MODULATION OF CERTAIN PHYSIOLOGICAL PARAMETERS TO SALINITY IN *CASUARINA EQUISETIFOLIA* SEEDLINGS INOCULATED WITH FIVE DIFFERENT *FRANKIA* STRAINS

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BALASUBRAMANIAN, A., SRINIVASAN, P.S. & KUMARAVELU, G. 1996. Modulation of certain physiological parameters to salinity in *Casuarina equisetifolia* seedlings inoculated with five different *Frankia* strains. The salinity tolerance of *Casuarina equisetifolia* inoculated with five *Frankia* strains was evaluated by measuring the needle transpiration, diffusive resistance and seedling water potential of the plant. The seedlings were inoculated in the form of nodule suspension and subjected to four salt treatments, viz. control (T_0), 0.15 mg (low) (T_1), 1.5 mg (medium) (T_2) and 15 mg (high) (T_3) of NaCl per gram of soil. The low salt concentration increased the transpiration and reduced the stomatal resistance but maintained a higher water potential. On the other hand, the medium and high concentrations drastically reduced the transpiration by increasing the stomatal resistance resulting in a low water potential and thus ultimately the death of seedlings. Besides, the good growth of *Casuarina* seedlings was observed with two *Frankia* sources, namely Sugar-cane Research Station [Tamil Nadu Agricultural University (Cuddalore)] and Marakanam.

Key words : *Casuarina equisetifolia* - *Frankia* - diffusive resistance - transpiration - water potential

BALASUBRAMANIAN, A., SRINIVASAN, P.S. & KUMARAVELU, G. 1996. Modulasi parameter fisiologi tertentu terhadap kemasinan dalam anak benih *Casuarina equisetifolia* yang diinokulasi dengan lima strain *Frankia* yang berbeza. Toleransi kemasinan lima strain *Frankia* dinilai dengan mengukur transpirasi jarum, kerintangan difusi dan keupayaan air biji benih tersebut. Biji benih tersebut diinokulasi dalam bentuk ampaian nodul dan dirawat dengan garam pada empat kepekatan iaitu kawalan (T_0), 0.15 mg (rendah) (T_1), 1.5 mg (sederhana) (T_2) dan 15 mg (tinggi) (T_3) NaCl segram tanah. Kepekatan garam yang rendah meningkatkan transpirasi dan mengurangkan kerintangan stomata tetapi mengekalkan keupayaan air yang tinggi. Di sebaliknya kepekatan garam yang sederhana dan tinggi mengurangkan transpirasi secara mendadak dengan meningkatkan kerintangan stomata yang menyebabkan keupayaan air yang rendah dan akhirnya kematian biji benih. Selain itu, pertumbuhan biji benih *Casuarina equisetifolia* yang baik diperhatikan dari dua sumber *Frankia* berbeza iaitu Pusat Penyelidikan Tebu [Universiti Tamil Nadu (Cuddalore)] dan Marakanam.

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Introduction

Casuarina equisetifolia, which is native to Australia, can be planted for the reclamation of problem areas like salt affected soils. The observations of Corner (1952), and Somasundaram and Jagadees (1977) have suggested that *Casuarina equisetifolia* is mostly a salt-tolerant species. However, very little work has been carried out to explain the physiological basis for its tolerance. In tree crops, salinity generally impairs physiological processes leading to their death. Gates (1972) stated that salinity reduced the transpiration in *Acacia harpohylla.* Studies of Wallace and Kleinkopf (1974) in *Eucalyptus citriodora* indicated that salinity lowered the water potential. According to Sands and Clarke (1977), NaCl treatment reduced the transpiration and water potential in *Pinus radiata* seedlings. Cromer *et al.* (1982) in their pot culture experiment showed that growth reduction in *pinus radiata* was associated with lower water potential under saline conditions. Increasing salinity reduced the stomatal conductance and CO_2 assimilation rate in mangroves (Clough & Sim 1989).

