Influence of Tripartite Interactions of VAM Fungi, *Frankia* and *Casuarina equisetifolia* on Growth and Nutrient Accumulation in Host under Nursery Conditions

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Abstract

Pot culture experiments were carried out in non-pasteurised soil to determine the effects of six different VAM fungi such as *Gigaspora margarita*, *Glomus deserticola*, *G. fasciculatum*, *G. mosseae*, *G. monosporum* and *G. versiforme* individually as well as in combinations with *Frankia* sp. on the seedling growth of *Casuarina equisetifolia*. Inoculation of VAM fungi or *Frankia* sp. increased the growth and biomass. Among the different VAM fungi, *Glomus fasciculatum* followed by *G. versiforme* significantly enhanced the growth, stem girth and biomass. Inoculation with *G. monosporum* increased mycorrhizal infection and spore number in the soil. Co-inoculation with *Gigaspora margarita* and *Frankia* sp. significantly increased root colonization and spore number. Nodule number and nodular biomass were increased with the inoculation of VAM fungi. They were further increased by inoculations with *Frankia* sp. with a maximum in *G. mosseae* and *Frankia* sp. inoculated seedlings. Inoculation with *Gigaspora margarita* individually or in combination with *Frankia* sp. significantly increased tissue P and K concentration. Significant increase of N concentration was observed in *Glomus monosporum* inoculation.

সারসংক্ষেপ

Casuarina equisetifolia-এর চারার বৃদ্ধিতে ছয়টি ভিন্ন ধরনের VAM ছত্রাক যথা : *Gigaspora margarita*, *Glomus deserticola*, *G. fasciculatum*, *G. mosseae*, *G. monosporum* and *G. versiforme* -এর প্রতিটি আলাদাভাবে অথবা *Frankia* প্রজাতির সাথে একত্রে প্রয়োগের ফলাফল নির্ণয়ের জন্য পাস্তুরিত নয় এমন মাটি দিয়ে পট কালচার পরীক্ষা চালানো হয়। VAM ছত্রাক অথবা *Frankia* -এর ইনোকুলেশানের ফলে চারার বর্ধন এবং বায়োমাস বেড়ে যায়। বিভিন্ন VAM ছত্রাকের মধ্যে *G. fasciculatum* সর্বাধিক এবং পরবর্তীক্রমে, *G. versiforme* চারার বর্ধন, কাণ্ডের বেড় এবং বায়োমাসের পরিমাণ উল্লেখযোগ্যভাবে বাড়িয়ে দেয়। *G. monosporum*-এর ইনোকুলেশান মাইকোরাইজাল সংক্রমণে এবং মাটিতে বীজাণু বৃদ্ধিকে তরান্বিত করে। *Gigaspora margarita* এবং *Frankia* প্রজাতির যৌথ-ইনোকুলেশন শিকড়ে এদের বেশি হারে উপস্থিতি (colonization) এবং বীজাণুর সংখ্যা উল্লেখযোগ্যভাবে বৃদ্ধি করে। VAM ছত্রাকের ইনোকুলেশনের ফলে নড্যুলের সংখ্যা এবং নড্যুলের বায়োমাস বৃদ্ধি পায়।

G. mosseae এবং *Frankia* প্রজাতি দ্বারা যে সব চারা ইনোকুলেশন করা হয়েছিল সেগুলোতে পুনরায় *Frankia* প্রজাতি ইনোকুলেশন করে নড্যুলের সংখ্যা ও বায়োমাস বৃদ্ধি সর্বোচ্চ পরিমাণে হয়। *Gigaspora margarita* স্বতন্ত্রভাবে কিংবা *Frankia* প্রজাতির সাথে যৌথভাবে ইনোকুলেশনের ফলে টিস্যুতে ফসফরাস এবং পটাসিয়াম-এর পরিমাণ উল্লেখযোগ্যভাবে বৃদ্ধি পায়। *Glomus monosporum* ইনোকুলেশন-এর মাধ্যমে নাইট্রোজেনের পরিমাণ উল্লেখযোগ্য বৃদ্ধি পায়।

Key words : *Casuarina equisetifolia*, *Frankia* sp., inoculation, VAM fungi

Introduction

Actinorrhizal plants such as *Casuarina* form symbiosis with the N_2-fixing actinomycete *Frankia* and with vesicular-arbuscular mycorrhizal (VAM) fungi (Vasanthakrishna *et al.* 1994, Sampavalan *et al.* 1995). The importance of interactions between these associations for promotion of growth of *Casuarina* in soils deficient in mineral nutrients is well established from experiments with inoculated plants in the laboratory and field (Diem and Dommergues 1990). Different species of VAM fungi vary widely in their ability to stimulate crop production (Bagyaraj *et al.* 1989). Vasanthakrishna *et al.* (1994) screened several VAM fungi in order to pick an efficient fungus for inoculating *Casuarina equisetifolia*. However, there are some reports emphasizing the need to select suitable VAM fungi in different agro-climatic zones. Hence, the present study was undertaken to find out the possibility of getting efficient VAM fungi and *Frankia* in *Casuarina equisetifolia* Forst. for sandy loam soil.

