Published July 1990

Salinity Tolerance in F_1 Hybrids of Pigeonpea and a Tolerant Wild Relative

G. V. Subbarao, C. Johansen,* J. V. D. K. Kumar Rao, and M. K. Jana

ABSTRACT

Salinity in soil or water presents a stress condition to crop plants that is of increasing importance in the sustainability and stability of crop production in the semiarid regions of the world. This study determined the potential for genetic introgression of salinity tolerance from a wild relative, *Atylosia albicans* (W. & A.) Benth., to cultivated pigeonpea, *Cajanus cajan* (L.) Millse. A sand culture system was used to grow the salt-tolerant *A. albicans*, salt-sensitive *C. cajan*, and their F_1 hybrids at various precisely maintained salinity levels. The results demonstrated the feasibility of transferring salinity tolerance from *A. albicans* to *C. cajan*. The high level of salinity tolerance in *A. albicans* was expressed as a dominant genetic factor in both the reciprocal F_1 hybrids of this species and *C. cajan*. The physiological attributes conferring salinity tolerance in *A. albicans* and the F_1 hybrids include Na and Cl retention in the roots and limited translocation to the shoots, high K selectivity, and maintenance of transpiration rate under saline conditions.

SOIL SALINITY in arid and semiarid regions of the world is a major detrimental factor for crop production (Epstein, 1978). To achieve an integrated approach toward economic utilization of saline soils, the traditional approach of drainage and reclamation should be supplemented with genetic improvements in salinity tolerance of crop plants (Epstein and Rains, 1987). Little is known about the genetic control of the physiological mechanisms involved in salinity tolerance; however, this knowledge is essential for planning an efficient breeding strategy for genetic improvement of salinity tolerance in crop plants (Tal, 1985).

Salinity tolerance is considered a complex trait (Ramage, 1980; Woolhouse, 1981), but much of this complexity is due to lack of knowledge, which needs to be resolved by coordinated physiological genetic research (Tal, 1985). After screening 14 wild relatives (belonging to *Atylosia, Dunbaria,* and *Rynchosia*) and 150 cultivated genotypes of pigeonpea, Subbarao (1988)

G.V. Subbarao, C. Johansen, and J.V.D.K. Kumar Rao, Crop Physiology Unit, Legumes Program, ICRISAT, Patancheru, A.P. 502 324, India; M.K. Jana, Dep. of Agric. Engineering, Indian Inst. of Technology, Kharagpur, India. Joint contribution from International Crops Res. Inst. for the Semi-Arid Tropics (ICRISAT) and Indian Inst. of Technology. Submitted as Journal Article no. 926 by the ICRISAT. Received 7 July 1989. *Corresponding author.

Published in Crop Sci. 30:785–788 (1990).

showed that *A. albicans* is one of the most salt-tolerant wild relatives of pigeonpea. Fortunately, there is no incompatibility barrier for hybridization of *A. albicans* and the cultivated pigeonpea (van der Maesen, 1986, p. 18). The present investigation was aimed at understanding the nature of inheritance of salinity tolerance in pigeonpea.

MATERIALS AND METHODS

Seed of *A. albicans*, *C. cajan* (ICP 3783), and F_1 hybrids of *A. albicans* × *C. cajan* (reciprocal crosses) were obtained from the Genetic Resources Unit, ICRISAT, India. Seed of *A. albicans* and F_1 hybrids required scarification, to ensure germination. Seeds were surface sterilized with 2 g L^{-1} $HgCl_2$ solution for 5 min and then thoroughly washed with deionized water. They were then germinated in moistened blotting paper rolls placed in an incubator at 28 °C. The seedlings were transplanted into 180-mm-diam. plastic pots filled with washed and sterilized river sand. The sand surface in each pot was covered with 50 g of polythene beads, to minimize evaporation. Pots were arranged in four randomized blocks on bench tops in a glasshouse. Temperatures during the experiment averaged 28/22 °C (day/night) and relative humidity was 60 to 70% (mean day + night).

