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Response of Tea (*Camellia sinensis* (L.). Kuntze) to Arbuscular Mycorrhizal Fungi under Plantation Nursery Conditions

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ABSTRACT

Response of tea (*Camellia sinensis* (L.) O. Kuntze) to inoculation with six species of indigenous arbuscular mycorrhizal (AM) fungi, *Acaulospora scrobiculata* Trappe, *Glomus aggregatum* Schenck & Smith emend. Koske, *G. fasciculatum* (Thatcher) Gerd. & Trappe emend. Walker & Koske, *G. geosporum* (Nicol. & Gerd.) Walker, *G. intraradices* Schenck & Smith and *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders was studied under plantation nursery conditions. Tea seedlings raised in the presence of AM fungi generally showed an increased growth and nutritional status over those grown in the absence of AM fungi. The extent of growth and nutritional status enhanced by AM fungi and the mycorrhizal dependency of the host varied with the species of AM fungi. However, AM association decreased nutrient use efficiencies. Seedlings inoculated with *S. calospora* had greater biomass and seedling quality than other mycorrhizal seedlings.

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is a commercially valuable plantation crop grown widely in the humid and subhumid tropics. Recently, green tea production has stabilized despite increased application of external inputs such as fertilizer and biocides. One reason for this static production has been attributed to the intense cultivation of this plantation crop, which has led to important changes in various physico-chemical and biological properties of the soil (Senapathi *et al.*, 1994, 1999).

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Tea, like most plantation crops, is associated with arbuscular mycorrhizal (AM) fungi. The host benefit from AM association is well documented for several crops and forestry species and includes increased uptake of immobile mineral nutrients especially phosphorus, improved water relations, seedling growth rates, vigour, transplantation shock and survival after transplantation (Smith & Read, 1997). Several studies, however, do indicate that different AM fungal species in a natural plant community may possess unique biological properties based on their biology (Bethlenfalvay *et al.*, 1989). However, there is little appreciation of the range of plant growth responses that can occur with native AM fungi. Studies have shown that native AM fungi can enhance the growth and nutrient uptake by plants (Muthukumar & Udaiyan, 2000; Muthukumar *et al.*, 2001). In spite of this, several studies have utilized exotic AM fungal species for the assessment of host performance (Guissou *et al.*, 1998; Koide *et al.*, 1999; Bâ *et al.*, 2000). The benefit of this approach is that AM fungi can be accessed very quickly, but unfortunately interactions between plants and 'exotic' AM fungi may have little relevance to the interactions that occur and the observed responses may not represent the suite of responses that plants would normally encounter locally. In contrast, the approach of using native AM fungi is often time and resource consuming for successful isolation and culture of individual AM fungi from the field. It is therefore important to screen different AM fungal species in a natural fungal community to ensure effectiveness of each species if it is to be utilized.

A major limitation of the use of AM fungal technology in agriculture is the huge quantity of the inoculum required to inoculate field crops. As AM fungi cannot be grown on laboratory media, production of a large quantity of the inoculum is difficult. On the other hand plantation crops are raised in nurseries during early stages before being transplanted to the main field. Thus, inoculation of seedlings in the nurseries not only results in saving the quantity of inoculum required, which in turn reduces the production cost, but also helps in the establishment and survival of the transplanted seedlings. Recent studies do indicate that clones of tea developed for higher yield, drought tolerance, disease or pest resistance vary in their ability to take up and utilize P (Zoysa *et al.*, 1999). AM fungi are also known to influence the host's uptake of P from the soil, as well as its utilization efficiency (Koide, 1991). Further, as different AM fungal species are known to affect a plant's P uptake to various extents, it is hypothesized that different AM fungi can influence the uptake of nutrient and their utilization to different extents. The first objective of the present investigation was to study the effect of indigenous AM fungal species on growth and nutrient uptake by tea seedlings under plantation nursery conditions. The second objective was to study the influence of AM fungi on the utilization efficiencies of various nutrients and seedling quality. This study was carried in order to harness a strategy for routine use of AM fungi in tea plantation nurseries.

