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Growth Response of *Dendrocalamus* Seedlings by Inoculation with Ectomycorrhizal Fungi

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Abstract: The response of out planted seedlings to inoculation may be positive, neutral, or negative depending on environmental factors or on the ECM species or host plant used. Growth responses following seedling inoculation of *Dendrocalamus* under controlled conditions with *Cantharellus tropicalis* have been observed in this study. Mycorrhizal roots of *laboratory grown seedlings* were longer than un-colonized roots, whereas in the *nursery grown plantlets* the effect of inoculation was discontinuous. Mycorrhizal colonization can also be viewed as "drought avoidance" in dry soil conditions as stressed conditions are avoided through hyphal penetration as shown in the present study.

Key words: *In vitro* mycorrhization · *Dendrocalamus* · *Cantharellus* · Drought tolerance

INTRODUCTION

Species of ECM mushroom have been used in a number of studies designed to evaluate the survival and growth of out planted ECM tree seedlings. The response of out planted seedlings to inoculation can be positive, neutral, or negative depending on environmental factors or on ECM species or host plant used. This symbiosis can increase plants acquisition of nutrients and water, improve plants ability to resist pathogens [1] and, ultimately, increase plant biomass production in a number of plants such as *Shorea*, *Pinus*, *Abies*, *Pseudostuga*, *Larix*, *Eucalyptus* due to mineral uptake by roots in presence of fungal associate with plant. Growth responses following seedling inoculation under controlled conditions with *Pisolithus* have been repeatedly observed [2]. Growth enhancement of seedlings in nurseries and *in vitro* experiments has also been repeatedly reported after inoculation with *Rhizopogon* species [3]. Plant growth responses have been extensively studied using different ECM mushrooms *viz. Tuber, Lactarius, Laccaria, Scleroderma, Cenoccocum, Thelephora, Cantharellus, Paxillus, Amanita, Hebeloma, Suillus* etc. Although, ECM are commonly assumed to enhance water uptake by their hosts [4], few researchers have addressed this experimentally. Some mycorrhizal fungi grown *in vitro* grow or at least survive at water potentials below the permanent wilting point of their host, although tolerance to low water potentials varies widely among species [5]. Controlled mycorrhization has already proved to be beneficial to other trees *viz. Eucalytus, Pinus* etc. Little information is available on the growth performance of monocot plants with ECM fungal association and utilization.

The bamboo plants cover large portion of tropical forests and show social and economic relevance by the exploitation of young culm as food and wood for handcraft, furniture, building houses, packing boxes etc. Bamboo seedlings are planted extensively on reforestation sites during rainy season in forests. First year mortality of these seedlings can approach as high as 100% on harsh sites. As a consequence, the main limiting factor for increase in productivity is mineral nutrition. *Cantharellus tropicalis* Rahi, Rajak, Pandey is a delicious edible species belonging to broad group of chanterelle mushrooms [6] forming ectomycorrhiza with *Dendrocalamus. C. tropicalis* is a high temperature species which appears in late rainy season from late August to September. An attempt has been made to evaluate the effect of ECM fungus on *D. strictus* and other members of bamboo group inoculating with *C. tropicalis* in pot experiments.

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MATERIALS AND METHODS

The experiments were conducted with bamboo plants. The plant growth and nutrient uptake with ECM fungal (*C. tropicalis*) inoculation was assessed in pot culture under glasshouse conditions. For the experiment sterilized soil and sand mixture (1:1) was used. It was taken in sterilized earthen pot of 12kg capacity. Sterilized seeds of *D. strictus* were germinated in earthen pots on steam sterilized soil and sand mixture (1:1v/v) in July 2007. Uncontaminated seedlings were then transferred into 1800cm^3 polythene bags containing the same sterilized soil mixture. The same experiments were also performed taking nursery grown one year old plantlets. Different parameters of each plantlet were measured when planted in fresh bags and again recorded at 4 months. All the data were statistically analyzed.

Two treatments were designed each for pot grown seedlings and nursery grown plantlets-

- Effect of ECM on growth of bamboo plants.
- Effect of ECM on drought tolerance of bamboo plants.