Materials and methods

Soil

The soil was sandy loam having pH 8.0 (1:2.5; Soil : water), electric conductivity 0.2 $mScm^{-1}$, nitrogen 10.9, phosphorus 0.5 and potassium 24.2 $mg.kg^{-1}$. The total N and available P were determined respectively by microkjeldahl and molebdenum blue method of Jackson (1973). Exchangeable K was extracted from the soil in an ammonium acetate solution (pH=7.0) and measured with a digital flame photometer (Jackson 1973).

Plant material

Seeds of *Casuarina equisetifolia* were sown for germination in autoclaved sand. Uniform, healthy 30-day-old seedlings were transplanted to 10 cm x 15 cm polythene bag containing 3 kg unsterilized soil. The soil had natural VA mycorrhizal (*Acaulospora scrobiculata*, *Glomus fasciculatum*, *G. geosporum* and *G. mosseae*) population of 72 spores 100 g^{-1} soil.

VAM fungi

Soil : sand mixture containing spores and infected root segments of *Sorghum vulgare* infected with *Gigaspora margarita* Becker & Hall ; *Glomus deserticola* Trappe, Bloss & Menge ; *G. fasciculatum* (Thaxter Sensu Gerd.) Gerd. & Trappe ; *G. mosseae* or *G. versiforme* (Karsten) Berch served as the inoculum. The inoculum potentials of the VAM cultures were determined by the most probable number (MPN) method outlined by Porter (1979). Inoculum was added at the rate of 24,000 infective propagules per polythene bag. The quantity of soil based VAM inoculum added for each species are as follows : *Gigaspora margarita* (20 g), *Glomus deserticola* (17.14 g), *G. fasciculatum* (30 g), G. *mosseae* (2.52 g), G. *monosporum* (8.88 g) and *G. versiforme* (26.66 g).

Frankia

Fresh *Frankia* nodules (5 g), collected from *Casuarina equisetifolia* growing in the field, were washed free of soil particles with tap water, surface sterilized with 70% alcohol for 1 minute and washed several times with sterile distilled water. The nodules were crushed in a mortar and pestle, filtered through Whatman No. 1 paper, and 10 ml of the suspension was added to each polybag.

Harvesting and measurements

Growth parameters such as root length, shoot length, stem girth, nodule number and plant dry weight were recorded after 150 days from the date of sowing. Root sub-samples were processed for microscopic observations following the procedure of Phillips and Hayman (1970), and the percentage mycorrhizal infection was determined by the root slide technique of Read *et al.* (1976). Spore population of each soil sample was estimated by a modified wet sieving and decanting technique of Gerdeman and Nicolson (1963). Total kjeldahl nitrogen was detected on a kjeltec Auto analyser (1030). Phosphorus determination was done by vanadomolybdate phosphoric yellow colour method (Jackson 1973). Potassium content was determined by Flame Photometer (Jackson 1973).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and the means were separated by Duncan's Multiple Range Test.

Results

Growth and biomass

Growth in *Casuarina equisetifolia* seedlings as measured by root and shoot lengths and their dry weights were invariably enhanced by VAM fungi and *Frankia* sp. inoculation, though the increases were not always statistically significant. Among the different VAM fungi, *Glomus fasciculatum* followed by *G. versiforme* significantly enhanced the growth, stem girth and biomass compared to control. Root to shoot ratio also significantly increased in *G. fasciculatum* and *G. versiforme* inoculation (Table 1). Seedlings co-inoculated with *Gigaspora margarita* and *Frankia* sp. had significantly enhanced growth, stem girth and biomass compared to single inoculation of either VAM fungi or *Frankia* sp. (Table 1).

Table 1. Interaction of different VAM fungi and *Frankia* sp. on the growth and biomass of *Casuarina equisetifolia*.

