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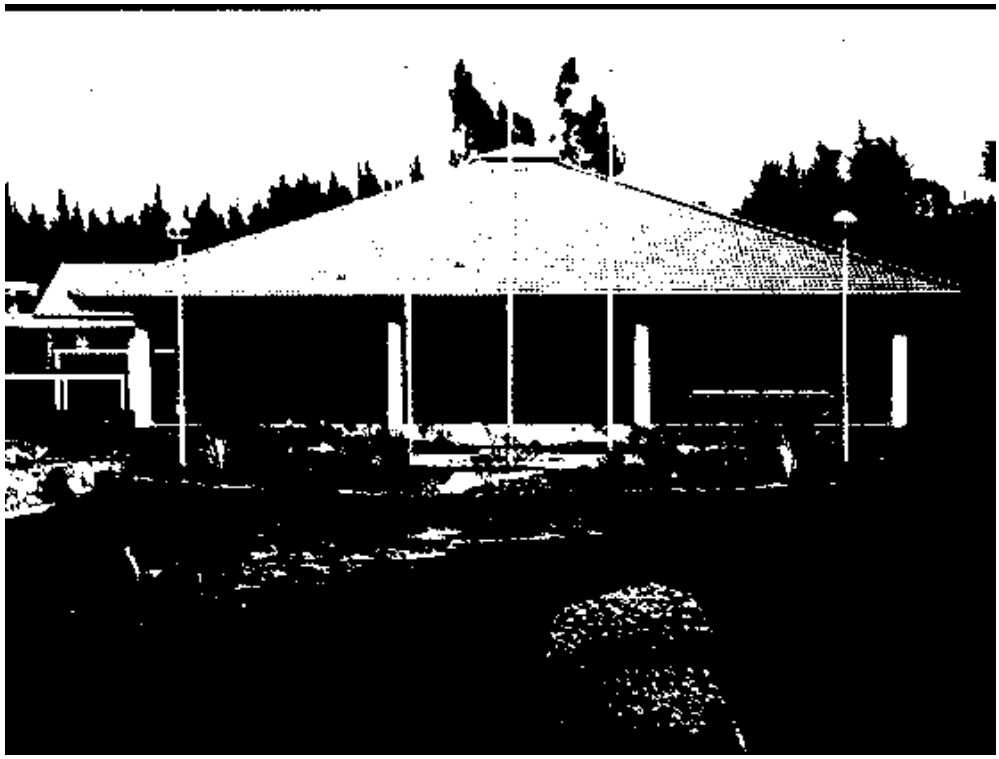

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RESPONSE OF CASUARINAS TO FRANKIA INOCULATION IN SALINE UNSTERILE SAND/VERMICULITE MEDIUM

ELLY J.M. MWANZA

Summary

The response of Casuarina cristata, C. cunninghamiana, C. equisetifolia, C. glauca, C. obesa and Allocasuarina decaisneana to inoculation with crushed nodule inoculum of Frankia collected from C. equisetifolia was studied under greenhouse conditions in unsterile field sand/vermiculite medium. Inoculation increased overall growth, nodulation, dry weight of shoot and root and the nutrient status of all the species except for Allocasuarina decaisneana. The interim results indicate that inoculation of five of the above species with the endophyte is beneficial in unsterile saline sand medium inspite of the presence of natural Frankia. The endophyte showed host specificity between Casuarina and Allocasuarina.

Introduction

Studies of symbiotic nitrogen fixation within the family Casuarinaceae have reported the beneficial effect of inoculation on plant growth in steam sterilized soils (Coyne 1983, **Zhang and Torrey 1985, Rosbrook and Bowen 1987).** Specificity at the generic **level** has **been found and it** has been **suggested that a Frankia source** highly **effective at nitrogen** fixation on one species of Casuarina may be ineffective on another (Reddel and Bowen 1986). This difference is of great practical **importance since successful establishment** of seedlings **in the field** may depend on the presence of infective and highly effective Frankia.

Most inoculation studies with Frankia on Casuarina have been undertaken in steamed or autoclave soils thus eliminating any indigenous endophyte that may be present in such soils. In Kenya,

work on Frankia began only recently and there is very little information on the response of various casuarinas to inoculation by locally collected Frankia. This study was undertaken to determine if inoculum of Frankia from **Casuarina** equisetifolia **was effective** in **nodulating C.** obesa, C. cristata C. **cunninghamiana and Allocasuarina decaisneana** in unsterile field **sandy soil.**

The results presented here mainly deal with the ability of Frankia nodule inoculum to promote growth, as evaluated by overall nodulation, plant dry weight, nitrogen concentration of roots **and shoots and sandy** medium nitrogen content.

Materials and Methods

Preparation of **inocula** and inoculation

Nodule material was collected from a 2 year old plantation of C. equisetifolia at Kwale, washed and stored on **silica** beads for upto 3 months. The dry nodules were severally washed in distilled **water and surface sterilized in 10% Hydrogen peroxide, rinsed** through **three** changes of sterile distilled water and crushed in 0.05M sucrose and made upto 100m1 suspension. 500m1 **plastic pots were** filled with mixture of sand and vermiculite (3:1 W/W) and watered to a moisture content of 20% (W/W) with distilled water.

Seeds of each species were surface sterilized in about 6% sodium hypochlorite for 20 minutes, rinsed three times and placed on the surface of a tray of saline **steam** sterilized sand (pH 8.7)

in the glasshouse. After six weeks, the seedlings were gently removed from the sand. For the uninoculated treatment, seedlings were directly transplanted. For the remaining treatments, seedlings were immersed in inoculum suspension for 20 min. They were then transplanted into the pots and excess inoculum suspension watered onto the seedlings (3ml/plant).