Mycelial inoculum of *C. tropicalis* in sand + tea leaves was prepared as follows: inoculum of fungus was grown aseptically in 250ml Erlenmeyer flasks containing 200ml sand + used tea leaves supplemented with 50ml distilled water. The flasks were incubated at 25±2^{O}C in dark. After 4 weeks, inoculum was removed from the flasks. Fungus free sand + soil mixture served as control. No fertilizers were applied throughout the study. Soil was filled in plastic bags (about 2kg/bag). Inoculation with the ECM fungus was done when the plantlets were transferred into the polythene bags 50cm^3 of inoculum was added in the transplanting hole, in contact with the roots.

To observe the effect of drought, one set of inoculated and un-inoculated seedlings were watered daily for four months. Four months after planting, randomly selected seedlings from each treatment were harvested, root system was rinsed free of soil and examined for percent root colonization by ECM fungus. The remaining set of non-mycorrhizal seedlings and seedlings inoculated with *Cantharellus tropicalis* were allowed to desiccate and then re-watered. Their ability to tolerate and recover from drought was compared. Seedlings were allowed to dry out for 4 days before being re-watered. Day 1 was the last day in which all seedlings were watered. The stressed seedlings were not watered until day 5 but were watered daily thereafter during the recovery period (days 6 to 10). At the end, growth parameters were recorded. The growth characteristics in respect of biomass increase and mean rate of dry matter production were calculated. The percentage mycorrhizal infection and colonization were measured after cleaning and staining the root bits by the method of Phillips and Hayman [7].

RESULTS

In the *laboratory grown seedlings* of *D. strictus,* the root examinations showed that *C. tropicalis* was able to form mycorrhizas with *D. strictus* covering entire or tip of root. Inoculations of *C. tropicalis* improved the seedling growth and mycorrhizal development when compared to un-inoculated seedlings. The percentage of mycorrhizal short roots was recorded in *C. tropicalis* inoculated seedlings. The shoot height and root length significantly (P<0.05) increased by inoculation of *Cantharellus* mycelia in soils when compared to un-inoculated seedlings. The shoot and root dry weights and shoot/root ratio was more in *C. tropicalis* inoculated seedlings (Table 1). Although, mycorrhizal plants were consistently larger than non-mycorrhizal ones, mycorrhizal development was 66% and 0% for seedlings grown in inoculated and un-inoculated pots. In the *nursery developed plantlets* of four bamboo species, the root examinations showed that *C. tropicalis* was able to form mycorrhizas with *D. strictus, D. asper* and *Bambusa nutans* Wall. ex Munro (and less effectively with *B. vulgaris*) as represented in Table 2. Although, the growth-promoting effect on the tree plantlets was discontinuous, this means that *C. tropicalis* was not able to establish stable symbiosis in the conditions of the experiment.

As shown in Table 4, comparison between stressed inoculated and non-inoculated laboratory grown seedlings indicates statistically significant (P<0.05) difference in shoot length, root length, shoot or root dry weight between seedlings grown in inoculated and un-inoculated treatments. The un-inoculated seedlings conditioned to cyclic drought were smaller than seedlings watered daily. Stressed mycorrhizal plants had greater root and shoot dry weight than stressed non-mycorrhizal plants. At 4 months, ECM inoculated (with *C. tropicalis*) and un-inoculated *nursery grown plantlets* watered daily showed significant difference in growth parameters

Table 1: Effect of ECM on growth and mycorrhizal development in seedlings.