Eucalyptus camaldulensis and *E. lesouefii* showed lesser stomatal conductance when irrigated with saline water (Moelzel *et al.* 1989). According to Golombek and Ludders (1989), salinity reduced stomatal conductance but at the same time increased the water use efficiency due to greater reduction of transpiration in *Ficus carica.*

The present study attempts to determine the effect of NaCl on the physiological processes of *Casuarina equisetifolia* when inoculated with five different *Frankia* strains.

Materials and methods

Collection of Casuarina root nodules

Casuarina root nodules were collected from the following different parts of Tamil Nadu where *Casuarina* predominantly grows:

Frankia source	Place	District	Age of plantation (months)
S_1	Karamanikuppam	Pondicherry	8
S_2	Thalankadu	Chengleput	18
S_3	Sugar-cane Research Station - Tamil Nadu Agricultural University (Cuddalore)	South Arcot	10
S_4	Varakalpet	South Arcot	6
S_5	Marakanam	South Arcot	13

Table 1. Some important chemical properties of the soil samples collected from different parts of Tamil Nadu

Source	EC m.mhos cm^{-1}	pH	Nutrient ($kg\ ha^{-1}$)		
			N	P	K
S_1	0.05	7.7	167.50	9.75	135.00
S_2	0.1	5.3	77.50	5.00	52.50
S_3	0.2	8.5	112.50	5.63	32.50
S_4	0.1	7.2	190.00	18.13	52.50
S_5	0.1	8.3	102.50	9.75	40.00

Values are presented without statistical analysis.

Preparation of Frankia nodule suspension

Nodules which were light in colour, appearing active and composing of young tissues were collected along with small subtending roots in all the above mentioned places. The collected nodules were packed separately for each place.Thirty grams of these nodules were washed with running tap water to remove all the adhering soil particles and surface sterilised with hydrogen peroxide (30% v/v) for 5 min. Under sterile condition, the sterilised nodules were washed three to five times with sterile distilled water and then crushed into suspension using sterilised pestle and mortar with 300 ml of sterile distilled water. The prepared nodule suspension was used as *Frankia* inoculum and the *Casuarina equisetifolia* seedlings were then inoculated by dipping the roots in the suspension for 5-10 min. The inoculated seedlings were planted in pots containing steam sterilised coarse river sand. The inoculation was done for all the sources of *Frankia* nodules. Three months after inoculation the light-coloured nodules were again collected from the inoculated seedlings and the *Frankia* nodule suspension was prepared by the above mentioned method. This prepared *Frankia* inoculum as a nodule suspension was used as a treatment source. This was done to favour a single dominant and effective *Frankia* strain within a particular nodule source and to counter more than one strain infection from a single nodule source.

Glasshouse experiment

The experiment was carried out at the glasshouse of the forestry nursery complex at the College of Forestry, Tamil Nadu Agricultural University, Coimbatore, during 1990-1992. Seedlings of uniform age of 15-20 days were collected from Cuddalore and inoculated with crushed nodule suspension of *Frankia* (collected from the five sources mentioned above) and planted into 12 × 12 cm size mud pots containing 2.5 kg of steam-sterilised coarse river sand. The different salt concentrations [control (T_0), 0.15 mg (low) (T_1), 1.5 mg (medium) (T_2) and 15 mg (high) (T_3)] were maintained as described by Reddell *et al.* (1985) in three replications. Every week each pot was supplied with 25 ml of complete nitrogenfree nutrient solution (Reddell *et al.* 1985), and the observations were made at 60 days intervals after planting/inoculation (DAI).

Needle transpiration rate and diffusive resistance were measured with a pre-calibrated LI-1600 steady state porometer, LI-COR Lincoln, Nebraska, USA and expressed in $\mu g\ H_2O\ cm^{-2}\ s^{-1}$, and $s\ cm^{-1}$ respectively.

The water potential was assessed by using whole seedlings with the help of pressure bomb apparatus (Meidner 1984) and was expressed in-bars.