A modified Arnon and Hoagland nutrient solution (0.25 strength) with 1.79 m*M* NH_4NO_3 amended with NaCl + $CaCl_2$ (1:1 w/w) was used for the five different salinity treatments (electrolytic conductivity of 0, 6, 8, 10 and 12 dS m^{-1}). The composition of the 0.25-strength nutrient solution in m*M* was: 0.23 KH_2PO_4, 0.52 KCl, 0.25 $MgSO_4$, 0.37 $CaCl_2$, 0.0015 $MnSO_4$, 0.00023 $ZnSO_4$, 0.00025 $CuSO_4$, 0.001 H_3BO_3, 0.00005 Na_2MoO_4, and 0.04 NaFeEDTA. The electrolytic conductivity of the nutrient solution (0.25-strength) without salt treatment was 0.33 dS m^{-1}. Plants were given deionized water up to 13 d after transplanting (DAT). On DAT 14, initial salt treatments were imposed by flushing each pot with 1 L of treatment solution. Thereafter, pots were flushed with treatment solutions (0.25 L pot^{-1}) once in 4 d to avoid salt buildup. For the 0 salinity treatment, 0.25-strength nutrient solution was used for all flushing operations. At the end of every day, the evapotranspirational water losses were adjusted by adding deionized water after weighing the pots. Pots were randomized every 4 d, to minimize spatial effects in the greenhouse. Transpiration rate from the first fully expanded trifoliate leaf was recorded between 1100 and 1200 h at 24 and 54 DAT using a LI-1600 steady state porometer (LI-COR, Inc., Lincoln, NB).

Plants were harvested at 75 DAT. Fallen leaves were collected and included for dry mass measurement and chemical analysis. Roots were carefully washed from pots in tap water. Plant tops and roots were dried at 70 °C for 48 h and dry mass was determined. Leaf, stem, and root samples were finely ground by a cyclone mill (UDY Corporation, Ft. Collins, CO) for chemical analyses. For determination of Na and K, finely ground plant samples of 200 to 300 mg were digested with 6 mL of tri-acid ($HNO_3/H_2SO_4/HClO_4$ at 10:0.5:2) in a sand bath at 250 °C for 6 to 8 h (Piper, 1952). Digested plant samples were diluted and analyzed for various elements by atomic absorption spectrophotometry. Chloride concentration in the plant samples was determined by Mohr's volumetric method (Blaedel and Meloche, 1960).

RESULTS AND DISCUSSION

There were considerable differences in the salinity tolerance of cultivated pigeonpea and the wild type, *A. albicans*. While the *Atylosia* could grow at 12 dS m^{-1}, symptoms of salt burning were visible in the cultivated pigeonpea at 6 dS m^{-1}, and at 8 dS m^{-1} growth was severely retarded and several plants began to wilt. At 10 and 12 dS m^{-1}, all ICP 3783 plants died. Both F_1 hybrids were able to grow at $\leq$ 12 dS m^{-1} without salt toxicity symptoms, although growth was reduced. The F_1 hybrids were morphologically intermediate between both parents. The leaf shape was similar to that of *A. albicans* and the growth habit was intermediate, neither twining like *A. albicans* nor erect like pigeonpea. Pigeonpea growth rate was greater than that of *A. albicans*, while the growth rate of F_1 hybrids was intermediate, as reflected in the total dry matter of the control treatment (Fig. 1).

Shoot dry matter decreased with increasing salinity in both parents and both F_1 hybrids (Fig. 1); however, the relative reduction was far greater in ICP 3783 than in *A. albicans* or the F_1 hybrids. The growth response of the F_1 hybrids was similar to that of *A. albicans*, and there were no significant differences between *A. albicans* and either of the F_1 hybrids at any of the salinity levels, indicating that the tolerance trait was due to a dominant gene or genes. There were no significant differences in growth between the F_1 reciprocals at any of the salinity levels, suggesting the absence of cytoplasmic-related factors. In other species also, salinity tolerance has been attributed to dominant genetic factors (Abel, 1969; Akbar and Yabuno, 1975; Venables and Wilkins, 1978; Tal and Shannon, 1983; Dvorak and Ross, 1986).

