

## INFLUENCES OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND MINERAL ELEMENT ABSORPTION OF CHENGLU HYBRID BAMBOO SEEDLINGS

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### Abstract

Arbuscular mycorrhizal (AM) fungi can enhance mineral nutrient and growth of host plants. In the paper, growth and mineral element absorption of Chenglù hybrid bamboo (*Bambusa pervariabilis* × *B. grandis*) seedlings inoculated with four AM fungi strains (*Glomus intraradices*, BEG Number193 and 141; *G. mosseae*, BEG Number167; *G. etunicatum*, BEG Number168) were studied. The results showed that AM fungi promoted the growth of bamboo seedlings. The biomass of Chenglù bamboo inoculated by AM fungi increased 1.84 (BEG167), 1.73 (BEG193), 1.59 (BEG168) and 1.54 times (BEG141) than that of the control respectively. Shoot number and diameter, total leaf area per plant, P and K concentration in plant as well were significantly increased by the inoculation of BEG167 and 193, which had better mycorrhizal effect and could make an important contribution to Chenglù bamboos production.

### Introduction

The majority (probably 70-80%) of terrestrial plants are capable of interacting with arbuscular mycorrhizal (AM) fungi in nature. AM fungi and plant roots are closely integrated as a result of co-evolution over at least 450 million years (Smith & Read, 2008). Mycorrhizas form a symbiotic association with the roots of host plants, where carbon flows to the fungus and inorganic nutrients move to plant. These fungi develop external hyphae approximately 3m in diameter (Simard *et al.*, 2002). Hyphae produce arbuscules and in most fungal genera vesicles they act as a bridge connecting the root with the surrounding soil (Allen, 1992). AM fungi are characterised by the formation of an extraradical mycelium and branched haustorial structures within the cortical cells, termed arbuscules (Hock & Varma, 1995). The beneficial effects of AM fungal symbiotic association on the growth of plants are well known, and they may be involved in improving uptake of macro- and micronutrient, increasing plant resistance against biotic and abiotic stress, and beneficial alternations of plant growth regulators (Smith & Smith, 1996; Liu & Li, 2000; Govindarajulu *et al.*, 2005; Kung'u *et al.*, 2008; Smith & Read, 2008). Nowadays, mycorrhizal fungi have been widely used in agriculture, horticulture, and forestry programs, as well as for environmental reclamation, to increase crop yield and health and to limit the application of agrochemicals (Johansson *et al.*, 2004).

Chenglù bamboo is a hybrid, using *Bambusa pervariabilis* as female and *B. grandis* as male parent. It is extensively cultivated as raw material for paper mills and also for a variety of purpose such as construction, agricultural implements and furniture. However, how to improve the afforestation quality of this bamboo and to optimize bamboo plantation ecosystem is still a serious problem. P nutrient is an important factor for the proper growth of bamboos, while little is known about effects of AM fungi on available P nutrient absorption of bamboos commonly cultivated. AM symbiotic efficiency is dependent on endophyte association and host plants (Monzon & Azcon, 1996). Therefore, it's necessary to understand the symbiotic interaction between AM fungi and Chenglù bamboo, and to improve Chenglù bamboo production. The present study evaluated the effect of

inoculation with four AM fungi on growth, biomass and mineral nutrient absorption in plantlets of Chenglù bamboo raised by conventional vegetative propagation in pots. It could provide the fundamental data for mycorrhizal application in Chenglù bamboos production.

### Materials and Methods

#### Mycorrhizal inoculum, host plant and soil properties:

Four AM fungi strains (*Glomus intraradices*, BEG Number193 and 141, *G. mosseae*, BEG Number167 and *G. etunicatum*, BEG Number168) were initially obtained from XiaoLing Li (Agriculture University of China). For inoculum preparation, a single colony of each AM fungus strain grown on sterilized sandy soil by white clover as host plant for three months, and the mixture of spores, mycelium, soil and plant root fragments were used as the AM fungal inoculum.

