Symbiotic and synergistic efficacy of endomycorrhizae with *Dendrocalamus strictus* L.

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ABSTRACT

The present investigation was undertaken to find out efficient strains of arbuscular mycorrhiza (AM fungi) alone or in combinations with *Trichoderma viride* for inoculation *Dendrocalamus strictus* L. seedlings. The inoculated seedlings showed good response having higher plant height, phosphorous ions content in root and shoot, AM spore number and root colonization than non-inoculated (control) seedlings in both single (alone) and co-inoculation (combined consortium) experiments. *T. viride* showed significant growth followed by *Glomus mosseae*, *G. fasciculatum* and mixed AM with single inoculation. In co-inoculation, the best growth responses were observed with *G. fasciculatum* + *T. viride* followed by *G. mosseae* + *T. viride*, mixed vesicular arbuscular mycorrhizas (VAM) + *T. viride*, *G. mosseae* + *G. fasciculatum* + *T. viride* + mixed VAM, *G. mosseae* + *G. fasciculatum* + *T. viride* and *G. mosseae* + *G. fasciculatum* after 120 days and also depicted maximum increase in phosphorus content of shoot and root when compared with other inoculated seedlings. However, all the inoculated seedlings showed significant increase in phosphorus content when compared with control seedlings.

Keywords: *Glomus mosseae*; *Trichoderma viride*; bio-inoculation; synergistic response; phosphorus ions content; VAM fungi

Bamboo (*Dendrocalamus strictus* L.) is a boon for a developing country like India. It serves as the backbone of the rural economy besides an ideal material for pulp and paper industry. Bamboo grows naturally in many types of forests. About 50% of the annual production of bamboo in India is used by various industries like pulp, paper, rayon, mat boards, besides agricultural implements. It is also used for making baskets, bridges, coffins, beds, toys and weapons and therefore, it is rightly called as 'poor man's timber'. In recent years bamboos are in great demand, but non-availability of sufficient quantity of saplings and seeds are the major problem. *Dendrocalamus strictus* L. is most commonly called as solid or lathi bamboo. It is widely distributed in dry deciduous forests and grows rapidly in all climatic conditions. It grows better in the drier parts and on sandstone, granite and coarse grained soils with low moisture- retaining capacity and soils with pH range 5.5–7.6. It grows more than 8 feet in 6–8 months. The major problem in the propagation of *D. strictus* is the erratic flowering and non-availability and low viability of seeds (Reddy 2006).

The maximum bamboo bearing forest area (22 261 km^2) is found in Madhya Pradesh and minimum in Haryana (km^2) (Singhal and Gangopadhyay 1999). The depleting resources of bamboo necessitate for immediate measures for their conservation and development. Therefore, it has become necessary to establish commercial timberlands by converting wastelands into productive ones and to maximize wood production from the limited forest areas. *D. strictus* have come up in different environmental zones including some areas for Haryana. But performance is comparatively less satisfactory in natural zones because of low soil nutrient status. Attempts have been made to enhance their growth using growth-promoting substances that include organic, inorganic and syn-

thetic fertilizers which accelerate the establishment of vegetation cover but it is not a feasible strategy due to their adverse effects on the environment, soil, plant, animal and human health.

Therefore, the use of microorganisms or biofertilizers has both more beneficial and economical gains. Mycorrhizal fungi can be used as fertilizers, therefore, referred as biofertilizers and can be substituted for substantial amounts of chemical fertilizers. Keeping all this in view, the present study was performed to select the efficient strains of VAM fungi for inoculation in *D. strictus* so that the juvenile seedlings should establish in the transplanted areas of Haryana state through more nutrients uptake from the rhizospheres.

MATERIAL AND METHODS

Experimental site. The experiment was performed at Botany Department, Kurukshetra University, Kurukshetra, Haryana. Kurukshetra and this site is situated in Haryana State (India) at 29°58'N latitude and 76°5'E longitude, about 250 m a.s.l. The climate of the area is tropical monsoonal. The area receives annual rainfall between 500–1500 mm mostly during tropical monsoon period (June to September). The mean maximum temperature varies from 17°C (January) to 38°C (May). Correspondingly, minimum mean temperature varies from 4.1°C (January) to 26°C (May). The experiment was performed from July to September.

AM endophytes and test plants. Different strains of AM fungi (*Glomus mosseae* and *G. fasciculatum*) and a bioagent (*T. viride*) were isolated from the rhizospheres of the test seedlings. The isolated strains of AM fungi were mass cultured in the rhizosphere of maize (*Zea mays* L.) and *T. viride* was mass produced on wheat bran: saw dust: distilled water (3:1:4) substrates, respectively. Four months old *D. strictus* seedlings were procured from Forest Research Institute, Dehradun, Uttarakhand, India.

