Fifth International Casuarina Workshop
Mamallapuram, Chennai, India, 03-07 February 2014

Screening of *Casuarina equisetifolia* Clones for Tolerance to Salinity and Identification of a Biochemical Marker

R.S.C. Jayaraj
Institute of Forest Genetics and Tree Breeding
Coimbatore 641 002, Tamil Nadu, India
**Email : rsc.jayaraj@gmail.com*

Abstract

Genetic improvement of a species can be carried out for three purposes- firstly for use in productive lands under normal plantation practices, secondly for use in marginal lands and thirdly for special uses, viz., pulpwood, fuelwood, timber, etc. Most of the genetic improvement programmes have concentrated only on the purpose first stated, so far. The second purpose is gaining importance due to emphasis on environmental amelioration and progress of forest plantations into degraded and marginal lands hitherto unused. Selection of *Casuarina equisetifolia*, for the purpose of afforestation of problem soils and marginal lands was carried out at the Institute of Forest Genetics and Tree Breeding, by Point Grading method, at a selection intensity of 1 in 10000, from 8- 10 year old plantations raised on stressed sites, under conditions of drought, salinity, disease and pests. After assessing 180 trees, 51 were selected. Of these only 45 could be rooted and these were assembled in a clone bank at the Institute (Jayaraj et al., 2001).

Studies have been made on the tolerance of Casuarinas to salinity and alkalinity besides other physico-chemical stresses. Several experiments have been conducted to rank the Casuarinas in terms of their tolerance to salinity, and a wide variation is reported among the species, in the following order of decreasing salinity tolerance : *Casuarina obesa* > *C. glauca* > *C. equisetifolia* > *C. cunninghaminana* > *C. cristata* (Clemens et al., 1983). Significant provenance variation has also been reported within the species, e.g., in *C. glauca* (Girgis et al., 1992). It has been suggested that there is high degree of genetic variation in salt tolerance in *Casuarina* and that it is possible to develop salt tolerant cultivars which could be used to reclaim salt damaged forests or to establish plantations with low quality brackish water (Allen et al., 1994).

With this background a study was conducted to screen 20 selected clones of *Casuarina equisetifolia* for their tolerance to salinity and also to identify a biochemical marker for

the tolerance. The screening was done in a glass house in sand culture, using sterilized washed sand as the medium in which the concentration of NaCl was increased at the rate of 0.2% once in 5 days. The concentration was increased from 0 to 2.4% over a period of 60 days. Growth in terms of height was measured with 24 ramets maintained under treatment and 11 ramets as control. Growth rate was estimated for all the ramets and using the average growth rate, the Relative Growth Rate was calculated using the formula RGR=(GRt/ GRc)x100, where GRt is the growth rate of the treatment and GRc is the growth rate of the control. Tolerance Index (TI) was calculated for each clone using the formula TI= RGR x Survival percentage. The clones were ranked on the basis of RGR as well as TI.

The symptoms of salt damage included succulence of the needles, necrosis of needle tips, colouration of the needles due to anthocyanin accumulation and drying of needles. Visual scoring of the symptoms was also done for the clones using the following scores: 1- No symptom; 3- symptoms visible on older leaves but plant is healthy; 5- symptoms visible on all leaves; 7- severe symptoms and 9- plant is dead (Jayaram et al., 2000). The clones were ranked on the basis of the scores also.

In order to identify a biochemical marker for the salinity tolerance, the method used by Dreier (1983 a) was tried. It has been found in crop plants that in stress due to NaCl, there is a concentration of NaCl, above which the proline content strongly rises, called as the "critical point". In salt sensitive plants this "critical point" is low, and the determination of the critical point by measurement of proline concentration could serve as a basis for analysis of salt tolerance. This has been confirmed in 19 cultivars of crop plants (Dreier, 1983b), *Brassica juncea* (Jain et al., 1991) and egg plant (Jain et al., 1987). The selected 20 clones were grown in water culture, and after rooting, they were subjected to different levels of NaCl stress using concentrations ranging from 0 to 350 mM NaCl. The concentration of proline was estimated in the needles and plotted against the concentration of NaCl, to determine the critical point, the concentration of NaCl at which proline starts accumulating rapidly. The critical point determined biochemically was correlated with the salt tolerance determined in terms of RGR, Tolerance Index as well as the Visual score, to determine the validity of the procedure of rapid screening for salinity tolerance in *Casuarina equisetifolia*.