One-year-old Chenglù bamboo seedlings (ramets) with stump were obtained from Chishui, Guizhou province of China. They were selected for uniformity (3.16cm culm diameter and 55cm cutting height) and were surface-sterilized in 75% ethanol for 1 min before transplanting (Fig. 1a).

The soil was collected from a crop field in the hilly region of Huaxi, Guiyang, South-west China. It is limestone with the following properties: pH 8.25, organic matter 43.41g/kg, total P 0.883 g/kg, Available P 46.2 mg/kg, Available N 132 mg/kg, Available K 385 mg/kg.

**Experimental design:** The experiment was single factorial design comprising 5 inoculation treatments (four AM fungal inoculation, control) with 12 replicates for each treatment (5×12). For the experiment, 60 plastic pots (30cm top diameter×25cm bottom diameter ×35cm height) and soil were fumigated by using 1% (v/v) formalin for 24h under airtight plastic sheets, and the fumigant was allowed to dissipate for 1 week. Each pot was filled with 25kg soil and all pots were arranged in a randomized manner. Bamboo seedlings were transplanted and buried into soil at a depth of approximately 6-8cm, with 2 culm nodes exposed above the ground (one seedling per pot) (Fig. 1a). Subsequently, the seedlings were irrigated enough. Mixed soil-based inoculum

(containing 450-500 infectious AM fungal spores in 250g soil) for mycorrhizal treatment was added to the appropriate pot close to root zone when rooting period (30 d after transplanting). The controls received the same amount of autoclaved inoculum. The experiment was conducted in the greenhouse from 28 March 2010 to 31 December 2010. The plants grew under natural light conditions without supplementary illumination and were watered to maintain 75% field capacity, and no nutrients were added.

**Growth measurement and biochemical analyses:** The roots of experimental plants were checked for root colonization every 2 months, and the final measurement was recorded after 6 months of treatment. Fresh roots randomly sampled were cleared and stained with acid fuchsin according to Phillips & Hayman (1970).

Percentage root colonization, intensity of the mycorrhizal colonisation, arbuscule and vesicle abundance were estimated by scoring mycorrhizal colonization in classes from 0 to 5 (Trouvelot *et al.*, 1986).

Shoots number in each treatment, and shoot diameter and height from all replicates were recorded after 6 months of plant growth. The average leaf quantity and area of one plant, from 5 seedlings randomly sampled in each treatment, were determined in the early shooting period (90d after inoculation), flourishing shooting period (150d after inoculation) and declining period (210d after inoculation), respectively. Ultimately, roots, shoots, branches and leaves were harvested separately, and the dry weight of each part was determined after oven-drying at 70°C until they reached constant weight. Then all samples were analyzed for nitrogen (N), phosphorus (P) and potassium (K) concentration.



Fig. 1. Cultivation design (a) and growth performance after 100 days (b) of Chenglù bamboo seedlings (photoed by W.X. Jiang).

The mycorrhizal dependency (MD) of the Chenglù bamboos was expressed as a percentage by calculating the difference between the total dry weight of the mycorrhizal plant (Tm) and the total dry weight of the control (Tck).

$$MD (\%) = (Tm - Tck) / Tm * 100$$

Photosynthetic rate (Pn) was measured by an infrared gas analyzer (Li-6400, Li-Cor, Lincoln, NE, USA) on three replications per treatment from 8:00 am to 18:00 pm at a sunny day before harvest. Measurements were recorded when the total coefficient of variation was less than 0.5%. Oven-dried sub-samples of shoots, roots, and leaves were milled and digested with a mixture of hydrogen peroxide and sulphuric acid for N, P and K analysis. Nutrient concentration in organs was determined by the methods of Cui (1998): Total N concentrations were determined using Kjeldahl analysis; P was measured by sulfuric acid-perchloric acid-water solution and Mo-Sb antispetrophotography methods; K was estimated by flame spectrophotometric methods. The data were expressed as percentage concentration. Ten grams of soil were collected from all replicates after bamboo harvest. Analysis of soil properties was determined with reference to "Soil Physical and Chemical Analysis" (Anon., 1978).

