

MAIZE-GAMAGRASS INTERSPECIFIC HYBRID, *ZEA MAYS X TRIPSACUM DACTYLOIDES*, SHOWS BETTER SALINITY TOLERANCE AND HIGHER Na^+ EXCLUSION THAN MAIZE AND SORGHUM

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Abstract - Two hybrids (1 and 2) originating from a cross between maize and gamagrass (*Tripsacum dactyloides* L.) were tested for salinity sensitivity, measured as the reduction in shoot dry weight of plants grown for one month in the range 50-300 mM NaCl, relative to plants grown in the absence of salt. The maternal parental maize line V182 (2n = 4x = 40Zm) and two forage sorghum species were included for comparison. Hybrid 1 (2n = 39; 30Zm + 9Td) and Hybrid 2 (2n = 46; 10Zm+36Td) showed much less biomass reduction at 150 mM and 250mM NaCl, respectively, compared to the moderately salt tolerant parental maize line and more salt sensitive forage sorghum (*Sorghum bicolor* L. Moench.) and sudangrass (*S. sudanese* Staph.) cultivars at the same levels. Na^+ accumulation in extract of leaves in both hybrids was very low, 7-8-fold lower than in the parental maize and in the growth solutions. It was concluded that the higher salinity tolerance of the two hybrids is attributable to a highly efficient Na^+ exclusion mechanism. These results can explain our previous findings that both hybrids produced 11- to 16-fold more forage biomass compared to the parental maize line in a field trial on moderately saline soil.

Keywords - gamagrass; interspecific hybrid; maize; Na^+ exclusion; salinity tolerance; sorghum.

I. INTRODUCTION

Salinity is one of the most significant factors limiting the yield of cereal crops. Globally, saline soils affect almost 50% of irrigated agricultural land [1]. Mechanisms for salinity tolerance in plants include: (1) Na^+ exclusion from shoots, where the ions are re-directed back into roots to achieve levels of Na^+ in photosynthesising tissues below the toxic range; (2) Compartmentalisation of toxic Na^+ ions into vacuoles in shoot cells; (3) Osmotic adjustment, where plant cells modify their cytoplasmic water potential by the production of solutes [1-4]. All three mechanisms are very important for plant responses to salinity, but each are utilised more or less effectively by different species. Plants with an effective Na^+ exclusion mechanism have reduced concentrations of Na^+ in leaves accompanied by higher biomass production in saline growth conditions. For example, such a mechanism is encoded by the *Nax2* gene, identified in the wild species *Triticum monococcum* and successfully introgressed into durum wheat with reports of 25% improved grain yields in salinized fields [5]. The compartmentalisation mechanism is most effective among halophyte species of plants [2-3]. Osmotic adjustment is also important for plant adaptation to a range of abiotic stresses, where proline can play a key-role as the mediator [6]. As we recently also reported within accessions of a wild tetraploid *Triticum dicoccoides* species, there is a wide range of Na^+ accumulation that is not always negatively correlated with a

decrease in relative biomass production under salinity [7]. This observation indicates exclusion, compartmentalisation or both mechanisms may be more or less effective in different genotypes within the same species.

In general, maize is more sensitive to salinity than sorghum [8]. The thresholds (maximum soil salinity) for maize (*Zea mays* L.), sudangrass (*Sorghum sudanese* Staph.) and sorghum (*Sorghum bicolor* L. Moench.) were determined as 1.7-1.8 dS/m, 2.8 dS/m and 6.8 dS/m (equivalent to about 25 mM NaCl, 35 mM NaCl and 90 mM NaCl), respectively [8]. However, the genetic variation to salinity tolerance among maize and sorghum genotypes is reported to be high [9-17].

Na^+ accumulation in leaves of salt-stressed plants is one of the most objective traits to measure, and is often associated with biomass production and salinity tolerance. A number of reports of salt stress responses in both maize and sorghum have identified a very strong negative correlation between Na^+ accumulation and plant growth under salt [9-10, 17-18]. However, not all genotypes conform to this relationship, with some salt tolerant lines accumulating high shoot Na^+ concentrations, and others accumulating low levels of Na^+ but growing poorly in saline conditions [12-15]. Cramer et al. [19] concluded that salt tolerance is not associated with the Na^+ accumulation in maize, despite their study including only two lines. Na^+ exclusion has become somewhat overlooked as a critical tolerance mechanism in maize and sorghum, with

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claims that osmotic tolerance may play a more important role [11].