MATERIALS AND METHODS

Soil and experimental design

The soil used was a vertisol gathered at the research farm of United Planters Association of South India (UPASI), Tea Research Institute, Valpari, Tamil Nadu, India. The soil had an initial pH of 5.2 (1:1 soil to water) and 14 mg kg^{-1} of total nitrogen (N); 1.7 mg kg^{-1} of total phosphorus (P) and 12.5 mg kg^{-1} of extractable potassium (K) according to Jackson (1973). The soil type used is similar to that of the plantation soil. The soil was air-dried and crushed to pass through a 2 mm sieve, steam sterilized (100°C for 3 h at 103.5×10^3 Pa) and incubated for 2 weeks to reduce potential phytotoxic effects of heating (Rovira & Bowen, 1966). The experiment consisted of seven treatments involving six AM fungal species plus control with ten replicates for each treatment.

Plant material

Tea seeds (cv. B.S.S. 5) procured from UPASI Tea Research Institute, Valpari, Tamil Nadu, India were sown in seed beds ($1.5 \times 0.5 \times 0.25$ m) containing sterile sand soil mixture (1:1, v/v) with the hylar scar pointing upwards. Care was taken to see that the seeds remained partially above the soil surface. The seeds germinated around 20–30 days after sowing. Uniform healthy 30 days old seedlings were transplanted in each polythene bag (23×15 cm) containing 2 kg of sterile soil with or without AM fungi according to treatments.

AM fungal inoculum

The AM fungal species used in this study were isolated from the rhizosphere of tea plants, multiplied using sterilized-soil-sand mix (1:1, v/v) as the substrate and cowpea (*Vigna unguiculata* (L.) Walp.) as host. After 90 days of growth, shoots of cowpea were severed and the substrate containing hyphae, spores and root bits was dried and used as the inoculum. The inoculum potential (IP) of each culture was estimated by the most probable number (MPN) method (Porter, 1979) prior to inoculation. Inoculum densities ranged between 129 and 1450 propagules g^{-1} of soil. AM inoculum was placed in the planting hole at different rates as to maintain an initial inoculum potential of 12000 propagules per bag at the time of transplantation (Table 1). The control soils were prepared by adding a mixture of 3 g of sterilized pot culture plus a composite AM fungal free soil filtrate. This composite AM fungal free soil filtrate was prepared by blending 20 g of each AM fungal inoculum in 1000 ml deionized water and passing the suspension through a Whatman No. 1 filter paper. All bags, including control, received 5 ml

of this composite microbial but AM fungal free filtrate in an attempt to equalize the background microbial flora.

Culture method and harvest

The seedlings were grown for 120 days in a randomized block design. Soil moisture was maintained near field capacity by watering as necessary throughout the experiment. At harvest, the shoot length was measured from the soil surface to the growing tip of the plant. Stem girth at 1 cm above the soil and root collar diameter were measured using a vernier caliper. Shoots were cut off at the soil surface and oven-dried (70°C). Root length was estimated according to Tennant (1975). The roots were later stained and examined for length and AM colonization using the magnified-intersect technique (McGonigle *et al.*, 1990).

Tissue N and P content in shoots and roots were determined after digestion by an automated photometric sodium salicylate procedure for N, and ammonium molybdate procedure for P using a Skalar Auto Analyser (SA 20/40, Holland). Tissue K was estimated using a flame photometer (Davis, 1962). Nutrient use efficiency was calculated as the dry weight of tissue produced per mg of nutrient. Although nutrient use efficiency is simply the inverse of nutrient concentration, it is used in the present study to determine the differences in plant requirement for nutrients (Chapin & Van Cleve, 1989). Efficiency of nutrient uptake was estimated according to Blair (1993) as the ratio of total nutrient uptake per plant divided by total root dry weight. Seedling quality index was calculated by using the formula of Dickson *et al.* (1960).

Seedling quality index = Seedling dry weight (g)/[Seedling height (cm)/Root collar diameter (mm) + Shoot dry weight (g)/Root dry weight (g)]

Statistical analysis

Each measured variable in the experiment was subjected to analysis of variance and mean separations were done using Duncan's Mulitiple Range Test. Pearson's correlation analysis was used to assess the relationships between plant and mycorrhizal variables whenever necessary.

RESULTS

Tea seedlings inoculated with different AM fungi developed moderate colonization levels ranging from 36 to 42% (Figure 1) and there were significant

TABLE 1

Different arbuscular mycorrhizal (AM) fungal species, number of infective propagules and the quantity of inoculum used (12000 propagules) to inoculate tea seedlings.

