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A QUANTITATIVE STUDY OF NODULATION AND ARBUSCULAR MYCORRHIZAL INFECTION IN *CASUARINA EQUISETIFOLIA* IN TAMILNADU

J. SEMPAVALAN and P. SANTHANAKRISHNAN

Department of Agricultural Microbiology
Tamilnadu Agricultural University,
Coimbatore - 641 003.

ABSTRACT

A survey was conducted in Tamil Nadu to examine nodulation and Vesicular Arbuscular Mycorrhizal colonization of *Casuarina equisetifolia* using soil samples and root nodules (for use as a *Frankia* inoculum) collected from 23 provinces. Nodulation occurred more frequently on soils of higher available phosphorus status. This experiment supported the hypothesis that low phosphorus supply and the absence of infective *Frankia* were two of the factors responsible for the low frequency of nodulation in some soils. The VA-mycorrhizal fungi exhibited distinct distribution patterns when associated with *Casuarina equisetifolia* host in different habitats. The reduction in mycorrhizal activity was attributed to the high-P availability in the soil. The greater effectiveness of some inoculum for increasing P concentration in the host plant may indicate adaptation in these mycorrhizal fungi to low P soil conditions. Thus differences in nodulation and mycorrhizal infection may occur among native *Frankia* and VA-mycorrhizal fungi when the native soils are different yet contiguous.

KEY WORDS: *Casuarina equisetifolia*, *Frankia*, Nodulation, Survey, Symbiosis, VAM

Natural populations of *Casuarina equisetifolia* are distributed over a wide latitudinal range in India. The *Casuarina* plantations are widespread along road sides, property lines, canal banks and around buildings and as wind breaks and village wood lots. The identification of specificity has important implications for the practical use of *C. equisetifolia* a species planted widely for poles, shelter belts, sand dune stabilization, river-bank conservation on nutrient deficient soils where the efficiency of the nitrogen fixing symbiotic system may be critical to the health and productivity of the plant (Torrey, 1983).

Nodulation of planted trees has been dependent on either the presence of native *Frankia* in the soil or inoculation with whatever inoculum has been conveniently available, neither of these techniques allowing control over the effectiveness of the association. It is highly probable that some do not nodulate but as the absence of nodulation in the field may reflect only the absence of an appropriate *Frankia*, or soil conditions unsuitable for nodulation of *C. equisetifolia* (Reddell *et al.*, 1986).

In addition, variation in infective capability and influence on host plant effectivity of arbuscular mycorrhizal fungi has been demonstrated between introduced species and the indigenous assemblages of arbuscular mycorrhizal fungi present in the native soils (Mosse, 1972, 1977). These studies have indicated the sensitivity of arbuscular mycorrhizal fungi to the soil environment that may influence both their capacity for infection as well as on the physiological response of the host plant. Comparisons of variation in infective capability among indigenous species are limited and, in general, have contrasted fungal communities only from widely separated locales.

The present study was undertaken to examine the differences in growth and nodulation under green house conditions of 23 provinces of *C. equisetifolia* each inoculated with nodule inoculum from each of the 23 sources. This study also taken up to compare the degree of infection and host influence of arbuscular mycorrhizal fungi from 23 province soils collected.

MATERIALS AND METHODS

Seed, Soil and Inoculum collection

Seed was collected from adult trees in each of 23 natural populations of *C. equisetifolia* from different locations throughout the natural range of the species and germinated in sand under controlled environment chamber. Root nodules and soil samples were collected from five plants at each site. Nodulated roots were excised from the main roots, placed in collecting bags, sealed and stored at 5°C. Soil samples were collected from the soil surface around the roots and stored in dark plastic bags at 5°C.

Pot establishment and maintenance

Soil samples (3 kg) collected from different sites were added to undrained pots. One month old *C. equisetifolia* seedlings were transplanted to pots and were progressively thinned to one per pot at the time of inoculation. Care was taken to prevent cross contamination of seedlings by using pots with no drainage holes and by sterilization of all inoculum equipment.

The nodule inoculum was prepared by grinding the surface sterilized nodule material in distilled water and 5 ml suspension containing approximately 0.25 g nodule tissue. Seedlings were inoculated by injecting 5 ml of the nodule suspension 1 cm below the soil surface on either side of the seedlings immediately following watering field capacity. The seedlings were maintained in a green house at a day-night temperature cycle of 30 and 25°C.

Measurement of growth and biomass

At 20 weeks, the height of each seedling was measured. Seedlings were carefully removed from the pots and the nodules were excised from the roots and counted. The plants and nodules were dried at 60° C for 48 h and weighed. Following dry weight determination, samples were processed in a Wiley mill and ground to a fine mesh consistency and used for the estimation of total nitrogen, phosphorus and potassium contents.