Eastern gamagrass (*Tripsacum dactyloides* L.) is a natural grass originating from Mexico and the southern states of the USA, areas that are affected by abiotic stresses including heat, drought and salinity [20-21]. *Tripsacum* is a wild genetic relative of cultivated maize, but it is considered a different species and has a different number of chromosomes to its domestic relative [22]. A range of interspecific hybrids between maize and *Tripsacum* with different combinations of chromosomes were developed [22-23]. Two maize-gamagrass hybrids were identified as being able to produce progeny plants with a stable number of chromosomes. Hybrid 1 ($2n = 39$) has a higher portion of introgressed chromosomes from the maize parent (30 chromosomes), and shows an annual growth habit and rapid rate of development [24]. In contrast, Hybrid 2 ($2n = 46$) has a higher portion of chromosomes from the male gamagrass parent (36 chromosomes), and displays a perennial growth habit, reduced growth rate and more tillers with much higher biomass production. However, the hybrids produced silage in a field trial under moderately saline conditions of 29.0 and 42.9 tonnes/ha, respectively, 11- and 16-fold higher than the parental maize line [25]. Nutrient composition, energy, protein content and digestibility of the silage from the hybrids were similar to those from parental maize. The hybrids also demonstrated a tolerance to high alkalinity, low soil nutrition and a combination of high salinity, strong drought and heat stresses [25], making them promising potential forage crops for farmers in marginal environments.

Another maize-gamagrass hybrid, originating from maize parental line N107B and *Tripsacum dactyloides* L. and regenerated from *in-vitro* cultures, was tested for tolerance to high salinity [26]. The report concluded that the hybrid plants actually produced more biomass under salt (250 mM NaCl during 20 days) than in non-saline conditions, but that this was primarily due to an increase in non-harvestable root biomass. Interestingly, there was a 2.5-fold higher accumulation of Na^+ in shoots of this hybrid, suggesting a high level of tissue tolerance to Na^+ [26].

The aims of this study were to: (1) Test the relative salinity tolerances (based on shoot dry weight) of two interspecific hybrids (*Zea mays* L. x *Tripsacum dactyloides* L.), compared with a parental maize line and two sorghum species; and (2) Investigate Na^+ accumulation / exclusion in leaves in the studied genotypes as a possible mechanism for salinity tolerance.

II. MATERIALS AND METHODS

Plant material

Tetraploid maize parental line V182 ($2n = 4x = 40$) and two maize-gamagrass hybrids, *Zea mays* L. x *Tripsacum dactyloides* L., were developed in Novosibirsk (Russia), registered and characterised by Dr. Victor Sokolov. The two hybrids were designated as Hybrid 1, originating from parental line V182, and Hybrid 2, originating from hybrid ICI-1993-F1, Imperial Chemical Industries (ICI), Iowa (USA). Both hybrids are apomictic and have different but stable numbers of chromosome. Hybrid 1 has 39 chromosomes ($30Zm + 9Td$) and Hybrid 2 has 46

chromosomes ($10Zm + 36Td$), where *Zm* and *Td* are the abbreviated genomes of maternal (maize) and paternal (gamagrass) species, respectively. Hybrids 1 and 2 also differ in growth habit, growing as an annual and perennial crop, respectively. Seeds of maize line V182 and the two hybrids for this study were harvested from plants grown at ACPFG, the University of Adelaide (Australia). Seeds of two other forage species (C_4 type photosynthesis), sorghum (*Sorghum bicolor* L. Moench.) cv. Sugargraze and sudangrass (*Sorghum sudanese* Staph.) cv. Sprint, were kindly provided by the Pacific Seeds Company (Queensland, Australia), and were used to compare plant growth and Na^+ accumulation traits with those of the parental maize line and two hybrids.

