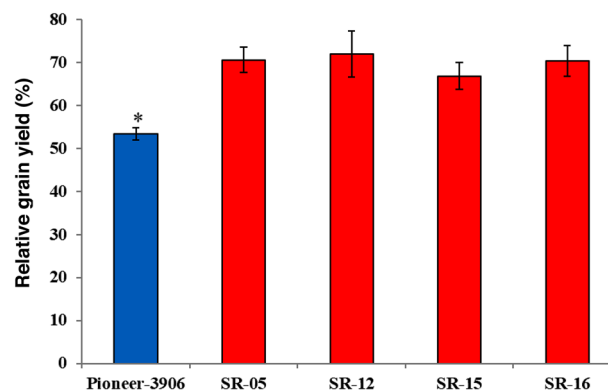


FIGURE 2 Relative grain yield of maize cv. Pioneer-3906 and salt-resistant maize hybrids (SR hybrids). Relative grain yield was calculated by dividing yield under saline by yield under nonsaline conditions multiplied with 100. Data show means of four replicates \pm standard errors of plants grown under saline conditions relative to nonsaline conditions. Significant decrease ($*p \leq 0.05$) for cv. Pioneer-3906 under saline conditions; no significant differences for the SR hybrids.

The maize hybrids used were SR-12 and the local hybrid EV-78. The planting densities were 5, 8, and 11 plants m^{-2} .

3 | RESULTS

3.1 | Experiment I

Due to artificial irrigation, plants were well supplied with water and therefore showed no wilting or leaf rolling, a typical symptom of drought stress for maize. Therefore, Phase 0 effects were not obvious during the growth period. Moreover, there were no toxicity symptoms excluding a major effect of Phase II problems. However, plant height of all genotypes was decreased significantly by about 30% under salt stress relative to nonsaline conditions indicating major Phase I effects (Figure 1).

With a maximum yield of 4.43 $t\ ha^{-1}$ for cv. Pioneer-3906 under nonsaline conditions, the yield performance was low (Table 1), but typical for the local conditions using a low plant density of 3 plants m^{-2} . Yields of the SR hybrids under nonsaline conditions were comparable to cv. Pioneer-3906. Salt stress significantly decreased the yield of cv. Pioneer-3906 by 53% but not significantly of the SR hybrids by about 30% (Figure 2).

As there was only one fully developed cob per plant and plant density did not vary (3 plants m^{-2}), yield was determined by the number of kernels per cob and the kernel weight. From Figures 3 and 4

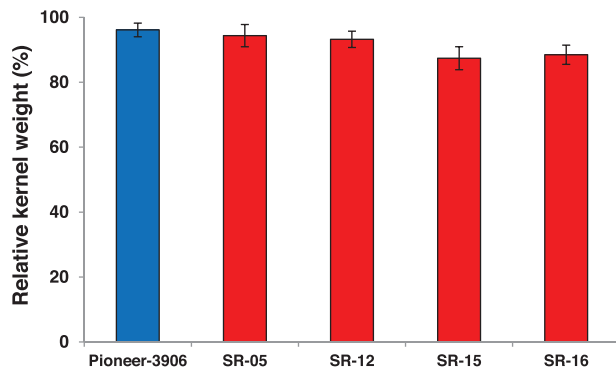


FIGURE 3 Relative kernel weight of maize cv. Pioneer-3906 and salt-resistant maize hybrids (SR hybrids). Relative kernel weight was calculated by dividing weight under saline by weight under nonsaline conditions multiplied with 100. Data show means of four replicates \pm standard errors of plants grown under saline conditions relative to nonsaline conditions. No significant differences.

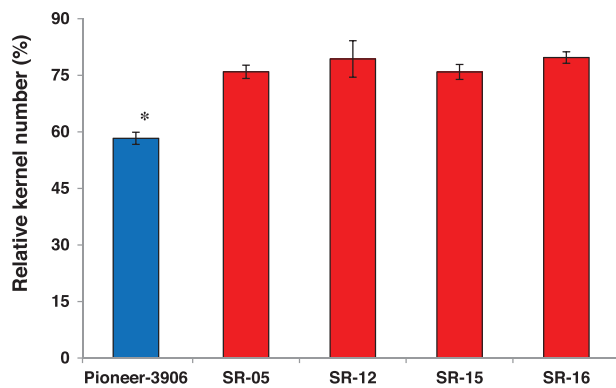


FIGURE 4 Relative number of kernels per cob of maize cv. Pioneer-3906 and salt-resistant maize hybrids (SR hybrids). Relative number of kernels was calculated by dividing number of kernels under saline by number of kernels under nonsaline conditions multiplied with 100. Data show means of four replicates \pm standard errors of plants grown under saline conditions relative to nonsaline conditions. Significant decrease ($*p \leq 0.05$) for cv. Pioneer-3906 under saline conditions; no significant differences for the SR hybrids.

it is apparent that the yield decrease of cv. Pioneer-3906 due to salt stress was not caused by poor grain filling but by kernel setting. This significantly decreased the harvest index of cv. Pioneer-3906 (Table 2) although straw yield was also decreased (not shown).