AM fungal species	Number of infective propagules (g^{-1} inoculum)	Quantity of inoculum used (g)
Acaulospora scrobiculata Trappe	530	22.60
Glomus aggregatum Schenck & Smith emend. Koske	1367	8.78
Glomus fasciculatum (Thatcher) Gerd. & Trappe emend. Walker & Koske	1450	8.28
Glomus geosporum (Nicol. & Gerd.) Walker	1060	11.32
Glomus intraradices Schenck & Smith	1280	9.38
Scutellospora calospora (Nicol. & Gerd.) Walker & Sanders	129	93.00

differences in the mycorrhizal dependency between seedlings inoculated with different AM fungi. The numbers of AM fungal propagules inoculated were not related to colonization levels ($r = -0.158$; $p > 0.05$; $n = 6$). Seedlings inoculated with different fungal species grew more vigorously than the uninoculated control seedlings. Mycorrhizal seedlings were 38–157% taller, with significantly higher stem girth and root collar diameter (Table 2). The dry weight of root and shoot of mycorrhizal seedlings were respectively 26–49% and 18–97% higher than non-mycorrhizal seedlings. However, greatest biomass (shoot + root) accumulation occurred in seedlings inoculated with *Acaulospora scrobiculata* and *Glomus intraradices*, which were 8–50% higher than other AM seedlings (Table 2).

AM inoculation significantly altered the root morphology of the tea seedlings. The total root length of AM seedlings were 177–397% higher with an 85–428% increase in lateral root production compared with non-AM seedlings. Similarly, AM inoculation also increased the leaf numbers and the leaf area of AM seedlings, which was 25 to 174% higher, compared with non-AM seedlings. Seedlings inoculated with *G. geosporum* and *G. aggregatum* had the lowest R/S ratios among AM seedlings and non-AM seedlings.

In general, AM inoculation significantly increased tissue NPK content of tea seedlings (Figure 2). AM seedlings had 200–305% higher shoot N, 39–70% higher shoot P and 33–79% higher K compared with non-AM seedlings. Among the AM seedlings, *G. fasciculatum* or *S. calospora* inoculated seedlings had 5–34% higher N, 9–19 higher P and 13–34% higher shoot K compared with other AM seedlings. Tea seedlings most dependent on AM association tended to have high nutrient concentration while the less dependent seedlings were among those having the lowest nutrient concentrations (Figure 3).

The efficiency of uptake of certain nutrients was significantly affected by certain AM fungi (Figure 4). The efficiency of phosphorus (EPU), nitrogen

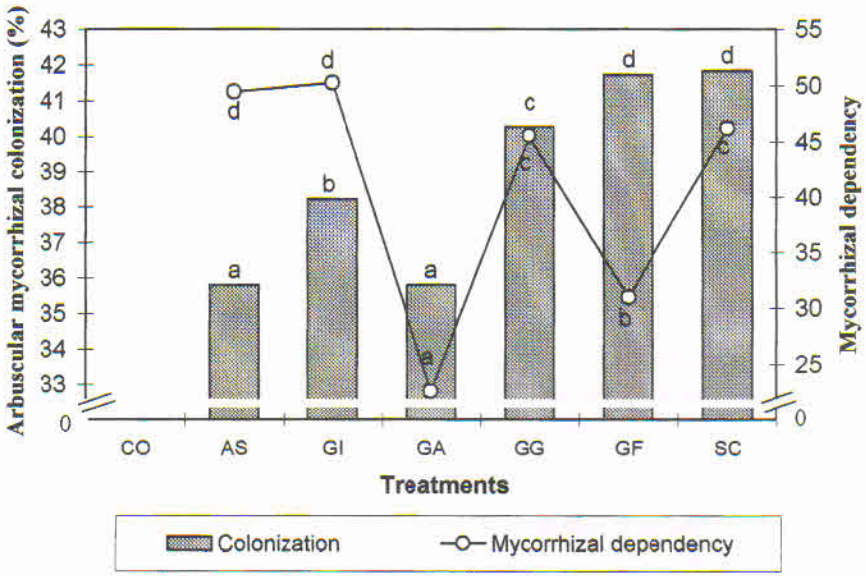

FIGURE 1. Arbuscular mycorrhizal colonization and mycorrhizal dependency of tea seedlings inoculated with different AM fungi.
CO Control, AS *Acaulospora scrobiculata*, GI *Glomus intraradices*, GA *G. aggregatum*, GG *G. geosporum*, GF *G. fasciculatum*, *Scutellospora calospora*. Means with same letter(s) do not differ significantly (Duncan's multiple range test, $p < 0.05$)