Arbuscular mycorrhizal colonization

To prepare the roots for assessment of mycorrhizal colonization, root segments were excised from the specimens collected, washed, cleared and differentially stained with 0.05% tryphan blue in lactophenol following a modification of the procedure of Phillips and Hayman (1970). The percentage of infection was

Table 1. Chemical properties of soil collected from different parts of Tamil Nadu

District/ Location	pH	EC (mmhos /cm)	Available nutrients (kg/ha)		
			N	P_2O_5	K_2O
Chennai					
Thiruvelangadu	7.6	0.16	132	16	154
Sadras	7.8	0.20	128	18	201
Thiruporur	7.9	0.15	129	18	204
Thiruvurkuppam	7.8	0.18	134	15	197
Kanchipuram					
Uthiramerur	7.9	0.19	130	20	123
Cuddalore					
Pudhusathiram	7.9	0.27	155	23	210
Nellikuppam	7.5	0.20	215	18	260
Parangipettai	7.6	0.23	201	19	261
Thiruvengadu	7.8	0.26	197	21	227
Neyveli	7.3	0.20	218	10	222
Villupuram					
Marakanam	7.8	0.22	220	10	280
Nagapattinam					
Velanganni	7.8	0.32	163	16	225
Vedharanyam	7.7	0.31	158	16	253
Seergazhi	7.9	0.36	175	18	234
Vaidheswarankovil	7.8	0.39	178	12	251
Trichy					
Srirangam	7.6	0.29	219	8	256
Thiruvanaikaval	7.7	0.30	221	9	267
Perambalur					
Perambalur	7.5	0.27	217	9	272
Pudukkottai					
Thirumayam	6.7	0.18	160	21	256
Northamalai	6.9	0.21	171	19	254
Coimbatore					
TNAU Campus	8.1	0.23	223	5	287
Karumathampatti	7.9	0.19	220	6	277
Mettupalayam	8.1	0.21	231	6	279

Table 2. Growth and nodulation of *Casuarina equisetifoliar* seedlings grown in soils collected from different parts of Tamil Nadu.

District/ Location	Plant height* (cm/pl)	Plant dry* weight (g/pl)	Nodule* No/pl	Nodule dry* weight (mg/pl)
Chennai				
Thiruvelangadu	39.6	0.478	35.70	1.490
Sadras	46.7	0.770	48.20	1.799
Thiruporur	39.0	0.527	36.20	1.420
Thiruvurkuppam	41.6	0.549	39.00	1.532
Kanchipuram				
Uthiramerur	52.1	1.306	62.60	3.213
Cuddalore				
Pudhusathiram	43.5	0.654	51.80	2.169
Nellikuppam	40.6	0.570	37.60	0.957
Thiruvengadu	45.6	0.552	40.00	1.813
Parangipettai	30.6	0.333	30.80	0.930
Neyveli	28.7	0.298	18.70	0.417
Villupuram				
Marakanam	26.4	0.301	14.20	0.380
Nagapattinam				
Velanganni	40.9	0.360	39.08	1.702
Vedharanyam	38.1	0.367	35.16	1.629
Seergazhi	29.9	0.245	37.30	1.266
Vaidheswarankovil	26.4	0.226	33.00	1.260
Trichy				
Srirangam	22.5	0.298	20.30	0.703
Thiruvanaikaval	24.4	0.216	11.90	0.210
Perambalur				
Perambalur	21.1	0.211	12.00	0.220
Pudukkottai				
Thirumayam	32.2	0.317	53.60	2.720
Northamalai	30.7	0.310	30.80	1.122
Coimbatore				
TNAU Campus	27.5	0.299	14.08	0.694
Karumathampatti	25.5	0.300	18.20	0.780
Mettupalayam	18.6	0.209	20.08	0.680
CD	8.63	0.147	5.13	0.675

* 20 weeks after transplanting

estimated by the root slide technique (Read *et al.*, 1976).

Recovery of mycorrhizal spores from the soil

Spores were recovered from each collection by wet sieving and decanting (Gerdemann and Nicolson, 1963). Total counts of the spore population were made by picking out the spores from the soil while examining the sample under a dissecting microscope at 20 times magnification.

RESULTS AND DISCUSSION

Infectivity of *Frankia* under natural conditions.

