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SHORT NOTE

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Effect of arbuscular mycorrhizal fungal isolates and organic manure on growth and mycorrhization of micropropagated *Dendrocalamus asper* plantlets and on spore production in their rhizosphere

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Abstract The effect of three different inocula of arbuscular mycorrhizal (AM) fungi was studied on the growth, biomass, P uptake, root colonization and AM spore production in tisssue culture-raised *Dendrocalamus asper* (bamboo) plantlets in two types of soil: (1) sand:soil 1:1 (2) sand:soil:organic manure 1:1:0.5 (v/v). The first two inocula were isolated from the bamboo rhizosphere and the third from teak rhizosphere soil. After 12 months, significant positive effects of inoculum on shoot P concentration, root colonization and spore production were observed in the sand:soil medium. In the organic manure-amended medium, these parameters further improved. Amendment with organic manure highly influenced spore production (5.3 to 17.8 fold increase) and enhanced height and dry biomass of *D. asper* plantlets.

Key words Bamboo · Dry biomass · Farmyard manure · P-uptake · Tissue culture

Introduction

Bamboos, one of the most useful groups of arborescent plants, belong to the grass family, Poaceae. In India, bamboos are spread over an area of 10.3 million ha (Tewari 1992). *Dendrocalamus asper* (Schult.) Backer ex Heyne and some other edible bamboos are being introduced into India for mass plantation under agroforestry and social forestry programmes. Fruit setting in woody bamboos is gregarious and periodical, ranging from 3 to 120 years, resulting in a paucity of seed. Therefore, micropropagation methods have been used (John et al. 1995). Phosphate fertilization is an important factor for the proper growth of bamboos (Prasad et al. 1990) and application of a mixed inoculum of AM fungi has been reported to significantly promote growth of three different bamboos in a nursery (Verma and Jamaluddin 1994). Micropropagated plantlets are highly dependent on arbuscular mycorrhiza. Branzanti et al. (1992), Fortuna et al. (1992), Gianinazzi et al. (1989), Salamanca et al. (1992), Schubert et al. (1992), Varma and Schuepp (1994), Vestberg (1992) and Vidal et al. (1992) demonstrated variable effectiveness of isolates/species of AM fungi.

Organic manure (farmyard manure) contains on a dry weight basis: 0.4–1.5% N, 0.3–0.9 % P_2O_5 and 0.3–1.9% K_2O. Of these half of the N or K, and one sixth of the P is readily soluble and available to plants. Organic matter improves soil fertility and also stimulates development of AM fungi in soil (Hepper and Warner 1983; Joner and Jakobsen 1992; St. John et al. 1983) and roots (Branzanti et al. 1992; Groaker and Sreenivasa 1994). Vejsadova (1992) observed a positive interaction between organic manure application and native AM frequency and intensity.

The present study evaluated the effect of different isolates of AM fungi and organic manure on growth, biomass and P-uptake in plantlets of *D. asper* raised from tissue culture and on AM spore production in their rhizosphere.

Materials and methods

The experiment was conducted as a two-factor randomized complete block design with 5 replicates in each treatment ($4 \times 2 \times 5$). There were 15 seedlings in each replication. The plantlets of *D. asper* were raised by axillary bud multiplication through tissue

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culture (Arya and Arya 1996). After root induction, they were transferred to a mist chamber and then to a shadehouse for hardening. The plantlets were placed in polyethylene bags containing 3 l steam-sterilized medium, i.e. sand:soil 1:1 or sand:soil:organic manure (made from buffalo dung) 1:1:0.5 (v/v). The physical and chemical properties of the sand/soil mixture were: loamy soil texture, pH 7.6, EC 0.35 mmho/cm, organic matter 1.3% and available N, P and K contents 144.8, 10.3, 18.8 mg/kg, respectively. The plantlets were kept on a polyethylene sheet in the shadehouse and provided with half sunlight for the first 2 months and subsequently full sunlight by removing them from the shade house. The plantlets were irrigated as and when required.

Plantlets were inoculated individually with AM fungi during their transfer from tissue culture bottles to polyethylene bags. The type, origin and composition of inoculum, inoculum potential and quantity used per plant are presented in Table 1. Inocula were prepared in sterilized substrate (sand:soil 1:1) in earthenware pots using *Panicum maximum* Jacq. as a trap plant. The voucher cultures are maintained at the Tropical Forest Research Institute. The roots of experimental plants were checked for root colonization every 2 months. The final measurements were recorded after 1 year of treatment. Height and both fresh and dry weights of shoot and root (root + rhizome) were recorded. Aliquots (100 g) of soil were taken from each replication and stored in a refrigerator for estimation of AM spores. Dry weights were recorded after oven drying shoots and roots for 72 h at 70 °C.