Experimental design. The experiment was performed in two designs i.e. single inoculation and combined or co-inoculation along with a control (non-inoculated) set. In single inoculation, only endomycorrhizal strains of *Glomus mosseae*, *G. fasciculatum*, *Trichoderma viride* and mixed AM consortium (spores of *G. geosporum*, *G. reticulatum*, *Gigaspora gigantea*, *Sclerocystis sinuosus*, *Acaulospora lacunosa*, *A. laevis* except *G. mosseae*, and *G. fasciculatum*) were used. There were only five treatments including control (6 plants in each replicate) with two growing rhizomatic seedlings in each pot under three replications (total 30 seedlings). The different combined treatments in co-inoculation were *G. mosseae* + *T. viride*, *G. mosseae* + *G. fasciculatum*, *G. fasciculatum* + *T. viride*, mixed AM + *T. viride*, *G. mosseae* + *G. fasciculatum* + *T. viride*, *G. mosseae* + *G. fasciculatum* + *T. viride* + mixed VAM consortium. There were seven treatments in co-inoculation trial including control (6 plants in each replicate) with two growing rhizomatic seedlings in each pot. Each treatment was having three replications (total 42 seedlings). In control set, no inoculum was added (without any inoculant).

Soil and inoculum preparation. Soil was collected from the top 20–30 cm of the soil profile. The soil properties were sand – 64.2%, silt – 21.81%, clay – 3.90%, pH – 8.08, total N – 0.042%, available P – 0.017%. Soil was sieved through a 2 mm sieve to remove large organic material and other debris. In each pot (size 35 × 25 cm) two seedlings were planted in the selected inoculum (20% w/w of soil taken in each pot). Plants were watered regularly. Hoagland's nutrient solution minus phosphorus (100 mL/pot) was given to plants after regular intervals of 15 days (Hoagland and Arnon 1950, Douds and Schenck 1990). Each treatment was replicated three times. The plantlets in pots were grown under natural illumination in a poly house.

Recording of data. The growth parameters like increase in plant height (cm), mycorrhizal root colonization (%), mycorrhizal spore number, fresh shoot weight (g), dry shoot weight (g), fresh root weight (g), dry root weight (g), phosphorus content in root and shoot (%) were selected for recording the data in the experimental seedlings after 60 and 120 days. Total phosphorus in plants was determined by vanadomolybdate phosphoric yellow color method in nitric acid system (Jackson 1973). AM fungal root colonization was studied by rapid clearing and staining technique (Phillips and Hayman 1970). VAM spores were isolated from rhizosphere soil of *D. strictus* using wet sieving and decanting technique (Gerdemann and Nicolson 1963) and the quantification of AM spores was done by grid-line intersect method (Gaur and Adholeya 1994).

Statistical analysis. A statistical analysis of the data was performed using analysis of variance. The significant difference among the means was tested by critical difference (*CD*) test at 5% level of probability. Least significant difference (*LSD*) test was performed for analyzing significant difference among the means of phosphorus content in different samples.

Table 1. Growth response in *Dendrocalamus strictus* L. after 60 and 120 days with single inoculation of different bioinoculants