The soil properties and growth and nodulation of *C. equisetifolia* seedlings grown in the soils from 23 provenances are summerized in Tables 1 and 2. The twenty three study sites differed in soil nutrient concentrations, pH and EC. The growth response of *C. equisetifolia* seedlings grown in Uthiramerur soil sample of Kanchipuram district displayed a significant increase in height (52.1 cm), plant dry weight (1.306 g/plant) and produced a relatively large number of nodules (62.6/plant). In contrast, seedlings grown in Mettupalayam soil sample of Coimbatore district showed a reduction in plant height (18.6 cm), plant dry weight (0.209 g/plant) and produced much fewer nodules (20.08/plant).

These results suggest that the host plant and inoculum source had some control over growth and nodulation of *C. equisetifolia* seedlings. Thirumayam inoculum of Pudukottai district was also notable despite abundant nodulation (53.6/plant) it was associated with the plant that averaged one of the lowest total dry weight. Thus it is an inefficient symbiont interms of plant dry matter produced per unit nodule mass. Nellikuppam inoculum of Cuddalore district, however was associated with plants that averaged the fourth highest total dry weight which is relatively high when compared with its sparse nodulation indicating a much higher degree of efficiency. The most generally effective inoculum source, in terms of nodulation was Uthiramerur soil sample. The least effective, averaged overall sources was Thiruvanaikaval of Trichy District.

Table 3. Relationship between soil properties and nodulation as well as per cent mycorrhizal colonization of *Casuarina equisetifolia* grown in soils collected from different parts of Tamil Nadu.

Soil factors	Y	Regression equation	Correlation co-efficient (r)
pH	Nodule number	38.62+0.877x	-0.101
	% VAM infection	37.797+9.9176x	+0.328
EC	Nodule number	40.068-32.838x	-0.149
	% VAM infection	28.79+41.150x	+0.216
N	Nodule number	87.505-0.3027x	-0.793
	% VAM infection	23.806-5.489x	-0.032
P_2O_5	Nodule number	0.600+2.181x	+0.849
	% VAM infection	35.785+0.9626x	-0.576
K_2O	Nodule number	87.091-0.2317x	-0.675
	% VAM infection	14.221+0.0331x	+0.223

The wide variation in the occurrence of nodulation probably reflects the effects of soil factors on host plant growth and the presence or absence of infective *Frankia* populations in the soil. Soil P level may be critical in regulating nodulation of *C. equisetifolia* seedlings. Moisture stress (Kant and Narayana, 1978), soil temperature (Reddell *et al.*, 1985), deficiencies of nutrients other than N and P (Robson and Honeragon, 1978) and low soil pH (Bond, 1957) may also inhibit nodulation. These factors may operate independently or in combination to limit host plant growth.

There was a significant negative correlation (P>0.05) between available nitrogen content of the soil and nodule number of the seedlings (Table 3). Such correlation suggests that the overall availability of nitrogen is important determinant of growth, and that plant preferentially assimilates combined nitrogen when it is available from soil over symbiotically fixed nitrogen (Arnone and Gordon, 1990). However, there was a significant (P<0.05) positive correlation between available phosphorus content of the soil and nodule number of the seedlings. This may explain that low soil P status prevents nodulation and indicate that the addition of P accelerates nodulation. This suggests soil P levels may be critical in regulating nodulation (Reddell *et al., 1986).*

The host, endophyte and their interaction were all factors responsible for the observed variation in plant growth and nodulation. Thus, the potential exists for improving the effectiveness of the association by selection of the symbiotic partner. The results suggest that if selection for Frankia is to proceed then isolates for testing should first be obtained from the same region and the performance of *C. equisetifolia* seedlings can be greatly enhanced by inoculation of nursery seedlings lacks *Frankia*. It is likely that the greatest benefits of any inoculation would be gained from the use of Frankia which can be introduced with minimal technology.

VA-mycorrhizal seedlings

As with soil fertility, the VA-mycorrhizal spore population and mycorrhizal colonization varied among the various soil samples (Table 4). The soil collected from Coimbatore locations was especially vigorous where it produced the highest spore population and percentage mycorrhizal colonization more than twice as great as those produced by the Cuddalore soil samples.

The extensiveness of infection and spore population recorded by Coimbatore soils suggest a greater capability for infection possibly because of lower available phosphorus in this soil or other factors involved in the association of the VAM fungi with its native soil (Porter *et al.*, 1987). The relatively poor mycorrhizal infection, spore population observed in *C. equisetifolia* seedlings grown in Cuddalore soils samples may suggest a negative effect due to the high available phosphorus content of the soil. Similar negative effects of high soil phosphorus on VAM infection have been noticed (Mosses, 1973). However some reports of high VAM infection at high concentration of available phosphorus do exist (Porter *et al.*, 1978). Similar results occurred in the present study for seedlings grown in Nagapattinam soil samples.