After acid digestion of samples on a Kjeldhal assembly (Lindner 1944), P concentrations in shoots and roots were determined by the method of Fiske and Subba Row (1925) using a spectrophotometer (Model 106 Systronic, India) and expressed as mg P/g dry weight.

Roots were stained with trypan blue according to the method of Phillips and Hayman (1970). The percent root colonization was assessed by the grid-line intersect method (Giovannetti and Mosse 1980). Spores were extracted from 50-ml soil samples by wet sieving and decanting followed by density gradient centrifugation (Sylvia 1994) and counted under a stereozoom microscope (Leica, Germany).

Treatment effects and interactions between them and soil groups were analysed using two way ANOVA. The means of each parameter were compared using Duncan's Multiple Range Test. A Pearson product (Moment Correlation Coefficient) among different parameters studied was also computed.

Results and discussion

Plantlets treated with AM fungi were significantly taller (39.1% in AM3 and 17.2% in AM1) than their respective controls in the sand/soil medium. Application of organic manure further enhanced height in plantlets treated with all three AM inocula (Table 2). Application of AM fungi significantly increased P concentration in shoots; the effect was greatest in AM3 followed by AM1 and AM2 in sand/soil. Use of organic manure further significantly increased shoot P concentration. In contrast to sand/soil medium, the highest shoot P concentration was observed in plantlets treated with AM1 followed by AM2 in organic manure-amended medium. Here, plants from the AM1 treatment contained 16.0% more shoot P than AM2 and 45.0% more than AM3. Both AM application and organic manure application significantly enhanced shoot P over their respective controls (Table 2). Soil contains more than 30 different organic P compounds (Barrow 1961; Dalal 1977) which are easily degraded and available to plants (Martin 1973). The higher shoot P concentrations found in AM plantlets is probably due to more efficient uptake of available P from the soil and manure and possibly to mineralization of organic phosphorus (Jayachandran et al. 1992) due to a higher phosphatase production by AM plants (Tarafdar and Marschner 1994).

AM treatments and organic manure application significantly increased both above and below ground dry biomass of plants. In the sand/soil medium, all three AM inocula produced statistically similar root dry biomass, which differed significantly from the control. AM1 and AM3 produced a higher shoot dry biomass than AM2 (Table 3). In the organic manure-amended medium, the highest shoot dry weight was produced in AM1-followed by AM2- and AM3-inoculated plantlets

Table 1 Details of the arbuscular mycorrhizal (AM) inocula used in the experiment (*TF* Tropical forest isolate, *vc* voucher culture)

AM inoculum	Origin (isolated from)	Species	Inoculum potential (Liu and Luo 1994) (propagule/ml)	Inoculum used per plant (ml)
AM1 (VC-13)	Bamboo rhizosphere, Jabalpur, India	*Acaulospora scrobiculata* Trappe (TF-19)	110	13.3
AM2 (VC-9)	Bamboo rhizosphere, Jagdalpur, India	*Glomus intraradices* Schenck & Smith (TF-8), *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (TF-5), *G. aggregatum* Schenck & Smith emend Koske (TF-7), *Scutellospora heterogama* (Nicol. & Gerd.) Walker & Sander (TF-9)	179	8.2
AM3 (VC-3)	Teak rhizosphere Jabalpur, India	*Acaulospora scrobiculata* Trappe, *A. delicata* Walker, Pfeiffer & Bloss, *Gigaspora* sp. *G. ramisporophora* Spain, Sieverding & Schenck, *Glomus intraradices* Schenck & Smith, *G. geosporum* (Nicol. & Gerd.) Walker, *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. etunicatum* Becker & Gerd., *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sander (TF-20 to TF-28)	292	5.0

Table 2 Height and shoot P contents after 1 year of *Dendrocalamus asper* plantlets treated with three inocula (AM1–3) of AM fungi and grown in two types of soil medium. Means within rows and columns followed by the same letter are not significantly different at the $P<0.05$ (*OM* organic manure)

Soil medium	Height (cm)					Shoot P concentration (mg/g dry wt.)				
	AM1	AM2	AM3	Control	Mean	AM1	AM2	AM3	Control	Mean
Sand:Soil 1:1	35.4d	31.0e	42.0c	30.2e	34.7β	2.1d	2.1d	2.2c	1.8e	2.1β
Sand:Soil:OM 1:1:0.5	49.0a	47.0b	47.6b	41.0c	46.2α	2.9a	2.5b	2.0c	1.9d	2.3α
Mean	42.2wx	39.0x	44.8w	35.6x		2.6w	2.3w	2.1w	1.8x	