A completely randomised design consisting of six plant species each inoculated with Frankia inoculum and an uninoculated control were employed. There were five replicate plants in each treatment. Single plants of the species were grown in pots.

Maintenance

Pots were watered to field capacity twice every week with distilled water. After 3 weeks, 40g of fine concrete ballast was placed on the surface of each

pot as mulch. A minus N nutrient solution (Norris and Date 1976) modified to contain 0.95 mg $CoSo_4$ $7H_20$ per litre and 0.22 mg Na2Mo4 **per litre** was applied to each pot fortnightly at the rate of 10ml/pot. To help sustain the seedlings prior to commencement of N-fixation, at 10, 24 and 38 days after planting all plants received 2, 5 and 2 mg N per pot respectively. The experiment was conducted in a glasshouse at temperatures of 26°C day and 17°C night.

Harvesting and Assessments

A preliminary assessment of nodulation was made 10 weeks after transplanting. Plants were harvested 30 weeks after inoculation. Height measurements of each plant were taken and **the plant removed** from the pot and roots washed carefully to remove adhering sand and vermiculite. Shoot and root dry weights were

determined following drying at 60°C for 48 hours. Shoots and roots were digested by Micro-Kjedhal procedure and analysed for N on an auto analyser (Mcleod 1982). The sandy medium was assayed for nitrogen percentage. The mean growth, number of nodules, biomass production, plant total N and N percentage in sand medium for the six species were compared.

Results and Discussion

Inoculation with Frankia remarkably increased height, number of nodules, biomass production, plant total nitrogen and sandy medium total nitrogen for the five species of Casuarina. However, it had little or no effect on Allocasuarina decaisneana (Table 1). There appears to exist host specificity with the endophyte between Casuarina and Allocasuarina The applied inoculum was only effective on the Casuarinas and not Allocasuarina.

These findings support earlier observations by Reddel and Bowen (1986) who suggested that Frankia inoculum from Allocasuarina, Casuarina and Gymnosperma could be genera specific.

Table 1: Effect of Frankia inoculation on Casuarina growth, nodulation, biomass production, plant nitrogen content and soil nitrogen status

	C.cristata	C.cunninghamiana	A.decaseneana	C.equisetifolia	C.glauca	C.obesea
Height (cm) inoculated (inoc)	55.62±7.751	51.6±8.7	18.92±4.21	36.25±4.21	83.64±11.21	74.88±6.59
control (ctr)	37.28±8.34	43.2±5.9	14.5±2.81	21.62±2.43	59.28±8.14	56.86±7.26
No.of nodules (inoc)	25.6±5.3	48.5±12.2	0	14.26±3.04	17.6±5.23	34.7±3.05
(ctr)	2.1±0.4	8.2±3.6	0	3.18±0.82	6.18±0.82	5.9±2.8
Biomass (gm) (inoc)	2.92±0.96	2.53±0.89	0.59±0.9	1.96±1.0	4.44±1.43	3.76±1.03
(shoot) (ctr)	1.55±0.65	1.96±0.58	0.43±0.14	0.97± 0.1	1.85±0.78	1.74±0.25
Biomass (gm) (inoc)	0.46±0.13	0.84±0.17	0.29±0.15	0.44±0.06	1.61±0 46	1.38±0 19
Root (ctr)	0.28±0.09	0.53±0.23	0.13±0.03	0.21±0.08	0.74±0.21	0.93±0.21
Plant Total N (inoc)	68.41±8.36	62.63±11.51	9.82±2.45	39.36±6.28	90.97±13.48	83.19±12.81
(mg/seedling) (ctr)	43.58±5.24	38.52±6.83	5.57±1.61	16.34±3.57	53.56±8.37	50.94±6.48
Sand medium (inoc)	0.14±0.01	0.14±0.01	0.07±0.009	0.07±0.00	0.21±0.03	0.21±0.03
Total N(%) (ctr)	0.02±0.005	0.02±0.005	0.02±0.005	0.02±.005	0.02±.005	0.02±.005

This is the first study in Kenya where inoculation studies with crushed nodule inoculum has been undertaken. Generally Casuarinas grow well and nodulate profusely at the Coast where the sandy soil contain Frankia in abundance. In other high potential areas of Kenya, the major cultivated species C. equisetifolia grows **well in fertile** soil but nodulation is generally poor. The latter observation is in line with findings in Australia (Reddel and Bowen, 1986) that nodulation and N-fixation is generally ineffective in soils with optimum nitrogen.

The present findings indicate that lack of optimum N in the soil and the presence of effective Frankia are pre-requisite to better growth and nodulation in infertile **soils.** Results of **this study suggest that inoculation** with effective Frankia from the coastal Region (Kwale) promotes growth despite the presence of indigenous

endophyte which was less effective as was evidenced by poorer growth and nodulation in the control (uninoculated) treatments.

In this experiment, using sandy medium (pH 8.7), C. equisetifolia gave lower growth and modulation than the lesser known Casuarinas in Kenya - C. glauca and C. obesa. In their native habitats the latter species are adapted to water-logged and saline soils respectively. Rosbrook and Bowen (1987) observed that C. lauca performed better than C. equisetifolia in sterile sand/vermiculite mixture under conditions of excessive watering.

Conclusion

Inoculation of casuarinas with nodule inoculum from C. equisetifolia is beneficial to species of C. glauca C. obesa, C. cunninghamiana and C. cristata which are not yet widely planted in Kenya. These species grew better than C. equisetifolia under sand/vermiculite mixture at a pH 8.7. There is need to undertake inoculation and field trials with the more promising species especially in water-lodged and saline soils. Work on isolation of individual strains of Frankia should be intensified to produce cultures for inoculation studies.

Acknowledgements

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