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CROSS INOCULATION STUDIES IN *Casuarina equisetifolia*

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ABSTRACT

An experiment was conducted to identify effective Frankia strain that established good nodulation for higher biomass production in *Casuarina equisetifolia* through cross inoculation of Frankia strains from four different hosts *viz., C. junghuhniana, c. cunninghamiana, Alnus nephalensis* and *Myrica gale*. The result of plant biomass production and nodulation assay revealed that the superior performance of *C. junghuhniana* source over other sources tested; on the other hand *A. nephalensis* and *M.gale* showed nil performance, but *C.cunninghamiana* source average performance over control.

KEY WORDS : Casuarina, , Nitrogen Fixation, Cross Inoculation

The actinomycete Frankia infect woody species, limited to eight non-leguminons plant families (Dixon and Wheeler, 1986). The infectivity of Frankia varies considerably from different plant genera. Based on cross inoculation studies Baker, (1987) identified four cross inoculation groups. The fast growing isolates of certain microbes help to nodule effectively in some other hosts (Basak and Goyal, 1980) which otherwise known for higher productivity. However, such evidence are limited and scanty in Frankia. A concerted effort was made to identify consolant strain for *C.equisetifolia.*

MATERIALS AND METHODS

Nodule collection

The experiment considered five typical actinorhizal trees age group of 2.5-3 year old as cross inoculation source. Active light colour nodules fully composed of young tissue were collected from *Casuarina cunninghamiana, C.junghuhniana, Alnus nephalensis, Myrica gale.* The collected nodules were immediately packed in plastic bags on ice and stored in the laboratory below -10^{0}C and subsequently used for inoculation.

Nodule suspension

Stored nodules were thoroughly washed in tap water to eliminate adhering soil particles and unwanted materials. Under aseptic condition, nodules were washed with sterile water and surface sterilized with 30 per cent hydrogen peroxide for 5 minutes and 3 minutes respectively. Thirty g nodule was crushed for nodule suspension using pestle and mortor with 100 ml sterile distilled water. Uniform aged aseptically grown *Casuarina equisetifolia* seedling roots were dipped in the nodule suspension for 5-10 minutes and subsequently planted in the steam sterilized pot mixture. The pot mixture was prepared by thoroughly mixing sand, soil and farm yard manure at 1:1:1 ratio. To have a better

Table 1. Cross inoculation effect on seedling productivity in Casuarina

Treatment	Shoot length (cm)	Root length (cm)	Total plant biomass g/plant (Dry weight)	Total chlorophyll mg. g-1 F.W.
Casuarina equisetifolia	92.53	38.99	12.39	5.62
C. junghuhniana	89.98	37.00	10.88	5.45
C. cunninghamiana	52.21	20.18	7,00	4.00
Alnus nephalensis	23.00	7.00	4.05	2.12
Myrica gale	22.30	8.99	4.28	1.82
Control	21.84	9.50	3.79	1.26
CD (5%)	2.60	1.92	2.43	1.20

Table 2. Cross inoculation effect on Casuarina nodulation

Treatment	Nodule number	Nodule dry weight (mg)	Nodule diameter (cm)	Nodule nitrogenase activity µ. mol. C3H3. g-1.h-1
Casuarina equisetifolia	16.21	4.62	0.89	2.89
C. junghuhniana	14.98	4.07	0.80	2.49
C. cunninghamiana	6.17	1.89	0.35	1.72
Alnus nephalensis	3.38	0.51	0.49	0.67
Myrica gale	2.93	0.38	0.18	0.29
Control	2.89	0.15	0.19	0.21
CD (5%)	1.5	0.62	0.12	0.50

understanding, *C.equisetifolia* nodules were also used besides uninoculated control.

Six months after inoculation, the cross inoculation effect and difference among cross inoculation sources were examined by estimating various plant growth characters, *viz.*, shoot root length, plant biomass and total needle chlorophyll content (Yoshida *et al.*, 1971). The infectivity capabilities of each source was evaluated interms of nodule number, nodule dry weight, nodule size, nodule nitrogenase activity (acetylene reduction method by Hardy *et al.*, 1968). Total nodule and plant nitrogen were estimated using mecrokjeldahl method as suggested by Humphries (1956).

