

Variability in salt tolerance mechanisms in sunflower

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ABSTRACT

- Salinity has always been considered a serious constraint to agricultural productivity and it is one of the criteria for classifying lands as marginal for agriculture. The purpose of this work was to assess sunflower salt tolerance at the vegetative stage in a group of sunflower lines that had been previously characterized in response to drought. Salt tolerance conceptually results from three concurrent general mechanisms: osmotic tolerance, ion uptake control and compartmentation, and tissue ion tolerance, which were evaluated in this study.
- Eleven non-branching sunflower inbred lines which had been previously characterized for their response to water deficit (Pereyra-Irujo et al., 2008) were used trials 1 and 2, and, from those, four were studied in trial 3: HA64, HAR1, HAR2 and HAR5. Experiments were carried out in the greenhouse or growth room, plants were grown in pots with sand and perlite irrigated with salinized (0.65 MPa) nutrient solution. Na⁺ and K⁺ concentrations, whole plant growth and leaf expansion kinetics were assessed. A Principal Components Analysis (PCA) was run on relative-to-control growth data to get a summary of the effects of salinity and establish a relative tolerance ranking. Osmotic tolerance was evaluated as the initial effects of the salt treatment on plant elongation, ion uptake control was assessed by determining Na⁺ concentration distribution among different organs, and tissue Na⁺ tolerance was deduced by comparing the percentage of dead leaves as a function of leaf blade Na⁺ accumulation.
- Lines HA64 and HAR2 were more tolerant to osmotic stress than the others, while lines HAR1 and HAR5 were the least tolerant. None of the lines showed leaf Na⁺ exclusion, only different degrees of Na⁺ accumulation: it was very low in HAR2, high in HAR1 and intermediate in the other two lines. Tissue Na⁺ tolerance was higher in HAR1 than in the rest. The analysis of leaf expansion kinetics indicated that, aside from expected decreased growth rates, growth duration tended to increase in several leaves of salt treated plants.
- A tolerance ranking based on relative growth responses indicated HAR2>HAR1=HA64>HAR5. Osmotic tolerance was observed in lines HA64 and HAR2, and tissue Na⁺ tolerance, in HAR1. The increase in leaf expansion duration could partially buffer the negative effects of salt stress on leaf area expansion. No association was apparent between these effects and leaf blade Na⁺ accumulation.
- The information derived from this study identified two salt tolerance mechanisms in four sunflower lines, which, if combined, could lead to genotypes with increased salt tolerance.

Keywords: Leaf growth - Na⁺ - osmotic tolerance - salt tolerance - sunflower

INTRODUCTION

Salinity affects about 800 million ha of soils and the surface is growing with the expansion of irrigation (Flowers and Yeo, 1995). Most crop plants are sensitive to this condition (Maas and Hoffman, 1977) and, thus, salinity has always been considered a serious constraint to agricultural productivity which, in turn, generates demands for stress tolerant plant genotypes.

Sunflower has been rated as moderately salt tolerant (Andrade et al., 1993; Katerji et al., 2000). Genetic variability for salt tolerance has been reported in this crop (Ashraf and Tufail, 1995), thus, sources of tolerance can be detected within it.

Salt tolerance conceptually results from three concurrent general mechanisms: osmotic tolerance, ion uptake control and compartmentation and tissue ion tolerance (Munns and Tester, 2008; Rajendran et al., 2009). In sunflower, tolerant accessions have been characterized by lower concentrations of Cl^- and higher K^+ in the leaves under saline conditions, and the maintenance of a relatively high leaf $\text{K}^+:\text{Na}^+$ ratio (Ashraf and Tufail, 1995).

Crop yield over a period of time is directly related to leaf area development, photosynthesis rate and assimilate partitioning (Hay and Porter, 2006). Variability in leaf area development and leaf expansion in response to drought was characterized in several sunflower lines by Pereyra-Irujo et al., (2008). The purpose of this work was to assess the response to salinity in vegetative plants of those lines, and determine its association with the three conceptual components of salt tolerance. In addition, the effects of salinity on leaf growth dynamics were assessed to determine whether salt stress exerted specific effects on lines with different Na^+ accumulation.