The increase in shoot (leaf and stem) Na was about 30 to 40 times higher in ICP 3783 at $\geq$8 dS m^{-1}, compared with *A. albicans* or the F_1 hybrids, which did not show a significant increase in the shoot Na with increasing salinity (Fig. 2). An increase in root Na was measured for both parents and F_1 hybrids under saline conditions. In ICP 3783, the increase in root Na occurred at 6 dS m^{-1}, followed by a significant decline at $\geq$8 dS m^{-1}. In *A. albicans*, root Na levels increased up to 8 dS m^{-1}, with no further increase at

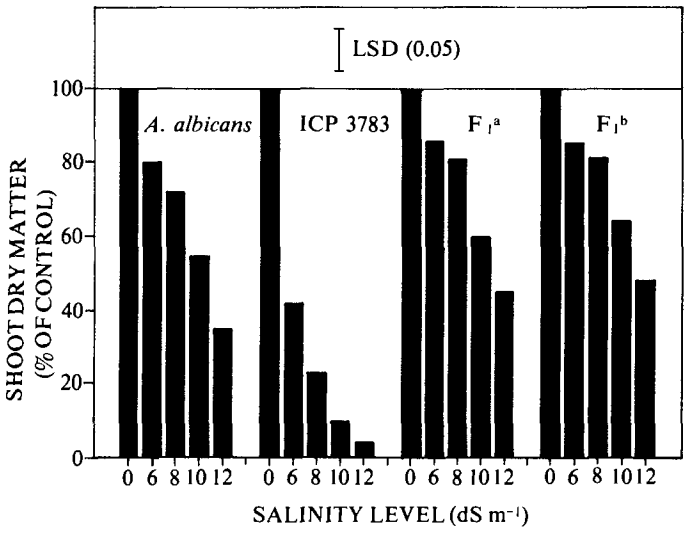

Fig. 1. Effect of salinity on growth of *A. albicans*, *C. cajan* (ICP 3783), and their reciprocal F_1 hybrids to 75 d after transplanting. Hybrid a = ♂ *A. albicans* × ♀ ICP 3783; Hybrid b = ♂ ICP 3783 × ♀ *A. albicans*. The 100% shoot dry matter values were 2.79, 8.04, 3.78, and 3.74 g $plant^{-1}$ for the respective entries. Data are means of four replications.

10 or 12 dS m^{-1}. In the F_1 hybrids, root Na increased with increasing salinity up to 12 dS m^{-1}. However, the differences among *A. albicans* and the F_1 hybrids in their root Na levels were not significant at any of the salinity levels. These results show that *A. albicans* regulates Na movement efficiently by retaining large quantities in the root and allowing a negligible amount to move into the shoot. This is in contrast to ICP 3783, where large quantities of Na accumulate in the shoot.

A. albicans was also able to maintain higher levels of K in leaf, stem, and root at salinity levels up to 12 dS m^{-1} (Fig. 3). Potassium levels in leaf, stem, and root of ICP 3783 declined at $\geq$8 dS m^{-1}, indicating inability to maintain K/Na selectivity at high salinity levels. In the F_1 hybrids, leaf, stem, and root K levels increased with increasing salinity. Thus, the efficient K-uptake ability under saline conditions is a heritable trait.