RESULTS AND DISCUSSION

It is a pertinent (Table 1) to observe that the superior performance of *C.junghuhniana* source, effected good seedling growth by recording higher

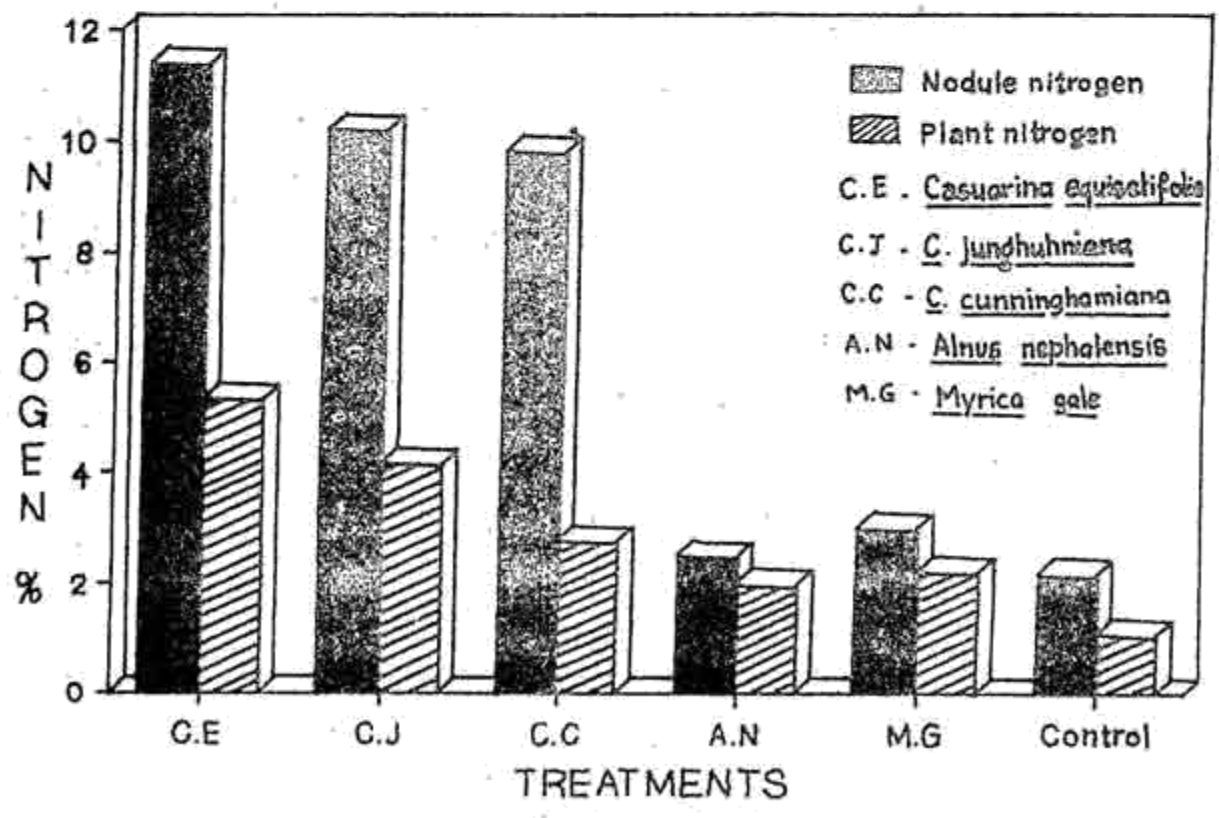

Fig.1. Cross inoculation and nitrogen builtup in Casuarina

shoot length (89.98 cm) root length (37.00 cm), higher dry matter production (10.88 g/plant) and thus showing on par performance with *C.equisetifolia* source. When compared to control *A.nephalensis* and *M.gale* sources had no special effect. However *C.cunninghamiana* source exhibited a recalcitrency trend between control and *C.equisetifolia* but had a definite infectivity effect, reflected advantageous performance over control. The infectivity assay (Table 2) also explained similar trend recording higher nodulation (14.98), nodule dry weight (4.07 mg) and nodule diameter (0.80 cm) by *C.cunninghamiana* source. All Frankia inoculation ultimately helped higher biomass built up in plants. The result (Fig.1) indicated higher nitrogen fixation in root nodule (10.19%) and subsequently helped higher nitrogen build up in the plant (3.82%) by *C.junghuhniana* source which is on par with *C.equisetifolia* source. The result proved wider infectivity variation among cross inoculation groups, further confirmed the heterogenity infectivity existence of Frankia strains (Wheeler *et al.*, 1991). Similar family effect (kohls *et al.*, 1994) might have helped higher cross- infectivity in the *C.cunninghamiana* source besides their similar infection pattern (Miller and Baker, 1986) on the other hand the average performance of *C.junghuhniana* source possibly due to lower proportion of high infectivity Frankia cells (Van Dijk and Sluimer-Stolk, 1990) coupled with possession of partial compatible Frankia strains (Torrey, 1990) towards *C.equisetifolia*.The conservative response from *A.nephalensis* and *M.gale* source may contemplated as geographical reason (Collected from temperate region) the lack of infection further emphasised the need to understand the infection process in this group (Torrey, 1988). This study investigated some pattern of microsymbiont-host specificity for *C.equisetifolia* and subsequently recommended the *C.cunninghamiana* host as a alternate for *C.equisetifolia* helping for higer productivity.

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