MATERIALS AND METHODS

Plant culture and growth conditions

Eleven non-branching sunflower inbred lines which had been previously characterized for their response to water deficit (Pereyra-Irujo et al., 2008) were used in two successive trials, and, from those, four were studied in trial 3. Seeds were germinated on moist tissue paper at 28°C in the dark and seedlings were transferred to 3 L pots containing a mixture of sand and perlite (3:1, V:V), and irrigated with half-strength Hoagland solution (Hoagland and Arnon, 1950). Salinization was initiated as assessed from apex dissections. Gradual salinization began when the first leaf reached 1 cm long (corresponded to approximately 80 °Cd from the initiation of leaf 8) and was provided by daily increments of 35 mM NaCl in the nutrient solution until the final concentration of 150 mM NaCl was reached, similar to the -0.65 MPa used by Pereyra-Irujo et al., (2008) to characterize the lines for drought tolerance.

Thermal time (°Cd) from seedling emergence was calculated as the daily integral of the difference between temperature and base temperature 4.8°C (Granier and Tardieu, 1998). Average temperatures during the day were $25.5 \pm 1^\circ\text{C}$ and $19 \pm 1.1^\circ\text{C}$ at night, and mean irradiance was $14.52 \pm 0.44 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$.

Na^+ and K^+ concentration

Na^+ and K^+ were determined by flame photometry in samples obtained from fully expanded leaf 7 blades and petioles, and first internodes and hypocotyls.

Growth measurement

Plant height was measured and plant relative elongation rates (PRERs) were calculated as the slopes of regression lines of the logarithm of height against °Cd.

The comparison of slopes in control and salinized plants estimated the response to the osmotic component of salinity (Rajendran et al., 2009). An additional measure of sensitivity to the treatment was assessed as the earliest °Cd at which significant height differences could be detected between control and salinized plants. Fresh (FW) and dry weight (DW) of plant parts were recorded at harvest (918 °Cd from emergence), whole plant and individual leaf areas (LA) were measured with a LiCor™ area meter, and the proportion of dead leaves was calculated.

Leaf appearance rates and leaf growth

Leaf area (LA) calculated as $0.65 \cdot (\text{width} \times \text{length})$. Leaf absolute expansion rate (LER) was calculated as the slope of the relationship between LA and thermal time (°Cd), and leaf relative expansion rate (LRER) as the slope of the relationship between the logarithm of LA and thermal time $d(\ln\text{LA})/d^\circ\text{Cd}$, as described by Pereyra-Irujo et al. (2008). The leaf growth profile was fitted to a sigmoid curve as described by Aguirrezábal et al., (2006).

Relative salt tolerance

First, it was determined whether growth variables per line were affected above or below the average effect in all four lines. To that aim, the following ratio was calculated: $\text{VL}/\text{AVL}_{1..n}$, where V is the percentage of control value for a given growth variable for line L; $\text{AVL}_{1..n}$ is the average for that variable,

across all lines. Growth variables were leaf dry weight (LDW), shoot dry weight (SDW), total plant dry weight (TDW), leaf number (LN), total leaf area (LA) and the ratio between dead leaves dry weight and total leaf dry weight (DLW/LDW). The ratios obtained for all variables were then added per line, to give a relative ranking of the lines under stress.

Statistical analysis were performed with (InfoStat., 2009). Fisher's least significant differences (LSD) test was run for comparisons of means after ANOVA.

RESULTS AND DISCUSSION

Na⁺ and K⁺ accumulation

All lines accumulated Na⁺ throughout the plant (Figure 1), but leaf blade Na⁺ concentrations were significantly higher in lines HAR1.

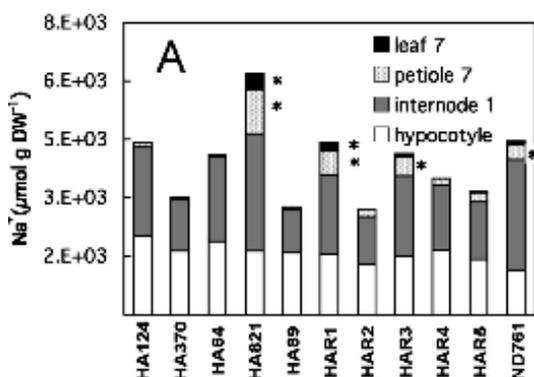


Figure 1: Na⁺ concentration in 11 sunflower lines treated with 150 mM NaCl. Asterisks next to blades and petioles indicate lines that had significantly higher Na⁺ concentration than the rest, in those organs. n= 3.

