EFFECTS OF SALT STRESS ON ROOTING OF *CASUARINA EQUISETIFOLIA* CUTTINGS

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Casuarina equisetifolia is a multipurpose tree species of tropical and subtropical countries. The species is widely planted in coastal areas of southern India as cash crop, mostly for poles and fuelwood. It is also planted for reforestation, shelterbelt, firebreak, shade, ornamental, soil fertility management and rehabilitation of lands. In recent years *C. equisetifolia* has become an important raw material for pulp and paper industries. Genetic improvement of the species is now an area of considerable research effort. Clonal propagation is an important component of tree improvement programmes for this species.

This species is salt tolerant and is recommended for plantations in saline and coastal areas (Tomar 1997). However, *Casuarina* spp. still suffer some salt-related stress including reduction in growth rate, tip necrosis, decreased nodule, shoot/root dry weight and nitrogen content of shoots, stunted growth, and even death (Clemens & Campbell 1982, Ng 1987, Bandyopadhyay 1988).

Use of saline water in nurseries for clonal propagation can seriously affect the success rates in the propagation of cuttings. Small amounts of salt in commercial nurseries can lead to huge economical losses over longer period of time. The amount of loss depends on the species ability to tolerate salt stress. The present study was conducted to determine the extent of damage of saline water on clonal propagation of *C. equisetifolia.*

Eight batches of *C. equisetifolia* from a single clone (CPCE890110), comprising 20 cuttings each, were rooted in vermiculite media soaked with different concentrations of NaCl solution. Cladode cuttings of length 5–7 cm were sterilized using 1% Bavistin Solution, a broad spectrum commercial fungicide containing cabendazim 50% w/w (BASF India Ltd. Mumbai). After treating with 2000 ppm indole 3-butyric acid (diluted with talcum powder), the sterilized cuttings were planted in 160 ml root trainers filled with vermiculite presoaked with 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4% w/v NaCl solution. For induction of roots, the treated cuttings and were maintained at 35–38 °C temperature and above 70–80% relative humidity inside poly-tunnels following Gurumurthi and Kumar (1996). Periodic water spray maintained the desired humidity. The number of cuttings that formed roots in each batch and the number of adventitious roots per cutting were observed after 30 days. The data was tabulated as a 2 × 8 contingency table and analyzed using chi-square test. To analyze the comparisons, pairs of response variables were tested. Average root length of cuttings was also measured. The Wilcoxon-Wilcox test (which is based on ranks) was used to compare multiple treatments of a series of subjects.

Our results showed that rooting media with NaCl concentrations higher than 1.0% (171 mM) had significantly low rooting percentages of 10–35% with $p < 0.05$ (Table 1). All cuttings in the control treatment and those treated with 0.2% NaCl formed roots. Root length also decreased with increasing salinity, reaching as low as 0.4 cm at 1.4% NaCl.

Received September 2002

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Decreases in root biomass of *Dalbergia sissoo* and rooting percentage and root length of *Populus teemula* have been reported with increased salinity (Singh *et al.* 1996, Evers *et al.* 1997). Complete inhibition of rooting was reported under *in vitro* conditions in *Helianthus annuus* at salt concentrations as low as 50 mM in MS medium (Vieira-dos-Santos *et al.* 2000).

The survival of cuttings was very low in rooting media with higher salt concentrations. More than 50% cuttings died in the 1.2 and 1.4% NaCl treatments. The death of cuttings at higher salinity stress can be attributed to the inability of cuttings to root under stressed environment and absorption of toxic amounts of Na^+ and Cl^- through cut ends (Kuligod *et al.* 1995). In the present study, roots developed at salt stress of 0.6% and higher showed almost no root hairs. Root hairs are important for absorption of water and essential elements, provide anchorage to plant, aid in microbe interaction and improve survival of seedlings after field transplantation.

Cuttings which were rooted in high salt concentrations (> 1%) also showed hypertrophy (increase in thickness of internode) of articles and chlorosis of cladodes. Hand sections of the swollen article showed deformation and increase in the size of cortical cells. Hypertrophy and chlorosis occur due to the synthesis and accumulation of ethylene under salt-stressed condition. The effect of ethylene (0.5 μl/l) on cell elongation and radial expansion was demonstrated by Stewart *et al.* (1974) in upper internode of *Pisum sativum* seedlings.

Casuarina equisetifolia, which is widely planted in coastal areas experiences reduction in growth due to salinity stress. The present study demonstrated the detrimental effects of saline water in production of quality planting material for clonal plantations of *Casuarina*. NaCl concentrations of 1.2 and 1.4% resulted in 85 to 90% reduction in rooting of cladode cuttings. Salt not only affected the rooting percentage but also prevented root hair development. Rooted cuttings with chlorotic and hypertrophied shoots and poor root system (less biomass and root hairs) will have lower chances of survival after field transplantation. Therefore, use of salt-free water in nurseries can improve success of clonal multiplication of elite planting stock of *C. equisetifolia*.

Table 1 Effect of NaCl on survival and rooting of *Casuarina* cladode cuttings

Treatments	Rooting (%)	Root length (cm)	Cuttings died No.	Roots/cuttings No. (Average)	Total root length (cm)
0.00%	100	10.89 ± 0.6	0	5.4 ± 0.5	58.81 ± 5.7
0.20%	100	10.38 ± 0.5	0	5.3 ± 0.4	51.94 ± 4.1
0.40%	85	11.24 ± 0.9	0	2.4 ± 0.4	26.98 ± 4.3
0.60%	80	10.87 ± 0.7	0	2.7 ± 0.3	29.89 ± 3.9
0.80%	60	10.80 ± 1.0	5	2.0 ± 0.2	21.60 ± 3.2
1.00%	35	12.21 ± 0.9	2	1.4 ± 0.2	17.09 ± 2.5
1.20%	15	06.55 ± 3.3	10	1.6 ± 0.7	10.48 ± 3.4
1.40%	10	04.15 ± 0.7	16	2.0 ± 1.0	08.30 ± 5.5

± SE mean; observations of rooted cuttings were used for mean calculation

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