Treatments	Root length (cm)	Shoot length (cm)	Stem girth (mm)	Dry weight (mg. plant $^{-1}$)		R/S ratio
				Root	Shoot	
Control	15.9 e	19.5 h	5.0 d	27 c	244 g	0.11 c
GMRG	24.2 bcde	31.0 efgh	10.4 bcd	83 bc	678 efg	0.12 bc
LDST	23.2 cde	29.9 fgh	10.0 bcd	83 bc	655 efg	0.12 bc
LFSC	28.3 bcd	36.3 bcdefg	12.4 abc	136 ab	796 cdef	0.17 a
LMSS	20.6 cde	29.4 fgh	9.4 bcd	67 bc	530 fg	0.12 bc
LMNS	25.1 bcd	32.0 defgh	11.6 abc	93 bc	730 def	0.12 bc
LVSF	26.8 bcd	33.8 cdefg	11.6 abc	128 abc	767 cdef	0.16 a
GMRG + *Frankia*	38.0 a	55.4 a	17.6 a	227 a	1499 a	0.15 ab
LDST + *Frankia*	30.0 abc	45.5 abc	15.6 ab	203 a	1230 abc	0.16 a
LFSC + *Frankia*	28.8 bcd	44.2 abcde	14.6 ab	163 ab	1029 bcde	0.15 ab
LMSS + *Frankia*	32.8 ab	48.4 ab	15.8 ab	205 a	1269 ab	0.16 a
LMNS + *Frankia*	29.0 abcd	45.0 abcd	14.8 ab	199 a	1200 abcd	0.16 a
LVSF + *Frankia*	28.4 bcd	40.8 bcdef	13.6 ab	152 ab	909 bcdef	0.16 a
Frankia	20.1 de	26.5 gh	6.8 cd	66 bc	463 fg	0.14 abc

GMRG-*Gigaspora margarita*; LDST-*Glomus deserticola*; LFSC-*Glomus fasciculatum*; LMSS-*Glomus mosseae*; LMNS-*Glomus monosporum*; LVSF-*Glomus versiforme*. Means within a parameter followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

VAM root colonization, spore number and nodulation

The number of mycorrhizal spores, root colonization and nodulation are presented in Table 2. Although the native mycorrhiza in unsterile soil caused 12% infection, inoculation with *G. monosporum* increased both mycorrhizal infection and the number of spores in the soil. However, the changes in the root colonization and sporulation in the experiment were not statistically significant. Whereas, co-inoculation with *Gigaspora margarita* and *Frankia* sp. significantly in creased root colonization and sporulation compared to single inoculations (Table 2). Nodule number and nodular biomass increased with VAM fungi inoculations. They were further increased by inoculations with *Frankia* sp. with a maximum in *G. mosseas* and *Frankia* sp. inoculated *Casuarina* seedlings (Table 2.).

Table 2. Interaction of different VAM fungi and *Frankia* sp. on nodule number, nodule mass, spore count and root colonization of *Casurina equisetifolia*.

Treatments	Nodule number (no. plant $^{-1}$)	Nodule mass (mg.plant $^{-1}$)	Spore count per 10 g. soil	Root colonization (%)
Control	2.0 f	8 f	65 h	12.0 e
GMRG	8.4 e	36 de	222 de	22.3 de
LDST	8.0 e	37 de	248 bcd	36.4 cde
LFSC	9.9 bcde	38 cde	236 cde	32.1 cde
LMSS	7.5 e	34 e	232 de	40.0 cde
LMNS	9.5 cde	37 de	250 bcd	41.3 bcde
LVSF	9.0 de	35 e	175 ef	38.6 cde
GMRG+ *Frankia*	15.0 ab	54 ab	325 a	78.5 a
LDST+ *Frankia*	13.8 abcd	52 abc	275 abcd	61.0 abc
LFSC+ *Frankia*	14.3 abc	50 abcd	307 abc	63.7 abc
LMSS+ *Frankia*	15.3 a	55 a	320 ab	75.2 a
LMNS+ *Frankia*	14.5 abc	53 abc	315 ab	72.8 ab
LVSF+ *Frankia*	14.0 abcd	48 abcde	293 abcd	53.9 abcd
Frankia	10.0 bcde	40 bcde	120 fg	17.1 e

GMRG-*Gigaspora margarita* ; LDST - *Glomus deserticola* ; LFSC-*Glomus fasciculatum;* LMSS-*Glomus mosseae;* LMNS-*Glomus monosporum* ; LVSF-*Glomus versiforme.* Means within a parameter followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

Table 3. Interaction of different VAM fungi and *Frankia* sp. on nutrient concentration of *Casuarina equisetifolia*.