**Statistical methods:** Analysis of variance was performed on all data to compare treatment effects (one-way ANOVA). Means significant difference were compared using Duncan's Multiple Range Test at  $p < 0.05$ . Pearson's correlation analysis was used to assess the relationship between AM fungal colonization, biomass and nutrient uptake. All the data were analyzed on SPSS Version 16.0.

## Results

**Mycorrhizal colonization, seedling growth and mycorrhizal dependency:** The roots of experimental plants were checked for root colonization every 2 months. External hyphae and entry points of AM can be observed in Chenglù bamboos roots after 60d of inoculation (Figs. 2a & b). Arbuscule developed after 90d (Fig. 2c). Mass vesicles and little arbuscule decomposer formed after 120d (Fig. 2d). No root colonization was detected in the control plants (Fig. 2e), while the colonization of inoculated plants was relatively high (Fig. 2f). The frequency of mycorrhizae by AM fungi ranged from 70.4 to 85.6% which was comparable from each other (Table 1). Among the four AM inocula, root colonization levels were the greatest in BEG193 and 167, followed by BEG141 and 168.

Application of AM inocula significantly enhanced the growth of Chenglù bamboo seedlings (Fig. 1b, Table 2). Generally seedlings inoculated with all AM inocula had significantly higher shoot number, shoot diameter, leaf number and area in different growth stages compared to control. Here, plants inoculated by BEG167 had 6 more shoots and 42.1% higher diameter than that of the control, while BEG 193 were 4 and 39.7%. BEG141 and 168 showed an increasing trend as well.

All mycorrhizal plants had higher dry weights than that of the control (Table 3). Among the different plant parts, the fine leaf and branch fraction were most affected by inoculation, followed by root and culm. Total plant dry weight of bamboo seedlings under AM inocula were 1.84 (BEG167), 1.73 (BEG193), 1.59 (BEG168) and 1.54 (BEG141) times than that of the control respectively, with MD between 35.22-46.35%.

**Table 1. Root colonization of Chenglù bamboo seedlings inoculated with or without AM fungi.**

Treatments	Frequency of mycorrhizae (F)%	Intensity of the mycorrhizae colonisation (M)%	Arbuscule abundance (A)%	Vesicle abundance (V)%
BEG167	80.68c	40.09c	13.82d	36.86d
BEG141	73.84b	22.81b	9.75c	27.44c
BEG168	70.4b	21.31b	2.77b	11.01b
BEG193	85.58c	45.49d	15.84d	38.54d
Control	1.75a	0.04a	0.00a	0.00a

Note: Mean difference of treatments identified by Duncan's test. Different letters in each column means significant at 5 % level, five plants respectively for every treatment, and the same symbol is used for other tables

**Table 2. Effect of inoculation with AM fungi on the growth of Chenglù bamboo seedlings.**

Treatments	Shoot number	Shoot ratio	Shoot height(m)	Shoot diameter (cm)	Leaf number			Leaf area (m <sup>2</sup> /plant)		
					90d	150d	210d	90d	150d	210d
BEG167	11	0.92c	1.87a	1.70b	132ab	139ab	126a	0.726b	0.840b	0.749b
BEG141	9	0.75b	1.62a	1.46ab	142abc	158ab	145ab	0.687b	0.826b	0.678b
BEG168	8	0.67ab	1.67a	1.65b	156bc	170ab	154ab	0.669b	0.794b	0.702b
BEG193	13	1.08c	2.04a	1.73b	175c	195b	181b	0.720b	0.837b	0.748b
Control	7	0.58a	1.75a	1.22a	105a	131a	117a	0.357a	0.483a	0.392a