It was found that, there was a significant negative correlation between available nitrogen content (r=-0.032) and available phosphorus content (r=-0.576) with percentage mycorrhizal

Table 4. Occurrence of VA-mycorrhizal fungal spore load and infection in seedlings of *Casuarina equisetfoliar* grown in soils colelcted from different parts of Tamil Nadu

District/Location	VA-mycorrhizal spores (No/100 ml)	VA-mycorrhizal infection (%)
Chennai		
Thiruvelangadu	36	16.2
Sadras	39	26.5
Thiruporur	42	20.3
Thiruvurkuppam	41	18.5
Kanchipuram		
Uthiramerur	39	25.4
Cuddalore		
Pudhusathiram	24	10.1
Nellikuppam	21	10.2
Thiruvengadu	23	9.8
Parangipettai	21	9.4
Neyveli	20	10.3
Villupuram		
Marakanam	26	12.7
Nagapattinam		
Velanganni	39	23.0
Vedharanyam	40	21.7
Seergazhi	49	29.9
Vaidheswarankovil	53	30.2
Trichy		
Srirangam	52	34.0
Thiruvanaikaval	49	26.6
Perambalur		
Perambalur	33	17.5
Pudukkottai		
Thirumayam	47	19.3
Northamalai	32	21.6
Coimbatore		
TNAU Campus	59	40.3
Karumathampatti	52	33.1
Mettupalayam	57	35.9
CD	3.881	3.433

colonization (Table 3). Here it is not clear whether this fungus is specific to host or to soil type and habitat. Many different considerations of soil characteristics may determine the infection capacity of VAM fungi. The central question posed in this study was whether nursery grown seedlings were adequately provided with symbionts at the time of outplanting. Seedlings from some nurseries were well endowed with VAM that have performed well in the field. Other seedlings, completely lacked mycorrhizae and inoculation might improve the field performance of these seedlings ; the degree of improvement depending on the planting site.

The result of this study suggests that Casuarina seedlings remain symbiont free for long periods of time in nurseries, a range of opportunities exist for artificial inoculation. Specific symbionts can be introduced into growing media prior to planting or inoculation delayed until the seedlings are moved outdoors where fertilization is reduced and the plants rendered more conducive to infection by appropriate VA-mycorrhizal fungi and Frankia.

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NEW JASSID RESISTANT COTTON VARIETY KC.2 (KOVILPATTI CAMBODIA-2)

D. KRISHNA DOSS, A. RAJAMANI, R. RAVIKESAVAN, N. MUPPIDATHI, R. SANKARA PANDIAN, S. SURESH and V. RAJARAM

Agricultural Research station,
Kovilpatti.

ABSTRACT

A new cambodia cotton variety KC.2 combining moderate resistance to leaf hopper, high yield and suitable for cultivation in rainfed black soil tract of Tuticorin, Tirunelveli and Virudhunagar districts of Tamil Nadu during North-East Monsoon was released in January 1997. This cultivar was developed from a cross involving MCU.10, a black arm resistant variety and KC.1, a jassid resistant strain. It has an average seed cotton yield of 772 kg/ha under rainfed condition which is 18% increase over LRA. 5166 and 11% increase over MCU.10. It has got a good ginning outturn of 37.5% and fibre lenght of 24.4 mm. It comes under medium staple category. The variety KC.2 has a good CSP value of 2412 for nominal 40's count as against standard CSP value of 2040. It has got a high yielding potential of 23.65 q/ha under favourable conditions.

KEY WORDS: Cambodia cotton, KC.2, Leaf hopper, Resistance

Cotton is an important commercial crop and it is considered as "Queen among fibres". Cotton earns foreign exchange of about Rs. 150 crores annually by export of sizable quantity of long and extra long staple cotton in India. But, our requirement of medium staple cotton is more. Though India occupies first place in world cotton area, it stands only in the third place with reference to world cotton production.

The reason for low production is mainly due to the fact that cotton crop is being cultivated as rainfed crop in about 60 to 70% of cotton area in India. In Tamil Nadu, cotton is cultivated in about 2.25 lakhs hectares with an annual production of about 5 to 7 lakhs bales of lint. We have to step up medium staple cotton production especially under rainfed condition to obtain the targetted produciton. In cotton, leaf hopper (Jassid) is a serious pest which limits the crop growth and yield considerably. Most of the farmers in rainfed cotton area are not taking up proper protection measures in the early stages of crop growth which result in low production.

Therefore, with a main objective of evolving a jassid resistant and medium staple Cambodia (*Gossypium hirsutum*) cotton strain with high seed cotton yield, tolerance to drought and suitable for