Leaf and stem Cl levels increased with increasing salinity in both parents and F_1 hybrids (Fig. 4). However, the increase in leaf Cl was very high in ICP 3783 (from 1.5 g kg^{-1} dry wt. in the control to 78.5 g kg^{-1} dry wt. at 12 dS m^{-1}) as compared with *A. albicans* (from 1.3 g kg^{-1} dry wt. in the control to 26.0 g kg^{-1} dry wt. at 12 dS m^{-1}). There were no significant differences among *A. albicans* and F_1 hybrids in leaf and stem Cl concentrations at different salinity levels. Further, in *A. albicans* and the F_1 hybrids, root Cl levels increased considerably with salinity up to 12 dS m^{-1}, unlike in ICP 3783, where this increase was only up to 6 dS m^{-1} and was followed by a decline at $\geq$8 dS m^{-1}. Washing roots with tap water may have contributed to the decline in root concentrations of Cl, as well as Na and K; however, the marked corresponding increases in shoot Na and Cl concentrations suggest that a breakdown in selectivity of long-distance transport was a contributing factor. The higher Cl retention in roots was similar in *A. albicans* and the F_1 hybrids. It is clear that *A. albicans* is able to regulate Cl movement into the plant system by effectively retaining Cl in the root. The sensitive pigeonpea, on the other hand, permits large quantities of Cl to move into the shoot system at high salinity levels.

Although we did not measure fresh weights, we have previously noted that dry-matter content of tissue increases with increasing salinity damage. Thus, if element concentrations were to be expressed on a tissue-water basis, the effects referred to above would be further exaggerated. There was considerable abscission of dead, dried leaves at high salinity levels in ICP 3783 but not in *A. albicans* or the hybrids. Abscised leaves were included for dry weight determination and chemical analysis.

Transpiration in the salt-sensitive ICP 3783 was markedly reduced by high salinity levels (Fig. 5). By

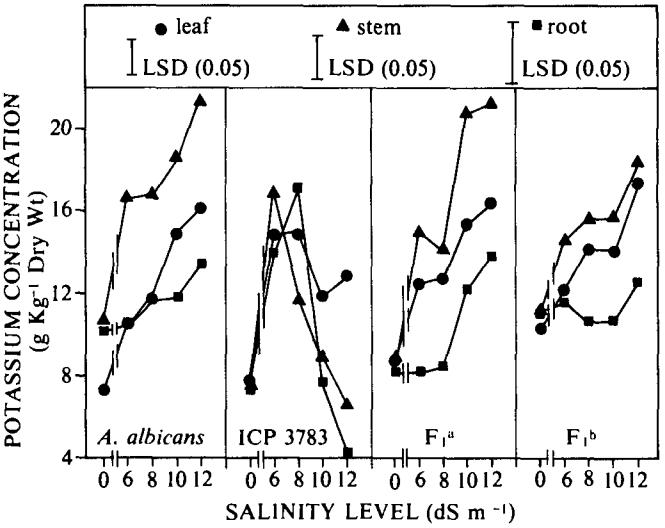

Fig. 3. Effect of salinity on tissue K concentration (g kg^{-1} dry weight) of *A. albicans*, *C. cajan* (ICP 3783), and their reciprocal F_1 hybrids (a and b), 75 d after transplanting. Data are means of two replications.

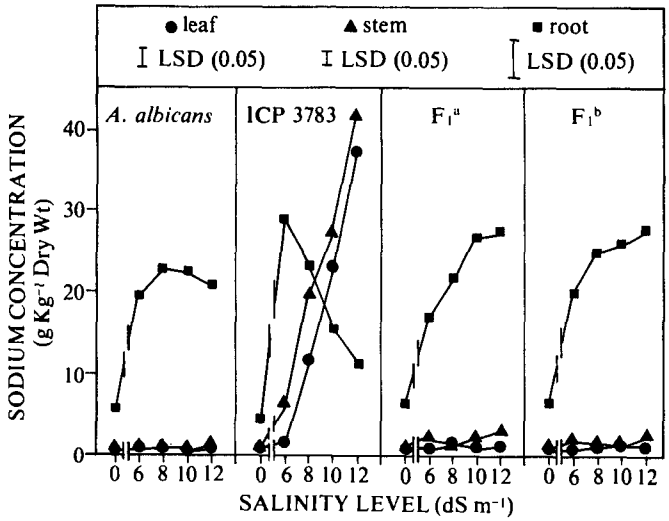

Fig. 2. Effect of salinity on tissue Na concentration (g kg^{-1} dry wt.) of *A. albicans*, *C. cajan* (ICP 3783), and their reciprocal F_1 hybrids (a and b), 75 d after transplanting. Data are means of two replications.