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	1.30 d	1.82 c	0.001 b	0.07 d	0.02 d	0.20 e
GMRG	1.38 bcd	2.08 abc	0.009 b	0.13 bcd	0.09 abcd	0.33 abcd
LDST	1.46 abcd	2.06 abc	0.003 b	0.11 bcd	0.08 abcd	0.31 abcd
LFSC	1.38 bcd	1.96 abc	0.006 b	0.13 bcd	0.06 bcd	0.26 cde
LMSS	1.37 bcd	1.91 bc	0.003 b	0.10 bcd	0.06 bcd	0.23 de
LMNS	1.47 abcd	1.99 abc	0.03 ab	0.14 abcd	0.07 abcd	0.28 bcde
LVSF	1.36 cd	1.89 bc	0.002 b	0.09 bcd	0.05 cd	0.21 de
GMRG+*Frankia*	1.61 a	2.25 a	0.100 a	0.26 a	0.16 a	0.51 a
LDST+*Frankia*	1.59 ab	2.26 a	0.08 ab	0.20 abc	0.15 ab	0.50 ab
LFSC+*Frankia*	1.58 ab	2.25 a	0.06 ab	0.16 abcd	0,13 abc	0.46 abc
LMSS+*Frankia*	1.57 abc	2.21 ab	0.06 ab	0.18 abcd	0.12 ab	0.46 abc
LMNS+*Frankia*	1.63 a	2.28 a	0.09 a	0.21 ab	0.14 abc	0.48 abc
LVSF+*Frankia*	1.56 abc	2.18 ab	0.03 ab	0.15 adcd	0.11 abcd	0.42 abcd
Frankia sp.	1.52 abcd	2.13 abc	0.002 b	0.08 cd	0.10 abcd	0.36 abcd

GMRG-*Gigaspora margarita* ; LDST-*Glomus deserticola* ; LFSC-*Glomus fasciculatum* ; LMSS- *Glomus mosseae;* LMNS- *Glomus monosporum* ; LVSF-*Glomus versiforme.* Means within a parameter followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

Tissue N, P and K concentration

Seedlings inoculated with *Gigaspora margarita*, individually or in combination with *Frankia* significantly increased tissue P and K concentration. Inoculation of *Glomus monosporum* caused significant increase in tissue N concentration (Table 3).

Discussion

Seedlings inoculated with VAM fungi generally had higher growth, dry weight and tissue nutrient concentrations. This response suggests that indigenous VAM fungi were present only in low numbers or that they were ineffective or both. However, the number of spores present in the soil under study (72 spores 100 g^{-1} soil) indicates that the response to inoculation can be mainly attributed to ineffectiveness of the indigenous endophytes. The present study also indicates that the plants varied in their response to inoculation with different VAM fungi, confirming earlier reports of host preferences towards VAM fungi (Vinayak and Bagyaraj 1990). Among the different VAM fungi, *Gigaspora margarita* in the presence of *Frankia* sp. produced significantly higher growth, root colonization and tissue nutrient concentration. The observed responses to inoculation with *Gigaspora margarita* can be ascribed to better compatibility and establishment of the endophyte with *Casuarina*. Mycorrhizal inoculation generally decreases the root to shoot ratio (Mathew and Johri 1989) ; however, several forage grass and crops have shown increased or unchanged root to shoot ratios when colonized by VAM fungi (Saif 1987). Similarly, in the present study, mycorrhizal inoculated plants had higher root to shoot ratios than uninoculated control.

The mycorrhizal incidence was considerably higher in endophyte inoculated seedlings than in uninoculated control. Both the extent of colonization and spore number varied with different fungal species. Seedlings inoculated with *Gigaspora margarita* and *Frankia* sp. had the highest root colonization and spore number. Similar results were reported in *Alnus* (Jha *et al.* 1993). Higher root colonization may favour more fungal-host contact and more exchange of nutrients which might result in better plant growth.

Increased nodulation was observed in plants inoculated with VAM fungi and *Frankia* sp. compared to plants inoculated with single endophyte. Further inoculation of *Frankia* sp. and exotic VAM endophytes favoured nodulation and colonization of indigenous endophytes. These results bring out a synergistic or additive interaction between VAM fungi and *Frankia* sp. with a consequential effect on nodulation and phosphorus uptake in *Casuraina equisetifolia*. Dual inoculation of *Gigaspora margarita* and *Frankia* sp. improved nodulation compared to inoculation of either symbiont alone. This is in accordance with the observations by Diem and Gauthier (1982). The enhanced nodulation found in the mycorrhizal plants can be attributed to the increased uptake by these roots, a situation analogous to that in nodulated mycorrhizal legumes (Bethlenfalvay and Yorder 1981).

The VAM fungi that improved plant biomass were also increased phosphorus concentration of the host. Inoculated plants had higher concentration of tissue P compared to uninoculated plants. Inoculation of *Gigaspore margarita* and *Frankia* sp. increased tissue P concentration. This can be attributed to the increased absorbing surface area due to the extensive extramatrical network of mycelium produced by the VAM fungi in association with the host root system (Howler *et al.* 1981). Stribley (1987) reported that P seems to be the most important nutrient involved, other nutrients such as N and K are translocated along VAM hyphae. The present study in addition to confirming above findings adds that uptake of N and K by plants is greatly influenced by the VAM fungal species colonizing the root. Besides, this increasing concentration of tissue N by direct absorption of extramatrical hyphae may also increase indirectly through association of *Frankia* sp.

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