Short-term effects of salinity. The osmotic component of salt tolerance.

Initial responses to salinity, when internal Na⁺ accumulation is still low, are attributed mainly to the osmotic component of salinity (Rajendran et al., 2009). Initial PRERs were differentially affected by salinity in the 11 lines in trial 1. PRERs were not changed in response to the salt treatment in HA124, HA64, HAR2 and HA89, and they decreased in all the rest. Susceptibility differences among the lines to decreased substrate water potential may be associated to osmotic adjustment, required to insure water uptake from a substrate with reduced water potential (Greenway and Munns, 1980). Variability in osmotic adjustment exists in sunflower (Chimenti and Hall, 1993) and has been shown to be positively associated to yield under drought (Chimenti et al., 2002).

Lines HAR1, HAR2, HA64 and HAR5 were selected for further studies. HAR2 and HA64 are parental lines incorporated in subsequent drought tolerance studies. HAR2 always exhibits low leaf Na⁺ concentrations, followed by HA64 and HAR5 (Figure 1), while HAR1 had high leaf Na⁺ concentration. This conformed a study group with a range of leaf Na⁺ concentrations, that included lines that were more (HAR1, HAR5) and less (HA64, HAR2) sensitive at the initial salinization stage.

Tissue Na⁺ tolerance

In wheat (Munns et al., 2006) and in tomato (Estañ et al., 2004) mechanisms that participate in the control of ion concentration among organs are linked to salt tolerance and exclusion of Na⁺ from the leaf blade was related to salt tolerance in sunflower (Ashraf and O'Leary, 1995). To check whether tolerance was related to Na⁺ exclusion in these lines, we plotted the proportion of dead leaves as a function of Na⁺ concentration in a representative leaf (12) (Figure 2). The lowest proportion of dead leaves was observed in HAR2, the line that had the lowest Na⁺ concentration in leaf blades. HAR1, HAR5 and HA64 had similar leaf Na⁺ concentrations, but the proportion of dead leaves was lower in HAR1, suggesting a higher degree of tissue Na⁺ tolerance in this line. Compartmentation of Na⁺ into vacuoles is a general mechanism for avoiding cytosolic Na⁺ toxicity (Flowers and Läuchli, 1983), which involves high metabolic costs in membrane transport mechanisms and intracellular osmotic balance provided by organic solutes (Tester and Davenport, 2003). Preliminary experiments (Senn, unpublished) suggest Na⁺ vacuolization in HAR1, and the contribution of this mechanism to the tolerance of this line should be experimentally tested.

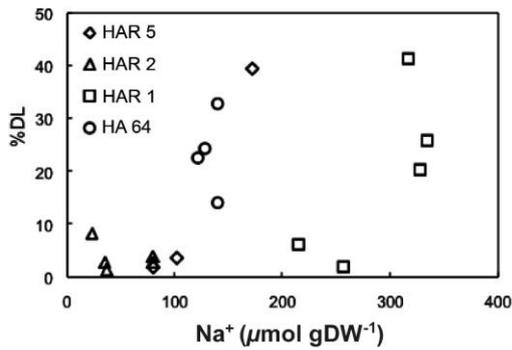


Figure 2. Proportion of dead leaves (% DL) as a function of Na^+ concentration (in leaf 12) in four sunflower lines grown under saline conditions (130 mM NaCl).

Salt tolerance in these lines and associated physiological mechanisms

A Principal Components Analysis (PCA) was run on relative-to-control growth data from the four lines included in trial three, to get a summary of the effects of salinity. All variables included in the principal components analysis contributed with almost equal weight to differentiate among cultivars, and were used to calculate a relative tolerance ranking, which indicated $\text{HAR2} > \text{HAR1} = \text{HA64} > \text{HAR5}$.