S. No	Plant species	Treatments	% colonization	No. of Shoot†	Shoot height†	Root length†	Shoot dry wt†	Root dry wt†	S/R ratio†	No. of leaves†	Leaves dry wt†
	Dendrocal	Inoculated	76.666	5^{i}	49.00^{k}	38.00^{a}	580.00^{d}	1339.33^{f}	0.454^{j}	23.333^{h}	540^{b} 1
	amus			(1.414)	(13.798)	(5.357)	(157.79)	(266.31)	(0.13)	(5.154)	(52.591)
	strictus	Control	0.00	3^{i}	25.00^{l}	35.00^{a}	400.00^{e}	1044.00^{g}	0.413^{j}	20.333^{h}	460^{c}
2	*Bambusa*	Seeds not	-	-		-	-	-	-	-	-
	nutans	obtained	-	-		-	-	-	-	-	-
3	*B. vulgaris*	Seeds not	-	-		-	-	-	-	-	-
		obtained	-	-		-	-	-	-	-	-
4	*D. asper*	Seeds not	-	-		-	-	-	-	-	-
		obtained	-	-		-	-	-	-	-	-

† Results are average dry weight/ length/ number with standard deviation (in brackets) for the experiment. Values within a column followed by same superscript letters do not differ significantly (P< 0.05).

LSD: least significant difference value= 3.816 (No. of shoot); 14.483 (Shoot height); 5.622 (Root length); 64.42 (Shoot dry wt); 279.53 (Root dry wt); 0.142 (S/R ratio); 2.571 (No. of leaves); 55.199 (Leaves dry wt).

Table 2: Effect of ECM on growth and mycorrhizal development in nursery plantlets.

			Shoot Height†*			No. of Branches†*			Number of leaves†*			Number of shoots†*		
S. No	Plant species	Treatments	Mean	±sd	LSD	Mean	±sd	LSD	Mean	±sd	LSD	Mean	±sd	LSD
1	*Dendroca*lamus	Inoculated	7.166^{a}	2.620	2.750	3.000^{c}	1.366	1.434	1.000^{d}	1.471	1.544	1.000^{e}	0.816	0.857
	Strictus	Un-inoculated	4.166^{b}			2.333^{c}			-2.00^{d}			0.333^{e}		
2	*Bambusa*	Inoculated	4.166^{f}	1.949	2.046	1.000^{g}	2.639	2.770	2.333^{h}	3.868	4.060	2.000^{i}	0.547	0.574
	nutans	Un-inoculated	2.833^{f}			3.333^{g}			0.000^{h}			1.000^{j}		
3	*B. vulgaris*	Inoculated	10.33^{k}	8.400	8.817	1.333^{m}	2.529	2.655	-2.00^{n}	3.932	4.127	0.666^{o}	0.516	0.542
		Un-inoculated	9.166^{l}			2.666^{m}			1.333^{n}			0.000^{p}		
4	*D. asper*	Inoculated	4.333^{q}	3.200	3.359	-0.33^{r}	2.957	3.104	0.666^{s}	5.585	5.862	0.666^{t}	0.547	0.574
		Un-inoculated	3.500^{q}			0.666^{r}			1.333^{s}			0.333^{t}		

† Results are average dry weight/ length/ number with standard deviation (in brackets) for the experiment. Values within a column for each plant species followed by same superscript letters do not differ significantly (P< 0.05).

Table 3: Effect of ECM on drought tolerance capacity of seedlings

S.No	Plant species	Treatments	% colonization	No. of Shoot†	Shoot height†	Root length†	Shoot dry wt†	Root dry wt†	S/R ratio†	No. of leaves†	Leaves dry wt†
1	*Dendrocalamus strictus*	Inoculated	67.333	4^{a}(1.643)	54.666^{b}(10.438)	35.000^{d}(3.444)	353.666^{c}(115.81)	990.000^{f}(105.014)	0.370^{g}(0.14)	15.666^{h}(2.581)	246.233^{i}(44.80)
		Control	0.00	5^{a}	39.200^{e}	35.666^{d}	303.666^{e}	877.66^{f}	0.382^{g}	14.000^{h}	254.00^{i}
2	*Bambusa nutans*	Seeds not	-	-		-	-	-	-	-	-
		obtained	-	-		-	-	-	-	-	-
3	*B. vulgaris*	Seeds not	-	-		-	-	-	-	-	-
		obtained	-	-		-	-	-	-	-	-
4	*D. asper*	Seeds not	-	-		-	-	-	-	-	-
		obtained	-	-		-	-	-	-	-	-

† Results are average dry weight/ length/ number with standard deviation (in brackets) for the experiment. Values within a column followed by same superscript letters do not differ significantly (*P*< 0.05).