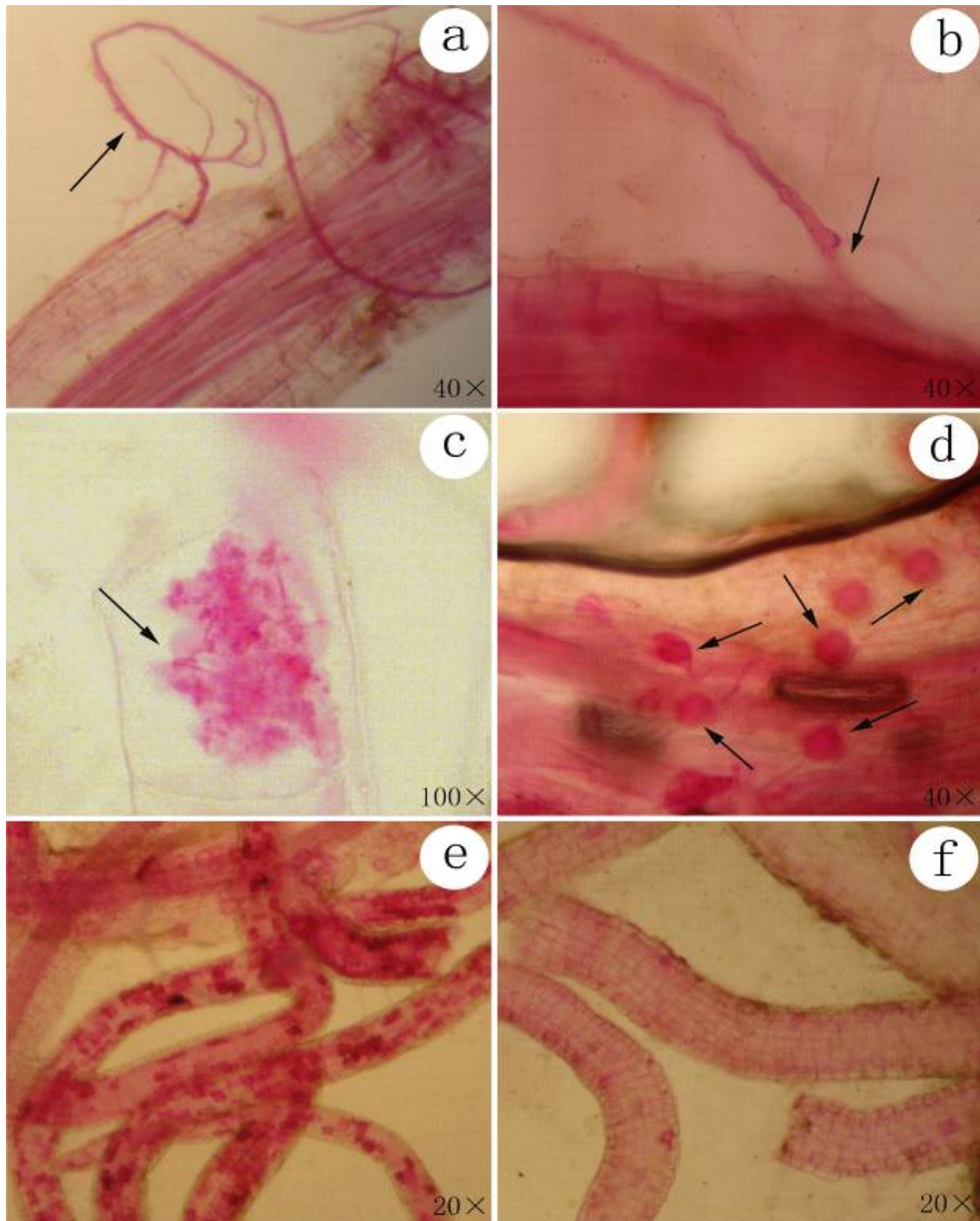


Fig. 2. AM fungi inoculation of Chenglù bamboo seedlings. a External hyphae of AM in Chenglù bamboos roots ; b Entry points of arbuscular mycorrhiza in Chenglù bamboos roots; c Arbuscular of AM in roots; d Internal hyphae and vesicles of AM in roots; e Roots structure of Chenglù bamboos inoculated by AM fungi; f Roots structure of the contro (photoed by W.X. Jiang).