HAR2 coincidentally, it was also the one with the lowest leaf Na^+ concentration, therefore, it is expected to express a high degree of osmotic tolerance as it achieved relatively high growth under salinity without incorporating Na^+ as an energetically cheap osmotic agent (Flowers and Läuchli, 1983). This agrees with the lower susceptibility to the initial, osmotic effects of salinity, observed in this line.

The second mechanism evaluated was leaf Na^+ buildup control. None of the lines showed leaf Na^+ exclusion, only different degrees of Na^+ accumulation. Buildup of high leaf Na^+ concentration was prevented in HAR2, HAR1 showed high leaf Na^+ values and the other two lines had intermediate Na^+ values.

Tissue Na^+ tolerance was the third mechanism evaluated, it was found to be higher in HAR1 than in the rest. It may be related to intracellular ion compartmentation in vacuoles.

Leaf growth

In salt-treated plants of all the lines, reduced leaf area was observed and leaf expansion duration tended to be prolonged (Figure 3). No association was apparent between these effects and leaf blade Na^+ accumulation. In water stressed sunflower plants, Pereyra-Irujo et al. (2008) linked prolonged leaf expansion to the increase in the duration of cell division. It is not known whether the same cause leads to leaf expansion prolongation under salinity.

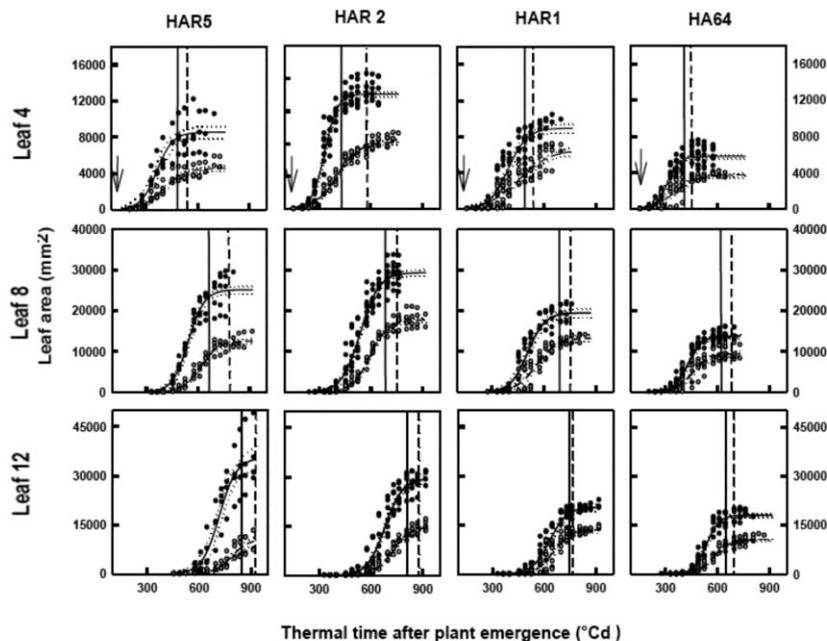


Figure 3. Expansion of leaves 4, 8 and 12 in four sunflower lines grown under control or saline (130 mM NaCl) conditions. Dots are individual leaves and solid lines are three parameter sigmoidal fits, dotted lines indicate 95% confidence intervals. Solid vertical lines indicate the end of expansion in controls and dashed ones in salinized plants. Arrows indicate the initiation of the salt treatment. $n=5-9$ plants from each sunflower line and growth condition.

An increase in leaf expansion duration can partially buffer the negative effects of stress on leaf area (Takami et al., 1981, Pereyra-Irujo et al., 2008). The contribution of the observed expansion time increases to counter-balance the negative effects of reduced expansion rates on final leaf areas was estimated to be approximately 10% in HAR2 and HAR5, half as much in HAR1 and even lower in HA64. Effects of water and salt stress on leaf expansion prolongation have often been reported in the literature (Aguirrezábal et al., 2006; Ortega and Taleisnik, 2003). It is likely that a common response to various stresses may have a common physiological basis, and it is interesting to speculate that the observed intraspecific variability for this response must have a genetic basis.

ACKNOWLEDGEMENTS

GC holds a CONICET fellowship and this work is part of his doctorate. Seeds for these trials were provided by Luis N. Aguirrezábal and Gustavo Pereyra Irujo, whose assessment in leaf growth analysis and critical comments of results are also gratefully acknowledged. The assistance of Paola Suárez and Matías Camisassa is thankfully appreciated. Financial support was from ANPCYT (PID 0066 and PICT 2007 00498) and INTA (PNOLE 031052).