LSD: least significant difference value= 1.724 (No. of shoot); 10.955 (Shoot height); 3.615 (Root length); 121.557 (Shoot dry wt); 110.223 (Root dry wt); 0.151 (S/R ratio); 2.71 (No. of leaves); 47.029 (Leaves dry wt).

Table 4: Effect of ECM on drought tolerance capacity of nursery plantlets

			Shoot Height[†*]			No. of Branches[†*]			Number of leaves[†*]			Number of shoots[†*]		
S. No	Plant species	Treatments	Mean	±sd	LSD	Mean	±sd	LSD	Mean	±sd	LSD	Mean	±sd	LSD
1	*Dendrocalamus*	Inoculated	1.333^{a}	1.892	1.986	1.000^{c}	5.567	35.063	0.000^{d}	2.338	2.454	0.333^{f}	0.408	0.428
	strictus	Un-inoculated	3.500^{b}			1.000^{c}			-2.66^{e}			0.000^{g}		
2	*Bambusa*	Inoculated	7.666^{g}	5.844	6.134	1.333^{h}	1.366	1.432	2.333^{i}	2.738	2.874	0.666^{k}	0.547	0.574
	nutans	Un-inoculated	6.833^{g}			2.000^{h}			-4.00^{j}			0.333^{k}		
3	*B. vulgaris*	Inoculated	2.333^{l}	1.934	2.030	0.666^{m}	0.752	0.790	2.000^{o}	4.320	4.534	1.000^{q}	1.211	1.271
		Un-inoculated	1.333^{l}			-0.33^{n}			-3.33^{p}			0.333^{q}		
4	*D. asper*	Inoculated	13.66^{r}	8.575	9.000	1.666^{t}	1.095	1.149	2.000^{v}	1.722	1.807	0.666^{y}	0.516	0.542
		Un-inoculated	1.500^{s}			0.333^{u}			-0.33^{x}			0.000^{z}		

† Results are average dry weight/ length/ number with standard deviation (in brackets) for the experiment. Values within a column for each plant species followed by same superscript letters do not differ significantly ($P< 0.05$)

Fig. 1: Efect of C. Tropicalis on growth of D. Stricyus.
a. D. Strictus seedlings grown in plastic pots colonized by C. Tropicalis mycelia, **b.** Enlarged colonized roots, **c.** Seedlings transferres to earthern pots, **d.** Infected seedling after 5-6 months, **e.** Infected roots harvested from pots, **f.** ECM roots,

(number of shoots, number of branches, number of leaves and height of plant) with *D. strictus, Bambusa nutans, D. asper* and non-significant with *B. vulgaris* (Table 4, Fig. 1).

DISCUSSION

In the present study, mycorrhizal roots of *laboratory grown seedlings* were longer, which led to increase in

absorptive surface area compared with non-mycorrhizal roots. Whereas, in the *nursery grown plantlets,* the effect of inoculation was discontinuous. Since the growth stimulation was not uniform, the introduced fungus did not form an effective symbiosis with trees except *D. strictus* and *D. asper*. This may be due to non-sterile soil of nursery bags and uneven age of plantlets as compared to controlled conditions in *laboratory grown seedlings*. However, it may be possible to use this indirect approach to produce plantlets for subsequent colonization by ECM fungi. Although, the rate of shoot/root extension of inoculated plantlets was not measured, it was almost similar to that of primary roots of non-inoculated plantlets. Comparing the results with mycorrhizal infection, at 4 months suggests that *C. tropicalis* is efficient but poorly competitive as compared to other ECM mushroom (*viz. Rhizopogon, Pisolithus, Scleroderma, Laccaria, Tuber* etc.) tested by other workers.