**Seedling nutrient concentration:** AM inoculations significantly enhanced the P and K concentrations except N in root, culm and leaf. The enhancing effects varied by AM species (Table 4). Here, culms from BEG193, 167 and 168 respectively contained 73.5%, 58.6% and 54.0% more P, and 27.0%, 19.3% and 7.2% more K than that of the control. The highest P and K concentration were

observed in root treated with BEG167 (35.3% and 24.6% more than that of the control), followed by BEG193.

**Diurnal variation of photosynthesis (Pn):** Diurnal variation of Pn of Chenglù bamboo seedlings didn't show significant differences between AM treatments and the control (Fig. 3).

**Table 3. Effect of inoculation with AM fungi on the biomass and mycorrhizal dependency of Chenglù bamboo seedlings.**

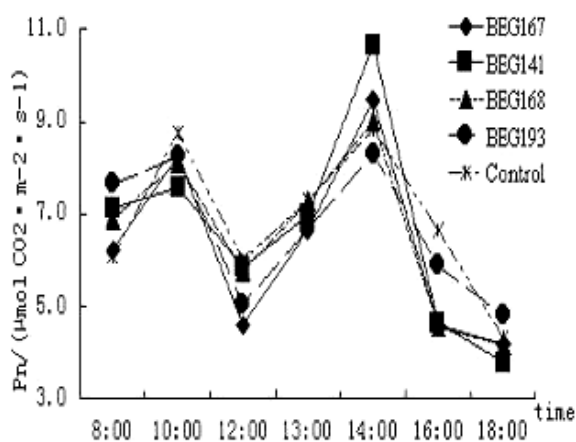
Treatments	Plant dry weight (g)					MD%
	Culm	Branch	Leaf	Root	Total	
BEG167	36.304ab	46.159b	29.898b	55.640b	168.009b	46.35
BEG141	33.956ab	37.704b	27.059b	37.864a	139.146b	35.22
BEG168	28.589ab	38.350b	30.391b	45.777ab	143.107b	37.01
BEG193	42.833b	40.286b	28.996b	44.822ab	156.940b	42.56
Control	20.876a	19.072a	18.270a	31.925a	90.143a	—

**Table 4. Effect of inoculation with AM fungi on the N, P and K concentration of Chenglù bamboo seedlings.**

Treatments	P concentration (%)			N concentration (%)			K concentration (%)		
	Root	Culm	Leave	Root	Culm	Leave	Root	Culm	Leave
BEG167	0.069b	0.151b	0.135c	0.800b	0.363a	2.361a	0.689b	2.106c	0.888ab
BEG141	0.057ab	0.099a	0.122ab	0.687a	0.388a	2.254a	0.605a	1.774ab	0.871ab
BEG168	0.058ab	0.134b	0.123abc	0.693a	0.362a	2.302a	0.612a	1.778ab	0.895ab
BEG193	0.066b	0.138b	0.126bc	0.749ab	0.403a	2.338a	0.644ab	1.980bc	0.954b
Control	0.051a	0.087a	0.111a	0.728ab	0.380a	2.282a	0.553a	1.659a	0.806a