Salinity experiment

Experiments were conducted in a temperature-controlled greenhouse ($22^\circ\text{C}/16^\circ\text{C}$ day/night) with 16 h natural daylight, in 6 inch (15 cm) diameter pots fitted with plastic bags. Each pot was half-filled with dry UC soil mix (equal portions of River sand, Redwood sawdust and Peat), weighed and mixed with measured quantities of NaCl powder. The top part of the pot was then filled with an equal weight of unamended dried UC soil mix. Water was added to attain a soil moisture content of 29%. Amounts of NaCl were calculated as 1.2, 2.4, 3.6, 4.8, 6.0 and 7.2 g per kg of dry soil in order to achieve concentrations of 50, 100, 150, 200, 250 and 300 mM NaCl, respectively, in solutes of soil mix in each pot (assuming no adsorption or precipitation of Na^+). Control pots contained no added NaCl but contained the same soil and level of moisture. Each treatment had three biological replicates (single plant in each separate pot).

Seeds were germinated in Petri dishes over 5 days and transplanted to the pots. Each pot was watered to weight using reverse osmosis water twice per week, to maintain constant moisture. Transplanted seedlings established root systems in the top part of the soil (without salt) and then penetrated the bottom part of the soil (with salt) with further growth. Plants were grown for one month. After recording images of the plants, all above ground material was harvested and shoot dry weights (SDW) recorded. The reduction in SDW of plants across a range of salinity levels was calculated using the formula: $\text{SDW}_{(\text{Control})} - \text{SDW}_{(\text{Treatment})} / \text{SDW}_{(\text{Control})} \times 100\%$, where $\text{SDW}_{(\text{Control})}$ and $\text{SDW}_{(\text{Treatment})}$ were the average SDW for Control and salt treatment, respectively, for three biological replicates for each species / hybrid.

Determination of leaf Na^+ concentration

Two youngest fully developed leaves were collected from an additional set of plants (three replicates) grown in the same conditions with 100 mM NaCl. One leaf was sampled in its entirety, whilst the second leaf was dissected into eight pieces of equal length to determine the distribution of Na^+ along the leaf. Fresh and dry weights of the leaf samples were recorded after harvest and drying in an oven at 85°C overnight, respectively. Na^+ concentrations were determined in samples digested in 20 ml of 1% nitric acid for four hours at 85°C using Flame Photometry, as described previously [27].