Owing to the high concentration of salt in T_2 and T_3 treatments, the seedlings died. The T_2 and T_3 treatment effects were therefore not included in the Tables 2, 3 and 4. The seedlings started drying after 60 days of salt treatment in the T_2 treatment and 90 days in the T_3 treatment.

Results and discussion

Effect of salinity on needle transpiration

Needle transpiration, a vital physiological parameter reflecting the plant water use efficiency, was greatly influenced by salinity. As shown in Figure 1, the reduction in transpiration rate was evident at treatments (T_2) and (T_3) with high salt concentrations and resulted in the death of seedlings, whereas T_1 with low salt concentration recorded high transpiration. This increase in transpiration rate was possibly due to higher water absorption from the soil under low saline condition. On the other hand, the higher salt treatments may counteract water absorption which in turn reduce transpiration ultimately leading to the death of seedlings. Besides, the higher transpiration rates in S_3 and S_5 corresponded to an enhanced growth of the *Frankia* which was observed both under the control and low salinity level.

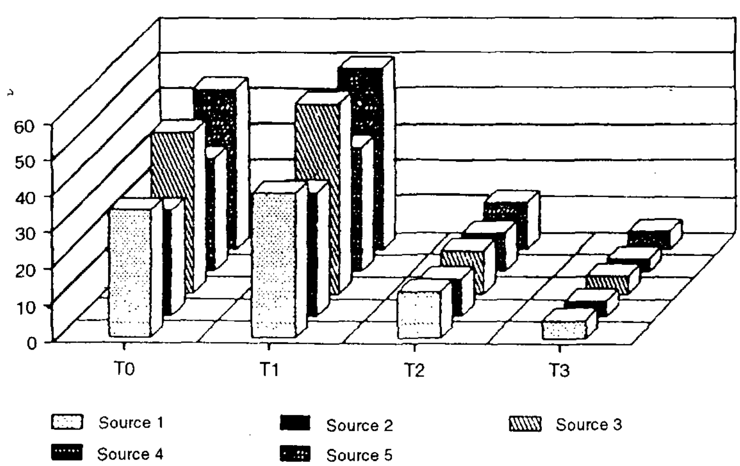

Figure 1. Effect of salinity on needle transpiration ($\mu g\ cm^{-2}\ s^{-1}$) of *Casuarina equisetifolia* at 150 days after inoculation (DAI)

Table 2. Effect of salinity on dry matter accumulation (g) of *Casuarina equisetifolia*

Treatment	Days after inoculation (DAI)	Source of inoculum					Treatment mean
		S_1	S_2	S_3	S_1	S_5	
	30	0.52	0.28	0.70	0.41	0.59	
T_0	90	3.24	2.43	5.73	2.78	4.92	
	150	6.00	7.84	10.32	5.00	8.93	
		3.25	3.52	5.58	2.73	4.81	3.98
	30	0.76	0.51	1.00	0.60	0.88	
T_1	90	5.94	4.94	9.38	4.19	8.51	
	150	9.63	9.38	15.88	8.87	14.09	
		5.42	4.94	8.75	4.55	7.83	5.35
Source mean		4.35	4.23	7.17	3.64	6.32	

	SE	CD (p=0.05)
Source	0.039	0.019
Treatment	0.035	0.097

Effect of salinity on needle diffusive resistance

Needle diffusive resistance reflects the inverse relation to needle transpiration. The data (Table 3) indicate that the low salt treatment (T_1) recorded a lower diffusive resistance of 0.27 s cm^{-1} than the control. The death of seedlings was noticed in T_2 and T_3 treatments due to high salt concentrations. Among the different sources of inoculum, S_3 and S_5 gave lower diffusive resistance of 0.22 s cm^{-1} and 0.25 s cm^{-1} respectively. Under salt stress especially in the medium and high concentrations, a rise in vapour pressure gradient between needles and the external air might have resulted in concomitant increase in transpiration leading to a needle water deficit which in turn increased the stomatal resistance. Moreover, the availability of water to seedlings at low salt concentration might have promoted water transport in the phloem thereby giving rise to higher transpiration and lower stomatal resistance. Begg and Turner (1976) while reporting similar results also suggested that a gradual increase in salt stress could cause water deficit leading to the reduction of photosynthesis which could in turn increase the stomatal resistance. The better performance of S_3 and S_5 *Frankia* sources under salinity may possibly contribute to good water regulation because of their salinity tolerance ability.