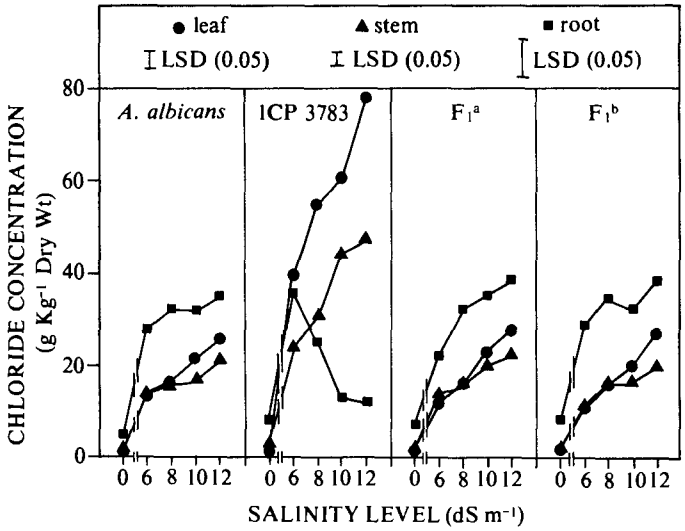

Fig. 4. Effect of salinity on tissue Cl concentration (g kg^{-1} dry weight) of *A. albicans*, *C. cajan* (ICP 3783), and their reciprocal F_1 hybrids (a and b), 75 d after transplanting. Data are means of two replications.

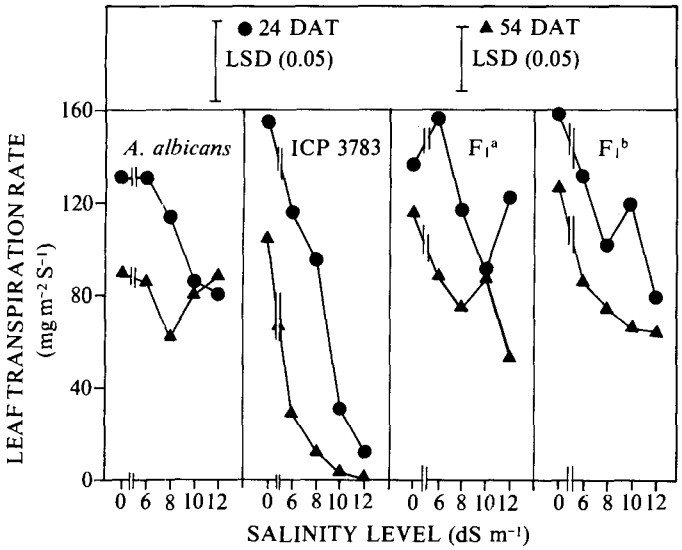

Fig. 5. Effect of salinity on leaf transpiration rate (mg m^{-2} s^{-1}) of *A. albicans, C. cajan* (ICP 3783), and their reciprocal hybrids (a and b), 24 and 54 d after transplanting. Data are means of four replications.

contrast, *A. albicans* and the F_1 hybrids were able to maintain near-normal transpiration rates at $\leq$12 dS m^{-1}. The wild type was able to maintain high transpiration rates, possibly because of the ability of the root system to meet the demands of water required for transpiration under salinity stress, or because of high K uptake facilitating normal stomatal opening. In wild tomato [*Lycopersicon peruvianum* (L.) Mill.], it has been suggested (Tal and Gavish, 1973; Gertal and Tal, 1986) that the low stomatal conductance of wild types at high salinity levels is responsible for high water-use efficiency, one of the attributes of high salinity tolerance; however, this does not appear to be the case for wild relatives of pigeonpea.