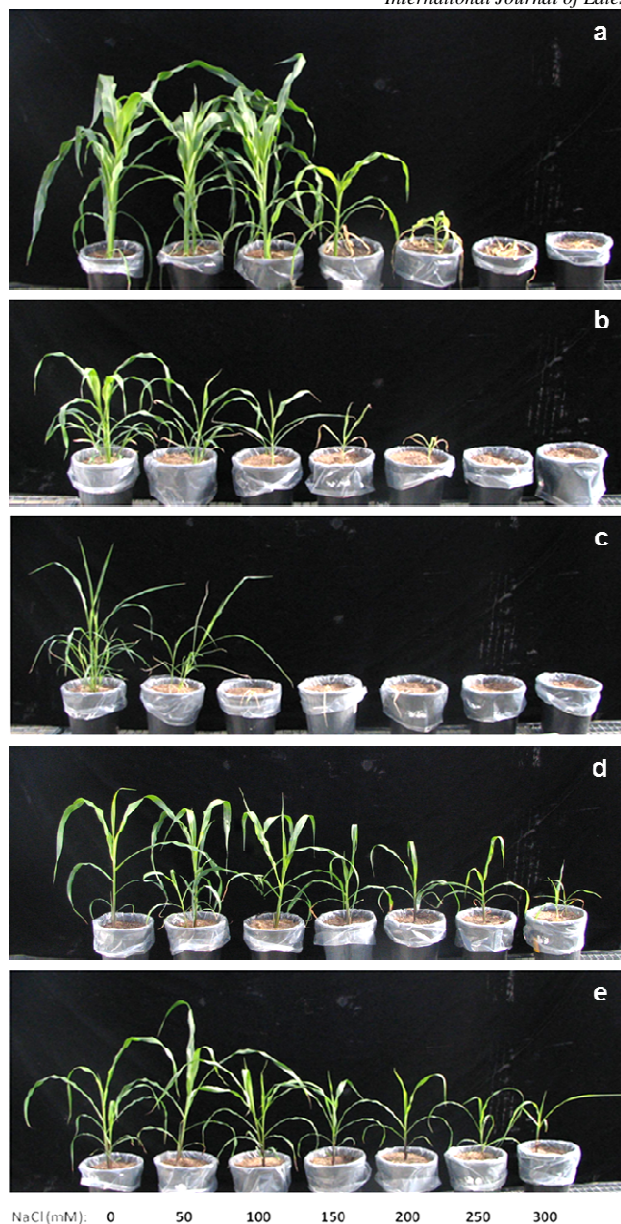


Figure 1. Seedlings of different forage grass species grown for one month in soil amended with a range of levels of NaCl: Controls (0, without NaCl) and 50-300 mM NaCl. Representative plants from three replicates were selected for these images: **(a)** Maize, parental line V182 (*Zea mays* L.); **(b)** Forage sorghum (*Sorghum bicolor* L. Moench.) cv. Sugargraze; **(c)** Forage sudangrass (*Sorghum sudanese* Staph.) cv. Sprint; **(d)** Hybrid 1, *Zea mays* L. x *Tripsacum dactyloides* L., with 39 chromosomes and annual habit; **(e)** Hybrid 2, *Zea mays* L. x *Tripsacum dactyloides* L., with 46 chromosomes and perennial habit.

Statistical treatment

Data were analysed using standard Microsoft-Excel software and presented as means for three biological replicates in all experiments \pm standard errors. Single-factor ANOVA was used to determine statistical differences between classes.

III. RESULTS

The five genotypes included in this study responded differently to salt stress, as shown in the images of plants taken prior to harvest (Fig. 1), and the measurements of average SDW (Fig. 2).

In this experiment, maize grew fastest, with plants producing about twice as much biomass in control conditions compared to the other species (Figs. 1 and 2). The parental maize line is well-adapted to growth in moderately warm temperatures. No significant difference was observed in maize SDW between 0 and 100 mM NaCl, but SDW was reduced by about half at 150 mM NaCl. Plant death was observed with higher salinity.

Forage sorghum and sudangrass plants grew more slowly in control conditions, possibly due to sub-optimal temperatures for this genus. The two species responded similarly to salt stress (Figs. 1 and 2), although the level of salinity at which plant death was noted was lower for sudangrass (100 mM NaCl) compared to sorghum (150 mM NaCl).

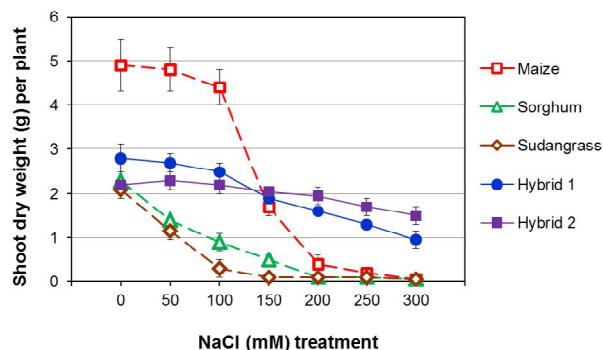


Figure 2. Shoot yields of plants grown for one month in pots with soil containing NaCl in the range 50-300 NaCl and in controls (0, without NaCl). Each data-point represents the mean of three biological replicates \pm standard error in three forage grass species (maize, sorghum and sudangrass; broken lines) and two hybrids (maize x gamagrass; solid lines).

By comparison, the two maize-gamagrass hybrids showed a very gradual decrease in SDW across the range of salt treatments (Figs. 1 and 2). Above-ground biomass production of Hybrid 1 was not affected up to 100 mM NaCl, while Hybrid 2 biomass production was unaffected up to 200 mM NaCl. Plant death was not observed at any NaCl level.

Table 1 shows the percentage DW reduction across salinity levels for each species used in the experiment. The critical level of salinity for the parental maize line was 150 mM NaCl with a SDW reduction of 65.3%. There was a strong effect on forage sorghum and sudangrass of even the lowest level of salinity (50 mM NaCl), with SDW reductions of 39.1% and 45.2%, respectively (Table 1). By contrast, the maize-gamagrass hybrids showed only a gradual decrease in SDW across the range of salt treatments. In particular, the growth reductions observed for Hybrid 2 were very small (Table 1).