Natarajan *et al.* [8] reported the ability of *Pisolithus tinctorius* and *Laccaria fraterna* to form ECM with *Acacia nilotica* under *in vitro* condition and nursery bags. Inoculation of these fungi improved the growth and mycorrhizal development in *Acacia nilotica* seedlings when compared to uninoculated control seedlings. In some instances, *Pisolithus* infection under controlled conditions has been associated with eucalypts and significantly reduced host growth [9]. Browning and Whitney [10] found that growth of *Pinus banksiana* increased when inoculated with both *Laccaria bicolor* and *L. proxima*. Osonubi *et al.* [11] reported the growth stimulation of *Acacia auriculiformis* by inoculation with *Boletus suillus*. Nezzar-Hocine *et al.* [12] observed that *Tricholoma tridentinum* Singer var. *cedretorum* Bon. had a significant influence on seedling height when this fungus was inoculated in soil as mycelial form. Growth improvement of plant with *Tuber* species was noted qualitatively in several studies [13]. *Scleroderma* species had been used to increase the early growth of a number of tree species [3, 14]. In South China, inoculation with spores of *Scleroderma* to non-sterile soil generally promoted the growth of eucalypts seedlings. Their nutrient acquisition increased the growth by 19–55% in shoot height and 25–41% in total biomass, compared to uninoculated plants in each soil [15]. Yazid *et al.* [16] have studied growth stimulation of *Hopea* spp. (dipterocarpaceae) seedlings following ECM inoculation with an exotic strain of *Pisolithus tinctorius.* Garbaye and Churin [17] have studied nursery-grown *Quercus petraea* and *Q. robur* seedlings for the effect of ECM fungi *Paxillus involutus*, *Hebeloma crustuliniforme* or *Laccaria laccata*. Various researchers have studied host plant growth responses to *Lactarius* species inoculation to various trees *viz. Picea sitchensis, Betula papyrifera, Pinus banksiana, P. sylvestris* [18].

Ahonen-Jonnarth *et al.* [19] have demonstrated the ability of ECM fungi to capture base cations and restrict their loss through leaching. Mechanisms for water transport are thought to function similarly. Recently, Khosla and Reddy [20] demonstrated the positive effect of *Pisolithus albus* on growth and survival of *Eucalyptus tereticornis* in bauxite mine soils. In another report, the inoculation of *Pisolithus arhizus* and *Scleroderma columnare* increased early growth of seedlings of *Shorea seminis* [21]. Pande *et al.* [22] have also shown that oak and pine seedlings when inoculated with ectomycorrhizal fungi showed significant more growth in all parameters (shoot length, root length, collar diameter). Ectomycorrhizal colonization and their effect on growth of *Acacia* tree has been demonstrated by Saravanan and Natrajan [23]. Appleton *et al.* [24] has also studied the effect of mycorrhizal inoculum on established street trees.

It is of interest that growth of mycorrhizal plants differed from non-mycorrhizal only when seedlings were subjected to water stress, indicating that when water is available, photosynthate is diverted to mycorrhizas at the expense of root and shoot growth. This is compensated by increased absorptive capacity, more rapid recovery of photosynthetic activity and increased chances for survival during drought. Stressed ECM inoculated plants had greater root and shoot dry weight than stressed non-inoculated plants. Rhizomorphs, or mycelial strands, were well developed among seedlings inoculated with *C. tropicalis* and may be important in water conductance. Gogala [25] correlated extent of colonization with *in vitro* IAA or ethylene production capacity of fungus and roots and their role in mycorrhizal formation.

Mycorrhizal colonization can be viewed as a kind of *drought avoidance* in that dry soil conditions are avoided spatially through hyphal penetration of deeper zones. Seasonal drought avoidance could be achieved by mycorrhizal fungi able to grow and colonize roots at cool soil temperatures when moisture is not limiting [26, 27]. Differences in host response to inoculations with ECM fungi suggest that drought tolerance should be considered as one of the more important criteria for selection of fungus species and ecotypes suitable for nursery inoculation. Moreover, mycorrhization increases the tolerance of host seedlings to drought and other environmental stresses, increasing the rate of successful reforestation of damaged or unfavourable sites.

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