**Table 5. Effects of different AM fungi on physical and chemical characteristic of soil.**

Treatments	Organic (g/kg)	Total P(g/kg)	Available-P (mg/kg)	Available-N (mg/kg)	Available-K (mg/kg)	pH
BEG167	52.94b	0.614b	25.44a	115.8a	229b	8.30b
BEG141	52.54b	0.621b	23.18a	119.5a	232ab	8.35ab
BEG168	52.91b	0.609b	22.32a	118.2a	222b	8.32ab
BEG193	53.14b	0.596b	24.16a	111.6a	226b	8.33ab
Control	49.89a	0.668a	22.12a	116.2a	252a	8.38a
Basal Soil	43.41	0.883	46.2	132	385	8.25

**Fig. 3. Effect of inoculation with AM fungi on the diurnal variation of photosynthesis of Chenglù bamboo seedlings.**

**Soil properties:** Mycorrhizal effect is also reflected in soil properties (Table 5). All AM inoculations significantly increased soil organic contents and available P, decreased total P and available K, and no significant

difference existed among AM treatments. Application of AM inocula showed the trend of lowering soil pH as well.

## Discussion

Symbiosis formation is a prerequisite for mycorrhizal efficiency or improvement of nutritional status of host plants, and root colonization rate reflected the compatibility between AM fungi and host roots (Li & Feng 2001). Our results showed that mycorrhizal colonization was well-established in the roots of Chenglù Bamboos. The root colonization rates differed among AM treatments. Reports that different species of AM fungi inoculated with the same host plant may possess different morphology, nutritional status, symbiotic efficiency and gene expression pattern (Wu *et al.*, 2009; Feddermanna *et al.*, 2010). It indicated that there were intraspecific differences in AM fungi with regard to the accumulation of mycorrhizal biomass. In addition, differences between BEG 193 and 141, isolated from the same species *Glomus intraradices*, may be explained by that not all isolates of the same species have identical properties, and it is now clear that multiple genomes can co-exist within individual AMF (Kuhn *et al.*, 2001; Rodriguez *et al.*, 2001).

AM fungi can enhance plant growth (Tang *et al.*, 2009; Manoharan *et al.*, 2010). The findings of this work showed that AM inocula by BEG 193 and 167 significantly increased shoot number, followed by BEG 168 and 141. The AM fungi, BEG 193 (*Glomus intraradices*) and 167 (*G. mosseae*) have been studied for various plant growth promoting activities and reported to positively influence the growth of various plant species (Xu *et al.*, 2008; Bedini *et al.*, 2009; Carretero *et al.*, 2009; Wu *et al.*, 2011). Chenglù bamboo seedlings in shooting period, after 100d of treatment, need to consume a lot of nutrients from mother bamboos, whose nutrient status determine the shoot quantity and quality (Zhou, 1998). Here, seedlings inoculated with AM had greater shoot number and diameter than non-AM seedlings, because mycorrhizal fungi are known to improve growth and survival of plants via increasing the absorption of water, and nutrients particularly P (Harley & Smit, 1983; Marschner & Dell, 1994; Al-Karaki, 2006; Miransari, 2008). It is documented that stem diameter and node number were defined completely before bamboo shoots sprouting out of the above ground. Shoot diameter, but node number, was seriously influenced by the environment in the soil when sprouting. Therefore, the more robust mother bamboos grew, the stronger shoots produced, and the greater spatial expansion capacity the young bamboos would have (Zhou, 1998).

The most intuitive indicator to reflect mycorrhizal efficiency, is changes in biomass of host plants. Our results showed that AM fungal inoculation significantly enhanced the dry weight biomass of Chenglù bamboo seedlings. This is in agreement with the results of other investigations. Verma & Arya (1998) reported that three different inocula of AM fungi enhanced dry biomass in tissue culture-raised *Dendrocalamus asper* plantlets. Muthukumar & Udaiyan (2006) also reported the promoting effects of AM fungi on dry masses of nursery-grown *Dendrocalamus strictus* seedlings. Mycorrhizal dependency (MD) has been defined as “the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility”. This work found that no significant relationship existed between MD and the root colonization rate. It may be explained since MD is mainly related to morphological and physiological properties of root systems (Mosse, 1973).