Table 1. Percentage of shoot dry weight (SDW) reduction under salt treatment compared to non-stressed conditions. Plants were grown for one month in pots of soil containing NaCl in the range 50-300 NaCl and in controls (0, without NaCl). Above-ground biomass was collected from individual plants and dry weights were recorded. Percentage reduction of dry biomass in each salt treatment relative to control was calculated using the formula described in the Material and methods section. The data correspond to SDW data in Fig. 2.

Plants	Concentration of NaCl (mM)						
	0	50	100	150	200	250	300
Maize	0	2.0	10.2	65.3	91.8	95.9	99.0
Sorghum	0	39.1	60.9	78.3	95.7	95.7	97.8
Sudangrass	0	45.2	85.7	95.2	95.2	95.2	97.6
Hybrid 1	0	3.6	10.7	32.1	42.9	53.6	66.1
Hybrid 2	0	-4.5	0.0	6.8	11.4	22.7	31.8

Na⁺ accumulation in leaves of plants grown in the 100 mM NaCl treatment varied greatly between the species and hybrids used in this study (Fig. 3). High Na⁺ concentrations were measured in the parental maize line while both hybrids (1 and 2) accumulated very low concentrations of leaf Na⁺, between 8- and 9-fold less than in maize. Leaf Na⁺ concentrations of sorghum and sudangrass were even higher than for maize. The distribution of Na⁺ concentration along leaves indicated higher concentrations in the younger, basal regions (Fig. 3b). This was a particularly clear trend in leaves of parental maize.

IV. DISCUSSION

Salinity tolerance is an important trait for selection towards improving plant growth and yield to meet the needs of our growing human population and changing climate. Plants with C₄ type photosynthesis, such as maize and sorghum, are very important for agriculture, but are described as only moderately sensitive and moderately tolerant to salinity, respectively [8]. However, genetic variation in salt tolerance is known to be very wide in these species. In our study, the parental maize line V182 had greater above-ground biomass production than the two selected sorghum genotypes, and SDW was reduced by only 2% and 10.2% in plants exposed to 50 and 100 mM NaCl, respectively, compared to control plants. However, with increased salt application maize growth was severely affected (Table 1). The maize line V182 appears to have good tolerance to low-moderate salt stress but not at higher levels. Unexpectedly, the forage sorghum and sudangrass cultivars were very sensitive to salt stress, where a low-moderate level of salinity (50 mM NaCl) caused significant reductions in SDW, of 39.1 and 45.2%, respectively (Table 1).

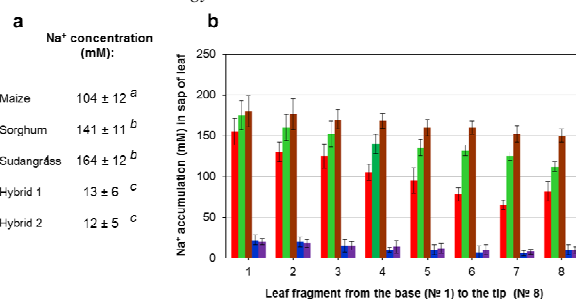


Figure 3. Na⁺ accumulation in extract of youngest fully developed leaf blade of five forage species grown for four weeks in pots of soil containing 100 mM NaCl; parental maize line V182 (*Zea mays* L.), forage sorghum (*Sorghum bicolor* L. Moench.) cv. Sugargraze, forage sudangrass (*Sorghum sudanese* Staph.) cv. Sprint and two hybrids, Hybrid 1 and Hybrid 2, originating from the interspecific cross (*Zea mays* L. x *Tripsacum dactyloides* L.). (a) Entire leaf blade Na⁺; (b) Distribution of Na⁺ along the leaf. Data are presented as means for three biological replicates ± standard errors. Letters represent ANOVA statistical classes for significant differences (p<0.05) for entire leaf blade Na⁺.

Two hybrids (1 and 2) were developed from the cross with parental maize, including line V182 in this study, and inherited 30 and 10 chromosomes, respectively, from the maternal parent. There is no evidence for possible recombination events between chromosomes from maize and gamagrass genomes, and thus it is assumed that entire non-recombinant chromosomes were introgressed from both genomes into the hybrids. Both hybrids showed reductions of SDW in salt treated conditions compared to control plants, but these were only significant at higher levels of salinity: 150 mM NaCl for Hybrid 1 (32.1% reduction) and 250 mM NaCl for Hybrid 2 (22.7% reduction) (Table 1). The reduction in biomass of the hybrids was much smaller compared to the parental maize line, shown here to be more salt tolerant than two sorghum species.