S. No.	Treatments	Increase in height		Mycorrhizal spore count		Mycorrhizal root colonization (%)		Fresh shoot weight (g)		Dry shoot weight (g)		Fresh root weight (g)		Dry root weight (g)	
		60	120	60	120	60	120	60	120	60	120	60	120	60	120
1	control	1.33^{b} ± 0.72	4.6^{c} ± 0.72	14.0^{d} ± 2.49	21.0^{c} ± 0.47	35.0^{c} ± 1.41	40.0^{b} ± 2.35	3.85 ± 0.12	6.0^{b} ± 0.24	3.24 ± 0.07	3.83^{c} ± 0.37	5.44 ± 0.5	7.36^{b} ± 1.1	4.43 ± 0.47	6.81^{b} ± 1.15
2	*G. mosseae*	3.16^{b} ± 0.59	$7.0^{c/b}$ ± 0.24	33.3^{b} ± 2.72	41.66^{b} ± 1.36	48.0^{b} ± 1.24	88.1^{a} ± 2.57	4.79 ±0.39	6.52^{b} ± 0.7	3.98 ± 2.26	5.23^{b} ± 0.51	7.87 ± 0.59	10.09^{b} ± 0.34	6.41 ± 0.12	7.78^{b} ± 0.36
3	*G. fasciculatum*	2.33^{b} ± 0.72	9.66^{b} ± 0.98	30.0^{b} ± 0	45.66^{b} ± 4.95	47.0^{b} ± 2.05	88.33^{a} ± 4.76	4.91 ± 0.47	6.25^{b} ± 0.79	3.80 ± 0.7	6.32^{b} ± 0.72	6.85 ± 0.98	10.15^{b} ± 0.97	6.10 ± 1.28	8.39^{b} ± 0.66
4	*T. viride*	7.50^{a} ± 0.84	20.33^{a} ± 1.18	44.33^{a} ± 0.72	68.66^{a} ± 3.56	57.0^{a} ± 1.24	88.33^{a} ± 3.6	4.95 ± 1.87	10.69^{a} ± 0.84	5.50 ± 0.84	9.62^{a} ± 0.96	8.78 ± 1.35	17.57^{a} ± 1.0	7.69 ± 0.41	13.9^{a} ± 0.67
5	mixed VAM	2.0^{b} ± 0	10.33^{b} ± 0.72	28.0^{b} ± 1.24	47.66^{b} ± 4.06	45.0^{b} ± 2.35	85.0^{a} ± 6.12	6.0 ± 0.71	7.46^{b} ± 0.77	3.83 ± 0.37	6.67^{b} ± 0.59	8.01 ± 1.41	11.32^{b} ± 1.79	6.03 ± 1.12	9.45^{b} ± 0.74
CD (5%)		2.5	3.84	6.83	12.89	6.65	15.94	NS	2.73	NS	2.50	NS	4.41	NS	2.94

Mean of three replicates ± standard error; *CD* – critical difference between means; NS – non significant at *CD* 5%

RESULTS AND DISCUSSION

Growth response in *Dendrocalamus strictus* with single inoculation. In *D. strictus* after 60 days of AM inoculation, *T. viride* showed the best results in all the observed growth parameters followed by *G. mosseae*, *G. fasciculatum* and mixed AM (Table 1). After 120 days of inoculation, again *T. viride* showed the best results followed by mixed AM, *G. fasciculatum* and *G. mosseae* (Table 1). The increase in height might be attributed to the increase in phosphorus content in inoculated seedlings (Liu et al. 2000) when compared with non-inoculated control seedlings (Table 2).

Growth response in *Dendrocalamus strictus* with co-inoculation (different combinations). In *D. strictus* after 60 days of inoculation with different combinations, the best results in terms of increase in all the growth parameters were seen in *G. mosseae* + *T. viride* followed by *G. fasciculatum* + *T. viride*, mixed VAM + *T. viride*, *G. mosseae* + *G. fasciculatum*, *G. mosseae* + *G. fasciculatum* + *T. viride* + mixed VAM and *G. mosseae* + *G. fasciculatum* + *T. viride* (Table 3). The best growth responses were observed with *G. fasciculatum* + *T. viride* followed by *G. mosseae* + *T. viride*, mixed VAM + *T. viride*, *G. mosseae* + *G. fasciculatum* + *T. viride* + mixed VAM, *G. mosseae* + *G. fasciculatum* + *T. viride* and *G. mosseae* + *G. fasciculatum* after 120 days (Table 3). *G. fasciculatum* + *T. viride* also depicted maximum increase in phosphorus content of shoot and root when compared with other inoculated seedlings. However, all the inoculated seedlings showed a significant increase in phosphorus content when compared with non-inoculated control seedlings (Table 4).

In single inoculation experiment, best growth response was seen with *T. viride* treatment. The increased growth by *T. viride* might be attributed to suppression of plant pathogens and also promoting nutrient uptake, especially phosphorus, which was higher than in other treatments. *T. viride* acted synergistically with the mycorrhizal population naturally present in the soil which might have increased the plant growth. Due to this reason,

Table 2. Phosphorus content in *Dendrocalamus strictus* L. shoot and root after 120 days with single inoculation

S. No.	Treatment	P (%) in dry weight	
		shoot	root
1	control	0.074^{c} ± 0.0007	0.113^{c} ± 0.002
2	*G. mosseae*	0.169^{a} ± 0.002	0.200^{a} ± 0.001
3	*G. fasciculatum*	0.170^{a} ± 0.001	0.200^{a} ± 0.002
4	*T. viride*	0.215^{b} ± 0.0004	0.210^{b} ± 0.0004
5	mixed VAM	0.20^{b} ± 0.002	0.200^{a} ± 0.0009
LSD 0.05%		0.006	0.007