TABLE 2 Effect of soil salinity on harvest index of maize cv. Pioneer-3906 and the SR hybrids

	Pioneer-3906	SR-05	SR-12	SR-15	SR-16
Nonsaline	0.40 \pm 0.01 A	0.40 \pm 0.01 A	0.39 \pm 0.01 A	0.40 \pm 0.01 A	0.39 \pm 0.01 A
Saline	0.33 \pm 0.01 B	0.39 \pm 0.01 A	0.38 \pm 0.01 A	0.38 \pm 0.01 A	0.38 \pm 0.01 A

Note: The values are means of four replicates \pm standard error. Values sharing different letters in a row differ significantly ($p \leq 0.05$)

3.2 | Experiment II

Under saline conditions, the plant height was not affected by varying the planting densities (not shown). The grain yield per plot of both the hybrids (SR-12 and EV-78) was increased significantly by increasing the planting density from 5 to 8 plants m^{-2} (Figure 5). An additional increase of planting density from 8 to 11 plants m^{-2} did not further increase the grain yield.

However, the grain yield per plant decreased significantly with an increase of planting density from 5 to 8 as well as from 8 to 11 plants m^{-2} (not shown). The straw yield of SR-12 was increased significantly by increasing planting density from 5 to 8 and (only for EV-78) from 8 to 11 plants m^{-2} (not shown). The maximum harvest index was observed at planting density of 8 plants m^{-2} and, for SR-12, it was significantly higher than the harvest index at planting densities of 5 and 11 plants m^{-2} (Figure 6).

4 | DISCUSSION

4.1 | Yield performance

Maize was grown on ridges with 3 plants m^{-2} following the local recommendations. This allows regular surface irrigation to avoid drought stress. The low planting density may be the main agronomic reason that the yield performance with 4.43 t ha^{-1} of cv. Pioneer-3906 was poor (Table 1). On the other hand, Pioneer-3906, which served as ancestor in the breeding program of the SR hybrids (Schubert et al., 2009), is an old cultivar that was grown as silage maize in Germany and grain maize in California in the early 1980s (Maas et al., 1983). Although breeding of the SR hybrids did not focus on yield performance (Schubert et al., 2009) their yields were comparable to cv. Pioneer-3906 under nonsaline conditions (Table 1).

4.2 | Salt resistance of the SR hybrids under field conditions

Salt stress strongly decreased the yield of cv. Pioneer-3906 (Figure 2). Throughout the growth period, there were no wilting symptoms or leaf rolling, indicating that Phase 0 effects were not responsible for the yield reduction (Schubert, 2011). Likewise, no ion toxicity symptoms were observed, which confirms earlier findings that sodium toxicity was avoided by strong sodium exclusion of cv. Pioneer-3906 (Fortmeier & Schubert, 1995; Maas et al., 1983; Schubert & Läuchli, 1990).

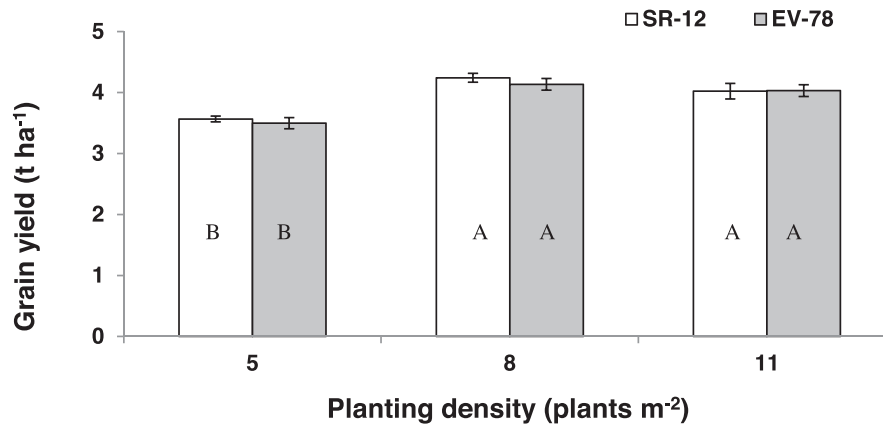


FIGURE 5 Effect of planting density on the grain yield of maize cv. Pioneer-3906 and cv. EV-78. Data show means of four replicates \pm standard errors of plants grown under saline conditions and nonsaline conditions. Different letters indicate significant differences ($p \leq 0.05$).