Above-ground biomass in both hybrids under non-stressed conditions was low (two-fold less) compared to the parental maize line. This may be due to the introgression of chromosomes from the paternal gamagrass genome. Hybrid 1 has an annual growth habit (like maize), whilst Hybrid 2 is a perennial (like gamagrass), illustrating that the characters inherited from each parent are considerably different for each hybrid. Nevertheless, both hybrids showed a similar response to salinity (Fig. 2).

This study, showing high salinity tolerance in the two hybrids, can explain our previously published findings, where both hybrids produced 11-16-fold more forage biomass compared to the parental maize line in a field trial with a moderate level of salinity [25]. In that study, silage biomass yields of 29.0 and 42.9 tonnes/ha were obtained for Hybrids 1 and 2, respectively, with similar quality and digestibility traits to the parental maize line. The two hybrids also showed tolerance to high alkalinity, low soil nutrition and a combination of high salinity and strong drought stress [25].

Measurements of leaf Na⁺ accumulation at a moderate level of salt stress (100 mM NaCl) showed that both hybrids accumulated very low concentrations of leaf Na⁺, 13 ± 6 and 12 ± 5 mM NaCl, for Hybrids 1 and 2, respectively. This was an 8-fold reduction in Na⁺ concentration compared to estimated soil solute concentration. By contrast, leaf Na⁺ concentration in the maize parental line (104 ± 12 mM NaCl) was equivalent to the external level of salinity. Both forage sorghum and sudangrass accumulated significantly higher leaf blade Na⁺ than maize, probably exceeding the critical level for Na⁺ ion toxicity, and likely associated with particularly low SDW and plant death in these genotypes.

There are conflicting reports on the role of Na⁺ exclusion for salinity tolerance in maize. Many studies show a strong association between Na⁺ exclusion and salinity tolerance [9-10, 17-18], while others suggest that such correlations are not applicable for all maize genotypes [12-13, 15]. Cramer et al. [19] concluded that salinity tolerance in maize was not determined by Na⁺ exclusion at all. Na⁺ exclusion can be a valuable mechanism for lower levels of salt stress, while osmotic regulation becomes more important at higher salinity [6]. It is possible that a maize Na⁺ exclusion mechanism is genotype-dependent as well as salinity level-dependent. Another explanation for the inconsistencies between studies may be that within the leaf blade there is vacuolar sequestration of Na⁺ ions in some genotypes.

Our results indicated that parental maize line V182 with moderately tolerance to salinity had less efficient Na⁺ exclusion mechanism from leaf blade at least at the studied 100 mM NaCl level. A dramatic reduction of leaf Na⁺ accumulation in both hybrids compared to the maize line was shown in this study, suggestive of a highly effective Na⁺ exclusion mechanism seems to be derived from the gamagrass parent. This conclusion is partly speculative because second parental maize line, ICI-1993-F1, as well as gamagrass plants were not included in this study.

It is suggested that the leaf sheaf can be a potential site for preferential accumulation of Na⁺ ions [19], restricting entry to the leaf blade. Our results, showing a gradient for the distribution of Na⁺ ions along the leaf blade in parental maize line V182 may indirectly support this hypothesis. Significantly higher Na⁺ accumulation was observed in the basal portions of the blade (close to leaf sheaf) compared to segments towards the leaf tip. A similar, although less evident trend was observed for the two forage sorghum species. By contrast, there was no obvious pattern of Na⁺ distribution along the leaves of Hybrids 1 and 2, probably due to very low levels of Na⁺ accumulation.

Another report of the salinity tolerance of a maize-gamagrass hybrid originating from maize parental line N107B [26] is in conflict with our findings. The authors reported a significant increase in root biomass and slightly higher (but not significant) shoot biomass at a very high level of salt treatment (250 mM NaCl) compared to plants of the same genotype grown under non-stressed conditions. In our study, SDW was reduced by 53.6% and 22.7% in Hybrid 1 and Hybrid 2, respectively, at the same estimated concentration. Pesqueira et al. [26] also report significantly higher (2.5-fold) Na⁺ accumulation in shoots in the hybrid grown at high salinity compared to non-stressed conditions.