Mean of three replicates ± S.E. Means in each column followed by the same letters do not differ significantly at $P = 0.05$

Table 3. Growth response in *Dendrocalamus strictus* L. after 60 and 120 days with co-inoculation of different bioinoculants

S. No.	Treatments	Increase in height		Mycorrhizal spore number		Mycorrhizal root colonization (%)		Fresh shoot weight (g)		Dry shoot weight (g)		Fresh root weight (g)		Dry root weight (g)	
		60	120	60	120	60	120	60	120	60	120	60	120	60	120
1	control	1.33^{d} ± 0.72	4.66^{e} ± 0.72	14.0^{e} ± 2.49	21.0^{b} ± 0.47	35.0^{e} ± 1.41	40.0^{b} ± 2.35	3.85^{c} ± 0.12	6.0^{b} ± 0.24	3.24^{d} ± 0.07	3.83^{c} ± 0.37	5.44^{b} ± 0.5	7.36^{d} ± 1.1	4.43 ± 0.47	6.81^{e} ± 1.15
2	*G. mosseae* + *T. viride*	14.16 ± 1.49	32.33^{b} ± 1.18	51.0^{a} ± 2.05	93.33^{a} ± 9.81	96.26^{a} ± 3.03	95.83^{a} ± 3.40	10.61^{a} ± 1.36	17.2^{a} ± 0.88	8.74^{a} ± 0.52	15.7^{a} ± 0.88	13.11^{a} ± 1.4	22.79^{a} ± 1.25	12.26^{a} ± 0.99	20.8^{a} ± 0.81
3	*G. mosseae* + *G. fasciculatum*	8.33^{b} ± 0.98	15.66^{d} ± 1.65	45.33^{b} ± 3.48	78.33^{a} ± 1.65	71.1^{c} ± 4.79	96.0^{a} ± 3.26	9.61^{a} ± 0.2	5.83^{b} ± 1.36	5.96^{b} ± 0.08	8.9^{b} ± 1.59	12.9^{a} ± 1.21	12.94^{c} ± 0.99	8.73^{b} ± 0.67	12.9^{d} ± 0.99
4	*G. fasciculatum* + *T. viride*	12.5^{a} ± 0.84	42.0^{a} ± 0.81	48.0^{b} ± 3.29	95.0^{a} ± 6.23	82.56^{b} ± 4.64	100^{a} ± 0	9.4^{a} ± 0.73	19.4^{a} ± 1.17	8.23^{a} ± 0.25	17.27^{a} ± 1.22	13.15^{a} ± 1.14	25.48^{a} ± 1.18	11.34^{a} ± 0.95	23.84^{a} ± 1.32
5	mixed VAM + *T. viride*	11.0^{a} ± 0.47	28.66^{b} ± 1.51	46.33^{b} ± 2.12	92.3^{a} ± 3.5	80.03^{b} ± 2.08	96.66^{a} ± 2.72	7.94^{a} ± 1.24	15.74^{a} ± 0.93	7.5^{a} ± 1.24	14.33^{a} ± 0.74	11.72^{a} ± 0.49	20.47^{a} ± 0.4	10.26^{a} ± 0.27	18.92^{b} ± 0.7
6	*G. mosseae* + *G. fasciculatum* + *T. viride*	3.6^{c} ± 0.27	22.33^{c} ± 1.18	31.0^{d} ± 0.47	87.33^{a} ± 2.17	50.0^{d} ± 2.35	92.86^{a} ± 0.05	6.41^{b} ± 0.57	14.66^{a} ± 4.0	4.37^{c} ± 0.22	11.58^{a} ± 3.51	7.36^{b} ± 1.1	21.25^{a} ± 2.06	6.81^{c} ± 1.15	15.97^{c} ± 2.06
7	*G. mosseae* + *G. fasciculatum* + *T. viride* + mixed VAM	7.0^{b} ± 0.94	22.66^{c} ± 1.18	41.6^{c} ± 1.36	89.33^{a} ± 4.74	66.33^{c} ± 3.06	92.83^{a} ± 3.37	6.19^{b} ± 0.75	11.5^{a} ± 0.9	5.23^{c} ± 0.51	10.8^{b} ± 0.9	11.11^{a} ± 0.89	18.01^{b} ± 0.97	7.87^{b} ± 0.33	17.14^{b} ± 0.97
CD (5%)		3.33	4.53	9.09	18.72	12.17	9.58	3.10	8.63	2.09	6.07	3.79	7.13	2.84	4.54