Results: RGR of the 20 clones ranged from 7.39 to 40.88 % and Tolerance Index ranged from 338 to 3666 indicating a high degree of variation, providing scope for selection

and use in plantation and breeding programmes. Mean score for the visual scoring of salt damage symptoms ranged from 3 to 7.4. A comparative evaluation of the three methods of screening showed that 11 clones could be classified as salinity tolerant by having Tolerance Index above average, while 13 could be identified having above average RGR and 10 having visual score above 5. 7 clones were common in the list of tolerant clones identified by all the three methods, indicating the reliability of all the methods. If all the three are used a rigourous screening can be done. In the present study clones CP 3903, CH 3001, TNPP-2, TNRM-4, TNAM-2, TNPP-4 and TNVM-3 were found tolerant of salinity. Clones TNCN-2, TNRM-3 and CP 5007 were found tolerant using Tolerance Index and RGR, but scored poorly in the visual scoring method. The clones that were found either below average in tolerance to salinity in terms of TI and RGR or scoring more than 5 in visual score were treated as intolerant to salinity and they are TNRM-8, TNRM-7, TNMT-6, TNAM-1, TNPV-2, TNPK-3, TNCN-1, TNCN-2, PY-157, PY-U2, CP-4805, CP-5007 and TNRM-3.

The comparison of these clones in terms of the pattern of proline accumulation, at different levels of salinity ranging from 0 to 350 mM NaCl revealed that there was no consistency in the pattern. The "critical point" or the point of NaCl concentration at which there is a spurt in proline accumulation could not be located in many clones and even when located did not correlate with tolerance to salinity. The symptoms of damage due to salinity also varied among the clones, some showing succulence of needles, some showing drying of needles and still some showing discolouration of the older needles which were dropped presumably to eliminate accumulated salts. Therefore, accumulation of proline may not be the only mechanism operating in the species, and the mechanism of tolerance may vary among sub-species and provenances, which will require an in-depth study.

Allen, J.A., J.L. Chambers and M. Stine (1994) Prospects for increasing the salinity tolerance of forest trees: a review, *Tree Physiology* 14(7):843-853.

Clemens, J., L.C. Campbell and S. Nurisjah (1983) Germination, growth and mineral ion concentration of *Casuarina* species under saline conditions. *Australian Journal of Botany* 31:1-9.

Dreier, W. (1983 a) La teneur en proline et la rèsistance des plantes aux sels, *Biologia Plantarum* 25(2):81-87.

Dreier, W. (1983b) Vèrification et utilisation d'un test èlabore de prèselection des plantes tolèrantes aus sels, a partir de la teneur en proline, *Biologia Plantarum* 25(2):88-94.

Girgis, M., Y. Ishac, H. Diem and Y. Dommergues (1992) Selection of salt tolerant *Casuarina glauca* and *Frankia. Acta Oecologica* 13:443-451.

Jain, R.K., R.S. Dhawan, D.R. Sharma and J.B. Chowdhury (1987) Salt tolerance and proline accumulation: a comparative study in salt tolerant and wild type cultured cells of egg plant, *Plant Cell Reports* 6:382-384.

Jain, S., H.S. Nainawatee, R.K. Jain and J.B. Chowdhury (1991) Proline status of genetically stable salt tolerant *Brassica juncea* somaclones and their parent cv. Prakash, *Plant Cell Reports* 9:684-687.

Jayaraj, R.S.C., A. Nicodemus, A. Balu, K.R.Sasidharan, M. Maria Dominic Savio, V.K.W. Bachpai, G. Ponraj, R. Hegde and R. Kamalakannan (2001) Selection programme of *Casuarina equisetifolia* for afforestation of problem soils of Tamil Nadu, In: Satyanarayana Rao, K., Surekha G. Sawant and Pankaj K. Aggarwal (Eds.)., Forestry, Forest Products and Coastal Population, Institute of Wood Science and Technology, pp. 59-67.

Jayaram, S., M. Ganapathy and G. Kuppuswami (2000) Problem and prospects to screen and identify multipurpose tree species to salinity, *Indian Journal of Forestry*, 23(4):357-359.