However, the hybrids from each study are derived from different interspecific crosses. Genotypes of the maternal maize lines (N107B and V182) as well germplasms of paternal gamagrass may play a crucial role for hybrid performance. Moreover, the hybrid tested in Pesqueira et al. [26] appears to be F₁ progeny grown initially in tissue culture, with rooted regenerants transferred to hydroponics for phenotyping. There is no supporting information about chromosome number for the hybrid they produced or its stability, or characteristics of F₂ plants derived from this hybrid. By contrast, the two hybrids in our study have stable genomes, of 39 and 46 chromosomes, and produce normal seeds [24]. Stable generations of hybrids can be generated and tested for more accurate comparisons of salinity tolerance.

Indeed, further research is needed to better understand what appears to be a highly efficient Na⁺ exclusion mechanism in the two hybrids between maize and gamagrass used in this study. Experiments utilising supported hydroponics to measure Na⁺ accumulation across a range of NaCl concentrations and in different tissue types (e.g. leaf sheath versus leaf blade) will facilitate more relevant comparisons with other studies.

V. CONCLUSIONS

Two interspecific hybrids between maize and eastern gamagrass showed higher salinity tolerance: both Hybrid 1 and Hybrid 2 had much less biomass reduction at 150 mM and 250mM NaCl, respectively, compared to the moderately salt tolerant parental maize line and more salt sensitive forage sorghum and sudangrass cultivars at the same levels. Na⁺ accumulation in extract of leaves in both hybrids was very low, 7-8-fold lower than in the parental maize and in the growth solutions. It was concluded that the higher salinity tolerance of the two hybrids is attributable to a highly efficient Na⁺ exclusion mechanism.

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REFERENCES

- [1] Hasegawa P.M. (2013) Sodium (Na⁺) homeostasis and salt tolerance of plants. *Environmental and Experimental Botany* 92, 19-31.
- [2] Parida A.K., Das A.B. (2005) Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety* 60 (3), 324-349.
- [3] Zhang J.L., Shi H. (2013) Physiological and molecular mechanisms of plant salt tolerance. *Photosynthesis Research* 115 (1), 1-22.
- [4] Gupta B., Huang B. (2014) Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *International Journal of Genomics* 2014, 701596.
- [5] Munns R., James R.A., Xu B., Athman A., Conn S.J., Jordans C., Byrt C.S., Hare R.A., Tyerman S.D., Tester M., Plett D., Gilliam