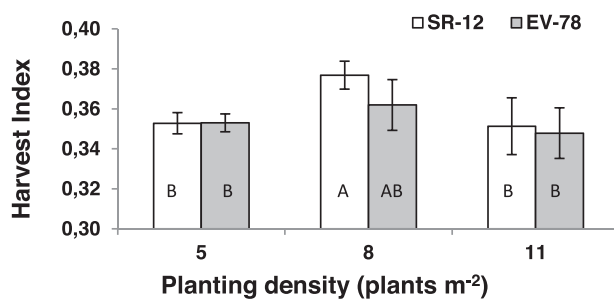


FIGURE 6 Effect of planting density on the harvest index of maize cv. Pioneer-3906 and cv. EV-78. Data show means of four replicates \pm standard errors of plants grown under saline conditions and nonsaline conditions. Different letters indicate significant differences ($p \leq 0.05$).

Therefore, also Phase II effects are unlikely to have significantly contributed to the yield reduction of cv. Pioneer-3906. There are two lines of evidence that Phase I effects were primarily responsible for the yield decrease. First, plant height was significantly reduced (Figure 1), probably because extension growth was inhibited (Pitann et al., 2009; Uddin et al., 2013; Zörb et al., 2013). Second, kernel setting was significantly hampered (Figure 4) most likely due to an inhibition of the concerted action of H⁺ ATPase and acid invertase activity. It has been shown that these are key enzymes in the establishment of sink strength and low activities of these enzymes lead to kernel abortion (Hütsch et al., 2014; Hütsch & Schubert, 2017; Jung et al., 2017; Li et al., 2013).

In contrast to cv. Pioneer-3906, grain yield (Table 1, Figure 2), and harvest index (Table 2) of the SR hybrids were not reduced by salt stress. This demonstrates the improved salt resistance of the SR hybrids under field conditions and corroborates data from pot experiments (Schubert et al., 2009). The major reason for the salt resistance at the tested salinity level may be the improvement of H⁺ ATPase activity in the kernels of the SR hybrids (Jung et al., 2017; Tscharn et al., 2022). Maintained H⁺ ATPase activity in the kernels acidifies the apoplast and thus optimizes *in vivo* acid invertase activity and additionally establishes a strong pH gradient which allows H⁺/hexose cotransport into the sink cells. Therefore, high H⁺ ATPase activity has

a dual function in the avoidance of kernel abortion under salt stress (Hütsch & Schubert, 2017).

It is interesting to note that the reduction in plant height (and straw yield, not shown) under salt stress was similar for cv. Pioneer-3906 and the SR hybrids (Figure 1) although during vegetative growth a better extension growth for the SR hybrids relative to cv. Pioneer-3906 had been demonstrated (Hatzig et al., 2010; Pitann et al., 2009; Uddin et al., 2013). This shows not only that extensive vegetative growth under salt stress is not a prerequisite for high grain yield but also that it is possible to improve the harvest index, which may improve water-use efficiency (Hütsch & Schubert, 2018).

4.3 | Planting density under saline conditions

Although in Experiment II we used 5 plants m⁻² instead of 3 plants m⁻² as in Experiment I, yield under salt stress was further enhanced with 8 plants m⁻² for both the cultivars tested (Figure 5). An additional increase of the planting density (11 plants m⁻²) did not further increase yield, indicating that 8 plants m⁻² was the optimum for the given conditions. It must be concluded that inhibition of vegetative growth due to Phase I effects requires that planting density must be increased under saline conditions to fully exploit the salt resistance. On the other hand, the optimum of 8 plants m⁻² increased the harvest index of SR-12 but not of EV-7. This may indicate that the relatively good performance of the local hybrid EV-73 under saline conditions was not due to high salt resistance per se (Ahmed et al., 2012) but to a higher yield potential realized under the specific regional conditions (Shannon, 1985).

5 | CONCLUSIONS

Salt resistance of the SR hybrids was demonstrated under field conditions. Superior performance under saline conditions relative to cv. Pioneer-3906 was primarily realized by the avoidance of kernel abortion. To fully exploit the potential of salt-resistant maize genotypes, it is necessary to increase the planting density relative to nonsaline

conditions. The optimum planting density under saline conditions must be evaluated in site-specific field experiments considering the degree of salt resistance.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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