Mean of three replicates. ± – S.E.; *CD* – critical difference between means

T. viride was tried in combination with other VAM strains and promising results were obtained with co-inoculation of *G. mosseae* + *T. viride* after 60 days. After 120 days, *G. fasciculatum* + *T. viride* showed best growth response as compared to single inoculation of VAM fungi strains. In all the treatments, promising growth of all inoculated seedlings were seen when VAM fungi were co-inoculated with *T. viride*. In both experiments (single and co-inoculation) best growth responses were observed after 120 days as compared to 60 days. In co-inoculation experiment, *G. mosseae* + *T. viride* (after 60 days) and *G. fasciculatum* + *T. viride* (after 120 days) have shown best

Table 4. Phosphorus content in *Dendrocalamus strictus* L. shoot and root after 120 days with Co- inoculation of different bioinoculants

S. No.	Treatment	P (%) in dry weight	
		shoot	root
1	control	0.074^{d} ± 0.0007	0.113^{e} ± 0.002
2	*G. mosseae* + *T. viride*	0.334^{c} ± 0.0004	0.248^{d} ± 0.001
3	*G. mosseae*+ *G. fasciculatum*	0.250^{a} ± 0.0009	0.225^{a} ± 0.003
4	*G. fasciculatum* + *T. viride*	0.250^{a} ± 0.001	0.235^{c} ± 0.002
5	mixed VAM + *T. viride*	0.245^{a} ± 0.001	0.226^{a} ± 0.001
6	*G. mosseae*+ *G. fasciculatum* + *T. viride*	0.115^{b} ± 0.0009	0.170^{b} ± 0.0009
7	mixed VAM + *G. mosseae*+ *G. fasciculatum* + *T. viride*	0.132^{b} ± 0.001	0.175^{b} ± 0.004
LSD 0.05%		0.004	0.01

Mean of three replicates. *LSD* – least significant difference; ± – SEM. Means in each column followed by the same letters do not differ significantly at $P = 0.05$

growth results. The succession of the AM fungi that varies with the increase in age of the host plant might be responsible for this change. Therefore, in *D. strictus*, early VAM strain was replaced by other efficient VAM strain after 120 days. When root and shoot phosphorus content in *D. strictus* were compared, the higher phosphorus content (P %) was observed in roots (Table 2). Parkash and Aggarwal (2009) and Parkash et al. (2009) also observed higher phosphorus content in roots than shoots of inoculated *Acacia catechu* seedlings. In this study, the inoculated seedlings have more percent in root colonization, spore number, fresh and dry shoot and root weights as compared with non-inoculated control. This suggests that these growth parameters are positively correlated, higher root colonization allows more nutrient uptake and more sporulation, hence, influence healthier seedling growth (Daft and Nicolson 1972, Hazarika et al. 1999, Parkash et al. 2009, Parkash and Aggarwal 2009). Prasad et al. (2000) also reported that *Gladiolus* plants inoculated with *Glomus fasciculatum* showed enhanced growth as compared to the controls. Their results also showed that VAM inoculation had a profound effect on the uptake of phosphorus by *Gladiolus* plants. Cooper and Tinker (1978), Hayman (1980), Koide and Li (1990) suggested that the mycorrhizal root colonization is regulated by phosphorus status of the root and not by phosphorus status of shoot. In the present investigation, increase in plant growth was observed in all inoculated seedlings, though the extent of plant growth was different in the inoculated seedlings. These differences could be due to the mechanism of mycorrhizal colonization and its development (Sanders et al. 1977) or the physiological differences between AM endophytes in rate of nutrient uptake, translocation and release (Gianinazzi-Pearson and Gianinazzi 1983) or interaction between mycosymbionts and soil environments (Mosse 1973). Thus, the results in the present study substantiate the hypothesis that not all combinations of host and endophytes have similar growth stimulating effects. Hence, the present screening trial clearly suggests that in *D. strictus*, *G. mosseae* + *T. viride* (after 60 days for early growth response) whereas *G. fasciculatum* + *T. viride* after 120 days are the most promising and the synergistic interacting VAM symbionts for inoculating *D. strictus* seedlings in the nursery. This technology is simple and can easily be adopted by nurserymen for obtaining healthy, vigorously growing planting stock that can establish better when planted at the field site. Also, this technique can be utilized for large-scale plantation of wastelands and saline soils to meet the growing needs of *D. strictus*.

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