- M. (2012) Wheat grain yield on saline soils is improved by an ancestral Na⁺ transporter gene. *Nature Biotechnology* 30 (4), 360-364.
- [6] Wani S.H., Gosal S.S. (2010) Genetic engineering for osmotic stress tolerance in plants - Role of proline. *The IUP Journal of Genetics and Evolution* 3 (4), 14-25.
- [7] Shavrukov Y., Langridge P., Tester M., Nevo E. (2010) Wide genetic diversity of salinity tolerance, sodium exclusion and growth in wild emmer wheat, *Triticum dicoccoides*. *Breeding Science* 60 (4), 426-435.
- [8] Maas E.V. (1985) Crop tolerance to saline sprinkling water. *Plant and Soil* 89 (1-3), 273-284.
- [9] Abbasi G.H., Akhtar J., Anwar-ul-Haq M., Ahmad N. (2012) Screening of maize hybrids for salt tolerance at seedling stage under hydroponic condition. *Soil and Environment* 31 (1), 83-90.
- [10] Eker S., Barut H. (2013) Interactive effects of cadmium and salinity stresses on growth and cadmium, sodium and potassium accumulation in two hybrid maize varieties. *Journal of Food, Agriculture and Environment* 11 (3-4), 1364-1368.
- [11] Hefny M.M., Metwali E.M.R., Mohamed A.I. (2013) Assessment of genetic diversity of sorghum (*Sorghum bicolor* L. Moench) genotypes under saline irrigation water based on some selection indices. *Australian Journal of Crop Science* 7 (12), 1935-1945.
- [12] Molazem D., Azimi J., Ghasemi M., Hanifi M., Khatami A. (2013) Is salinity tolerance related to Na accumulation in maize cultivars (*Zea mays* L.)? *Life Science Journal* 10 (1), 72-75.
- [13] Pitann B., Mohamed A.K., Neubert A.B., Schubert S. (2013) Tonoplast Na⁺/H⁺ antiporters of newly developed maize (*Zea mays*) hybrids contribute to salt resistance during the second phase of salt stress. *Journal of Plant Nutrition and Soil Science* 176 (2), 148-156.
- [14] Almodares A., Hadi M.R., Kholdebarin B., Samedani B., Akhavan Kharazian Z. (2014) The response of sweet sorghum cultivars to salt stress and accumulation of Na⁺, Cl⁻ and K⁺ ions in relation to salinity. *Journal of Environmental Biology* 35 (4), 733-739.
- [15] Hussain I., Ashraf M.A., Anwar F., Rasheed R., Niaz M., Wahid A. (2014) Biochemical characterization of maize (*Zea mays* L.) for salt tolerance. *Plant Biosystems* 148 (5), 1016-1026.
- [16] Kausar A., Ashraf M.Y., Niaz M. (2014) Some physiological and genetic determinants of salt tolerance in sorghum (*Sorghum bicolor* (L.) Moench): Biomass production and nitrogen metabolism. *Pakistan Journal of Botany* 46 (2), 515-519.
- [17] Abbasi G.H., Akhtar J., Anwar-ul-Haq M., Malik W., Ali S., Chen Z.H., Zhang G. (2015) Morpho-physiological and micrographic characterization of maize hybrids under NaCl and Cd stress. *Plant Growth Regulation* 75 (1), 115-122.
- [18] Ahmed K., Saqib M., Akhtar J., Ahmad R. (2012) Evaluation and characterization of genetic variation in maize (*Zea mays* L.) for salinity tolerance. *Pakistan Journal of Agricultural Sciences* 49 (4), 521-526.
- [19] Cramer G.R., Alberico G.J., Schmidt C. (1994) Salt tolerance is not associated with the sodium accumulation of two maize hybrids. *Australian Journal of Plant Physiology* 21 (5), 675-692.
- [20] Krizek D.T., Ritchie J.C., Sadeghi A.M., Foy C.D., Rhoden E.G., Davis J.R., Camp M.J. (2003) A four-year study of biomass production of eastern gamagrass grown on an acid compact soil. *Communications in Soil Science and Plant Analysis* 34 (3-4), 457-480.
- [21] Jatmiansky J.R., García M.D., Molina M.C. (2004) Response to chilling of *Zea mays*, *Tripsacum dactyloides* and their hybrid. *Biologia Plantarum* 48 (4), 561-567.
- [22] Blakey C.A., Costich D., Sokolov V., Islam-Faridi M.N. (2007) *Tripsacum* genetics: From observations along a river to molecular genomics. *Maydica* 52 (1), 81-99.
- [23] Molina M.D.C., García M.D., Chorzempa S.E. (2006) Meiotic study of *Zea mays* ssp. *mays* (2n = 40) x *Tripsacum dactyloides* (2n = 72) hybrid and its progeny. *Electronic Journal of Biotechnology* 9 (3), 276-280.
- [24] Kindiger B., Sokolov V., Khatypova I.V. (1996) Evaluation of apomictic reproduction in a set of 39 chromosome maize-*Tripsacum* backcross hybrids. *Crop Science* 36 (5), 1108-1113.
- [25] Shavrukov Y., Sokolov V., Langridge P., Tester M. (2006) Interspecific hybrid, *Zea mays* L. x *Tripsacum dactyloides* L., a new fodder crop with large silage biomass production under abiotic stresses. In: N. Turner, T. Acuna (Eds), *Groundbreaking Stuff. Proceedings of the 13th Australian Agronomy Conference. Australian Society of Agronomy*, Perth. Retrieved from http://www.regional.org.au/au/asa/2006/poster/environment/4578_shavrukovy.htm#TopOfPage.
- [26] Pesqueira J., García M.D., Staltari S., Molina M.D.C. (2006) NaCl effects in *Zea mays* L. x *Tripsacum dactyloides* (L.) L. hybrid calli and plants. *Electronic Journal of Biotechnology* 9 (3), 286-290.
- [27] Shavrukov Y., Langridge P., Tester M. (2009) Salinity tolerance and sodium exclusion in genus *Triticum*. *Breeding Science* 59 (5), 671-678.