AM fungal application significantly enhanced the P and K concentration of Chenglù bamboo seedlings. The higher P concentrations found in AM plantlets is probably due to more efficient uptake of available P from the mycorrhizal pathway (MP) via AM fungal hyphae into root cortical cells (Smith & Smith, 2011). MP assists with the delivery of nutrients (particularly P) to host plants, regardless of whether they respond positively or not (Smith *et al.*, 2009). Positive effects arise largely from the increasing P uptake via the MP, alleviating P efficiency (Smith & Read, 2008; Smith & Smith, 2011). The higher K concentrations observed in AM seedlings in the present study also coincides with the reports of maize (Wu *et al.*, 2005), *Medicago sativa* (Khan *et al.*, 2008), *Panax ginseng* (Cho *et al.*, 2009) and *Carthamus tinctorius* (Abbaspour, 2010). Besides, higher K from AM-plants may be related to either a direct absorption and transportation through extensive hyphae (Sieverding &

Toro, 1988) or indirect role of P concentration improved (Wilson, 1988). No significant differences were observed in N concentration between AM-plants and the controls, which results agreement with reports from *Atriplex nummularia* (Plenchette & Duponnois, 2005) and *Araucaria angustifolia* (Zandavalli *et al.*, 2004). Although studies using <sup>15</sup>N tracer techniques have shown that AM hyphae can transport N from soil to root (Tanaka & Yano, 2005; Jackson *et al.*, 2008), high mobility and rapid movement of mineral N to roots through mass flow has suggested that AM fungi could play little role in plant N nutrition (Tinker & Nye, 2000).

Data from present study showed that no significant differences were observed in Pn between AM-plants and non-AM-plants. The result is inconsistent with some studies which suggested that AM symbiosis can, through improving the water status and photosynthetic (Pn) capacity, alleviate the deleterious effect and protect *Zea mays* and other plants against low temperature stress (Zhu *et al.*, 2010), salt stress (Sheng *et al.*, 2008; Wu *et al.*, 2010) and diesel stress (Tang *et al.*, 2009). It is possible that mycorrhizae can enhance plants under stress to absorb the material required to synthesize certain enzymes necessary to maintain at a relatively stable level (He *et al.*, 2007; Tang *et al.*, 2009). It has been reported that the positive mycorrhizal effects on host photosynthesis is based on an improvement of water, nutrition, growth and phosphorus absorption through mycorrhizal formation to expand the host roots absorption area in the soil, indirectly promoting the photosynthetic rate (Kapoor *et al.*, 2008; Sheng *et al.*, 2008). Thus, our results suggested that the enhanced growth and total leaf area of Chenglù bamboo seedlings inoculated with AM fungi indirectly improved the solar energy use efficiency as well.

AM fungi are important factors contributing to soil quality through their effects on host physiology, soil ecology interactions, and their contributions to maintaining soil structure (Rillig, 2004; Rillig & Mummey, 2006; Cho *et al.*, 2009). Other studies, however, suggest that the presence of plants, regardless of AM fungi, appears to have a much greater impact on increasing soil stability, with measured changes in porosity and repellency potential explaining the mechanism (Paul *et al.*, 2009). Our work indicated that the application of inoculants improved soil organic contents and removed more nutrients, especially P and K from the soil as part of an intergrated nutrient management system. These results were in agreement with Adesemoye *et al.*, (2008). In addition, application of AM inocula showed the trend of lowering soil pH as well, which required further research.

## Conclusion

In summary, our results suggested that symbiotic relationship between Chenglù bamboo and AM fungi can be well established, and AM fungal inoculation can promote plant growth, biomass accumulation and mineral nutrients absorption. For 4 strains used in the study, BEG167 and 193 have the most significant mycorrhizal effect and could make an important contribution to Chenglù bamboos production.

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