Table 3 Shoot and root dry weights after 1 year of *D. asper* plantlets treated with three inocula of AM fungi and grown in two types of soil medium. Means within rows and columns followed by the same letter are not significantly different at the $P<0.05$ (*OM* organic manure)

Soil medium	Shoot dry wt. (g)					Root + rhizome dry wt. (g)				
	AM1	AM2	AM3	Control	Mean	AM1	AM2	AM3	Control	Mean
Sand:Soil 1:1	10.1c	7.2d	12.1b	5.7e	8.7β	9.9d	9.4d	9.3d	4.8e	8.3β
Sand:Soil:OM 1:1:0.5	15.3a	14.4ab	13.3b	9.3cd	13.1α	17.3b	20.1a	14.7c	9.5d	15.4α
Mean	12.7w	10.8w	12.7w	7.5x		13.7w	14.7w	12.0w	7.1x	

Table 4 Effect of the application of OM and three inocula of AM fungi on root colonization and AM spore production in *D. asper* plantlets after 1 year. Means within rows and columns followed by the same letter are not significantly different at the $P<0.05$ (*OM* organic manure)

Soil medium	Percent root colonization					Spores per 50 ml soil				
	AM1	AM2	AM3	Control	Mean	AM1	AM2	AM3	Control	Mean
Sand:Soil 1:1	62.8b	54.2c	73.0a	14.0f	51.0β	26.6f	13.0fg	96.2d	9.4g	36.3β
Sand:Soil:OM 1:1:0.5	72.8a	66.0b	73.0a	19.4e	57.9α	314.4b	231.4c	510.8a	69.8e	281.6α
Mean	67.8x	60.1y	73.2w	16.7z		170.5x	122.2y	303.5w	39.6z	

and significantly differed from the control. The highest root dry biomass was produced in the AM2 treatment followed by AM1 and AM3 (Table 3).

The inoculated plantlets were colonized by AM fungi within 2 months, whereas the control plantlets showed no colonization for up to 6 months. However, at the end of the experiment they showed a low level of root colonization by AM fungi which had contaminated the plantlets. The colonization of roots by the three test inocula varied significiantly and use of organic manure significantly affected root colonization by two of them (Table 4). Groaker and Sreenivasa (1994) also reported an enhancement of growth, yield and root colonization (especially by *Glomus fasciculatum*) in wheat as a result of organic amendment.

AM fungi isolated from bamboo rhizosphere soils (inocula AM1 and AM2) (Table 1) were more effective than those isolated from the teak rhizosphere (AM3) in enhancing root and shoot dry biomass and P concentrations in shoots in organic manure-amended media (Tables 2, 3). Although the AM1 and AM3 inocula produced the same level of root colonization, which was significantly higher than AM2, the single-species inoculum (AM1) led to a significantly higher shoot P concentration than both mixed inocula. Chavez and Ferrera-Cerrato (1990) also reported that AM effects differed widely with host-endophyte combinations in micropropagated strawberry. This may be due to the existence of some physiological host preference/specificity in these fungi. A positive significant correlation ($P<0.01$) between root colonization and height, dry biomass and P concentration ($r=0.52$, 0.77 and 0.66, respectively) reaffirmed that growth stimulation in the mycorrhizal plantlets was mediated through improved accumulation of nutrients, particularly P (Bolan 1991).

AM treatment along with organic manure application enhanced AM spore production many fold compared with the sand/soil medium. The highest spore numbers in AM3-, AM1- and AM2-inoculated organic manure-amended medium were 5.3, 11.8, and 17.8 times higher than in the soil/sand medium, respectively. This may be due to the diversity of AM species in the inocula (Table 1). Spore production differed amongst the isolates of AM fungi. However, in the sand/soil medium, the AM1 and AM2 inocula gave statistically similar numbers of spores (Table 4). Enhancement of spore production in organic manure-amended medium may be due to enhanced growth and spread of AM hyphae (Hepper and Warner 1983; Joner and Jakobsen 1992; St. John et al. 1983). Baby and Manibhushanrao

(1996) applied different organic manures to a rice crop and concluded that leaf manure amendment stimulated high arbuscule development and sporulation.

In conclusion, mycorrhization of in vitro-raised bamboo plantlets with suitable isolates/species of AM fungi and application of organic manure in small doses can have cumulative positive effects on plant growth. Combination of these two factors during nursery production of micropropagated bamboos could greatly promote plant supply for mass plantation and could be of interest in other micropropagated crops.

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