(ENU) and potassium (EKU) was 21–63%, 169–445% and 7–136% higher in AM seedlings than in non-AM seedlings. Among AM seedlings the EPU, ENU and EKU of inoculated seedlings were 40–118%, 44–65% and 42–122% higher than in other AM seedlings. There were also significant differences in nutrient use efficiencies among AM and non-AM seedlings (Figure 5). The nitrogen use efficiency (NUE) and potassium use efficiency (KUE) of non-AM seedlings were 12–279% and 27–166%, respectively, higher than in AM seedlings. However, the phosphorus use efficiency (PUE) of seedlings inoculated with *A. scrobiculata*, *G. intraradices* and *G. geosporum* was 101%, 126% and 25% higher than in AM seedlings. The lowest nutrient use efficiencies among treatments occurred in *S. calospora* inoculated seedlings.

Further, AM inoculation significantly affected the quality of the tea seedlings (Figure 6). Seedlings inoculated with *S. calospora* showed greater quality index than all other treatments and this increase was 103% compared with non-AM seedlings and 16–68% over those seedlings inoculated with other AM fungi.

TABLE 2

Influence of different arbuscular mycorrhizal fungi on growth of tea seedlings.

Treatments	Seedling height (cm plant^{-1})	Total root length (cm plant^{-1})	Lateral roots (no plant^{-1})	Stem girth (mm plant^{-1})	Root collar diameter (mm plant^{-1})	Leaf		Dry weight (g plant^{-1})		R/S ratio
						Number (plant^{-1})	Area (cm plant^{-1})	Root (R)	Shoot (S)	
Control	8.81 a	76.01 a	10.20 a	1.07 a	1.24 a	3.30 a	8.06 a	0.58 a	0.76 a	0.76 b
Acaulospora scrobiculata	22.62 a	354.05 c	44.71 a	2.04 b	2.09 b	5.30 b	10.04 b	1.02 d	1.63 d	0.63 b
Glomus intraradices	22.07 a	378.01 c	53.90 f	3.04 a	2.38 d	8.90 c	12.16 c	1.01 d	1.68 d	0.60 b
G. aggregatum	14.43 c	213.61 b	19.80 b	2.44 c	2.32 d	4.90 b	14.30 d	0.83 c	0.90 b	0.92 b
G. geosporum	22.32 a	237.14 b	33.10 c	2.46 cd	2.06 b	8.30 c	17.32 f	0.73 b	1.73 a	0.42 a
G. fasciculatum	12.18 b	221.63 b	18.90 b	2.69 d	2.15 be	8.00 c	22.07 g	0.77 b	1.17 a	0.65 b
Scutellospora calospora	17.57 d	210.62 b	38.40 d	2.41 c	2.18 c	7.90 c	15.65 a	1.07 d	1.42 c	0.76 b

Means in a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p < 0.05$).