REFERENCES

- Aguirrezábal, L., Bouchier-Combaud, S., Radziejwoski, A., Dauzat, M., Cookson, S.J. and C. Granier. 2006. Plasticity to soil water deficit in *Arabidopsis thaliana*: dissection of leaf development into underlying growth dynamic and cellular variables reveals invisible phenotypes. *Plant Cell Environ.* 29, 2216-2227.
- Andrade, A., Wolfe, D.W. and E. Fereres. 1993. Leaf expansion, photosynthesis, and water relations of sunflower plants grown on compacted soil. *Plant Soil* 149, 175-184.
- Ashraf, M. and J.W. O'Leary. 1995. Distribution of cations in leaves of salt-tolerant and salt-sensitive lines of sunflower under saline conditions. *J. Plant Nutr.* 18, 2379-2388.
- Ashraf, M. and M. Tufail. 1995. Variation in salinity tolerance in sunflower (*Helianthus annuus L.*). *J. Agron. Crop Sci.* 174, 351-362.
- Chimenti, C.A. and A.J. Hall. 1993. Genetic variation and changes with ontogeny of osmotic adjustment in sunflower (*Helianthus annuus L.*). *Euphytica* 71, 201-210.
- Chimenti, C.A., Pearson, J. and A.J. Hall. 2002. Osmotic adjustment and yield maintenance under drought in sunflower. *Field Crop Res.* 75, 235-246.
- Estañ, M.T., Martínez-Rodríguez, M.M., Pérez-Alfocea, F., Flowers, T.J. and M.C. Bolarin. 2004. Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. *J. Exp. Bot.* 56 703-712.
- Flowers, T.J. and A. Läuchli. 1983. Encyclopedia of Plant Physiology, New Series, in Läuchli, A., Bieleski, R.L. Springer Verlag, New York, pp. 651-681.
- Flowers, T.J. and A.R. Yeo. 1995. Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.* 22, 875-884.
- Granier, C. and F. Tardieu. 1998. Spatial and temporal analyses of expansion and cell cycle in sunflower leaves. A common pattern of development for all zones of a leaf and different leaves of a plant. *Plant Physiol.* 116, 991-1001.
- Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* 31, 149-90.
- Hay, R. and J. Porter. 2006. The physiology of crop yield. Second ed. Blackwell Publisher, Singapore.

- Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. U. Calif. Agric. Exp. Stn. Circ.347 pp 1-32.
- InfoStat., Grupo InfoStat, 2009. Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba. Córdoba, Argentina.
- Katerji, N., van Hoorn, J.W., Hamdy, A. and M. Mastrorilli. 2000. Salt tolerance classification of crops according to soil salinity and to water stress day index. *Agricult. Water Manag.* 43, 99-109.
- Maas, E.V. and G.J. Hoffman. 1977. Crop salt tolerance. Current assessment. *J. Irr. Drain. Div.-ASCE* 102, 115-134.
- Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 59, 651-81.
- Munns, R., James, R.A. and A. Lauchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57, 1025-1043.
- Ortega, L. and E. Taleisnik. 2003. Elongation growth in leaf blades of *Chloris gayana* under saline conditions. *J. Plant Physiol.* 167, 517-522.
- Pereyra-Irujo, G.A., Velazquez, L., Lechner, L. and L.A.N Aguirrezábal. 2008. Genetic variability for leaf growth rate and duration under water deficit in sunflower: analysis of responses at cell, organ, and plant level. *J. Exp. Bot.* 59, 2221-2232.
- Rajendran, K., Tester, M. and S.J. Roy. 2009. Quantifying the three main components of salinity tolerance in cereals. *Plant Cell Environ.* 32, 237-249.
- Takami, S., Turner, N.C. and H.M. Rawson. 1981. Leaf expansion of four sunflower (*Helianthus annuus* L) cultivars in relation to water deficits. I. Patterns during plant development. *Plant Cell Environ.* 4, 399-407.
- Tester, M. and R. Davenport. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.* 91, 503-527.