Effect of salinity on seedling water potential

Water potential of the seedlings was altered by salinity (Table 4). Low salinity treatment (T_1) recorded a water potential of -18.07 bars as against -14.18 bars in the control (T_0). Again S_3 and S_5 sources gave higher values in water potential than the others. This result infers that better soil moisture condition and low salinity level help to maintain higher water potential in *Casuarina* seedlings. Sands and Clarke (1977) in studying the response of pine trees to salt stress observed that the decreased water potential may be due to excess concentration of sodium chloride in the soil.

Table 3. Effect of salinity on seedling diffusive resistance (s cm^{-1}) of *Casuarina equisetifolia*

Treatment	Days after inoculation	Source of inoculum					Treatment mean
		S_1	S_2	S_3	S_4	S_5	
T_0	30	0.29	0.28	0.30	0.40	0.31	
	90	0.27	0.34	0.22	0.46	0.25	
	150	0.35	0.32	0.19	0.33	0.25	
		0.30	0.31	0.24	0.40	0.27	0.30
T_1	30	0.26	0.21	0.28	0.38	0.31	
	90	0.27	0.30	0.18	0.44	0.21	
	150	0.37	0.26	0.13	0.34	0.17	
		0.30	0.26	0.20	0.39	0.23	
Source mean		0.30	0.29	0.22	0.39	0.25	0.27

	SE	CD (p = 0.05)
Source	0.0007	0.002
Treatment	0.0006	0.002

Table 4. Effect of salinity on seedling water potential (WP) (-bar) of *Casuarina equisetifolia*

Treatment	Days after inoculation	Source of inoculum					Treatment mean
		S_1	S_2	S_3	S_4	S_5	
T_0	30	- 15.20	- 14.60	- 14.10	- 15.20	- 11.94	
	90	- 16.77	- 14.01	- 12.38	- 17.70	- 10.22	
	150	- 17.50	- 16.00	- 10.00	- 17.00	- 10.15	
		- 16.49	- 14.87	- 12.16	- 16.63	- 10.77	- 14.18
T_1	30	- 19.10	- 16.90	- 17.00	- 16.90	- 15.80	
	90	- 24.34	- 22.31	- 16.14	- 19.34	- 17.42	
	150	- 18.00	- 21.00	- 10.50	- 25.34	- 11.00	
		- 20.48	- 20.07	- 14.50	- 20.53	- 14.74	- 18.07
Source mean		- 18.49	- 17.47	- 13.35	- 18.58	- 12.76	

	SE	CD (p = 0.05)
Source	0.065	0.181
Treatment	0.058	0.162

The study indicated that the low salt level (T_1) positively altered the transpiration, diffusive resistance and ultimately , water potential which promoted the growth and development of *Casuarina* seedlings up to 150 days after inoculation. On the other hand the medium (T_2) and high (T_3) salt concentrations affected all the physiological factors and ultimately caused the death of seedlings. The two *Frankia* sources, namely Sugar-cane Research Station-TNAU (Cuddalore) (S_3) and Marakanam (S_5), have tolerated the saline conditions better than the other three sources. Thus this study showed that different strains of *Frankia* can be isolated and retested at nursery level for salinity tolerance. It may also be advantageous to isolate such salinity tolerant *Frankia* strains as pure culture for afforestation work in saline areas. The isolation work is under progress in our institute.

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