The expression of wild-type physiological traits, such as efficient Na and Cl regulation, high K absorption capacity, and maintenance of stomatal conductance during saline conditions, in the F_1 hybrids shows that these physiological traits are heritable and controlled by a dominant gene or genes. Further studies on the segregating F_2 and F_3 generations, including the analysis of the ionic constituents, can establish the inheritance pattern of these physiological traits. If this trait is shown to be controlled by a limited number of genes, it seems feasible to improve salinity tolerance in pigeonpea by a simple backcrossing procedure.

ACKNOWLEDGMENTS

The authors thank Drs. F.B. Lopez and D.L. Oswalt, ICRISAT, for their suggestions on the manuscript.

REFERENCES

Abel, G.H. 1969. Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans. Crop Sci. 9:697–698.

Akbar, M., and T. Yabuno. 1975. Breeding for saline resistant varieties of rice: III. Response of F_1 hybrids to salinity in reciprocal crosses between Jhona 349 and Magnolia. Jpn. J. Breed. 25:215–220.

Blaedel, W.J., and V.W. Meloche. 1960. Elementary quantitative analysis. Harper & Row, New York.

Dvorak, J., and K. Ross. 1986. Expression of tolerance of Na, K, Mg, Cl and SO_4 ions and sea water in the amphidiploids of *Triticum aestivum* × *Elytrigia elongata*. Crop Sci. 26:658–660.

Epstein, E. 1978. Crop production in arid and semi-arid regions using saline water. A report for the National Science Foundation–Applied Science and Research Applications. Dep. of Land, Air and Water Resources, University of California, Davis.

Epstein, E., and D.W. Rains. 1987. Advances in salt tolerance. Plant Soil 99:17–29.

Gertal, E.T., and M. Tal. 1986. Potassium utilization and fluxes in wild salt-tolerant relatives of the cultivated tomato. Physiol. Plant. 67:415–420.

Piper, C.S. 1952. Soil and plant analysis. Hans Publ., Bombay, India.

Ramage, R.T. 1980. Genetic methods to breed salt tolerance in plants. p. 311–318. *In* D.W. Rains et al. (ed.) Genetic engineering of osmoregulation: Impact on plant productivity for food, chemicals and energy. Plenum Press, New York.

Subbarao, G.V. 1988. Salinity tolerance in pigeonpea (*Cajanus cajan*) and its wild relatives. Ph.D. diss., Indian Inst. of Technology, Kharagpur, India.

Tal, M. 1985. Genetics of salt tolerance in higher plants: Theoretical and practical considerations. Plant Soil 89:199–226.

Tal, M., and U. Gavish. 1973. Salt tolerance in the wild relatives of the cultivated tomato: Water balance and abscisic acid in *Lycopersicon esculentum* and *L. peruvianum* under low and high salinity. Aust. J. Agric. Res. 24:353–361.

Tal, M., and M.C. Shannon. 1983. Salt tolerance in the wild relatives of the cultivated tomato: Responses of *Lycopersicon esculentum, L. cheesmanii, L. peruvianum, Solanum pennellii* and F_1 hybrids to high salinity. Aust. J. Plant Physiol. 10:109–117.

van der Maesen, L.J.G. 1986. *Cajanus* DC. and *Atylosia* W. & A. (Leguminosae). Agric. Univ. Wageningen Pap. 85-4.

Venables, A.V., and D.A. Wilkins. 1978. Salt tolerance in pasture grasses. New Phytol. 80:613–622.

Woolhouse, H.W. 1981. Crop physiology in relation to agricultural production: The genetic link. p. 1–21. *In* C.B. Johnson (ed.) Physiological processes limiting plant productivity. Butterworths, London.