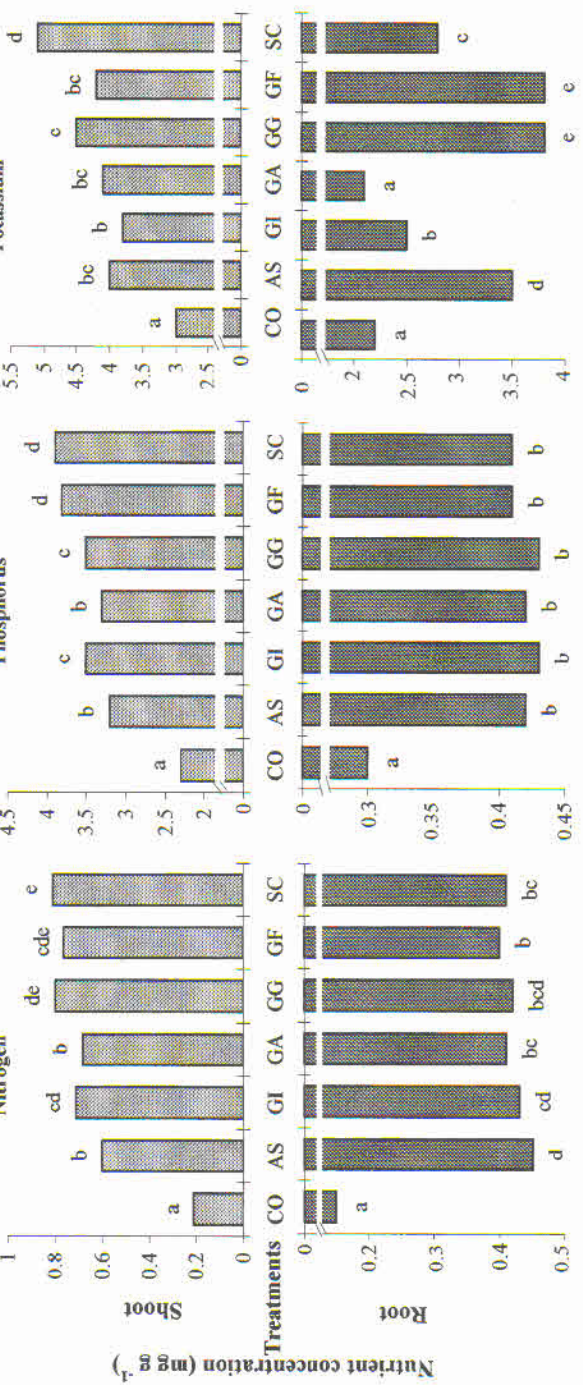

FIGURE 2. Influence of arbuscular mycorrhizal colonization on shoot and root nutrient concentration of tea seedlings. Legends are as in Figure 1.

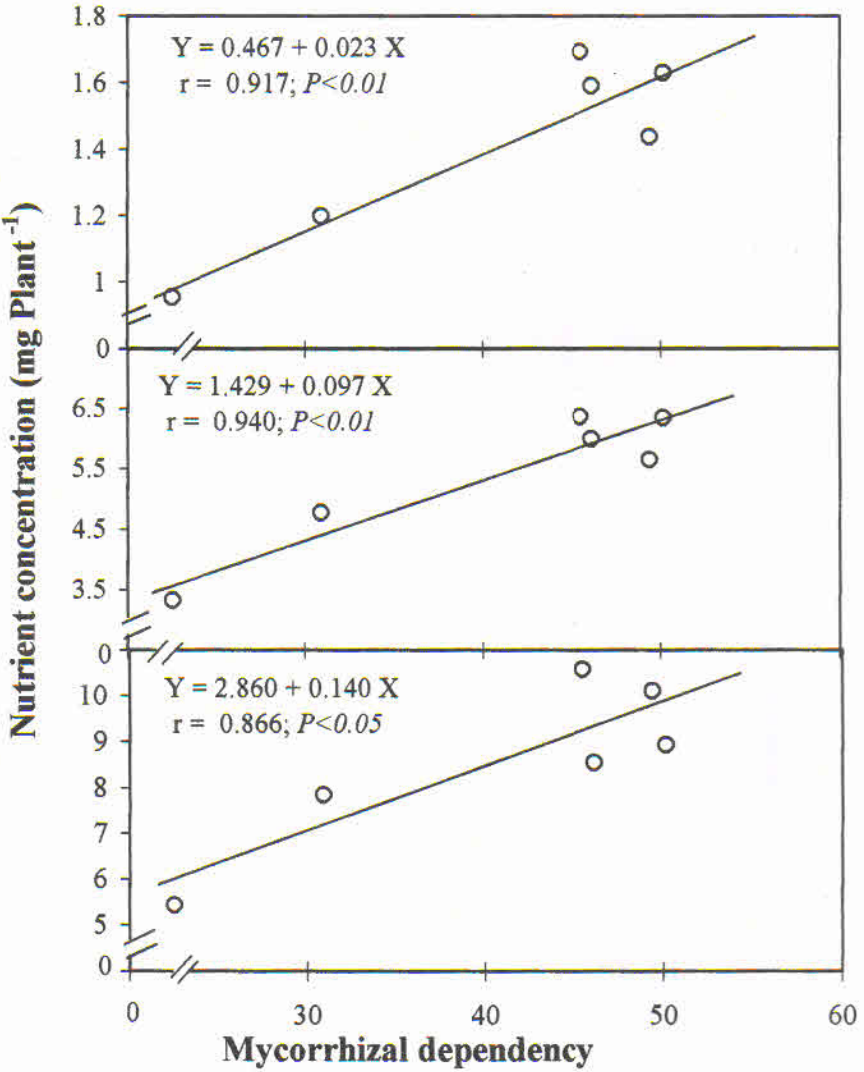

FIGURE 3. Relationship between seedling nutrient content and mycorrhizal dependency of tea seedling inoculated with different AM fungi.

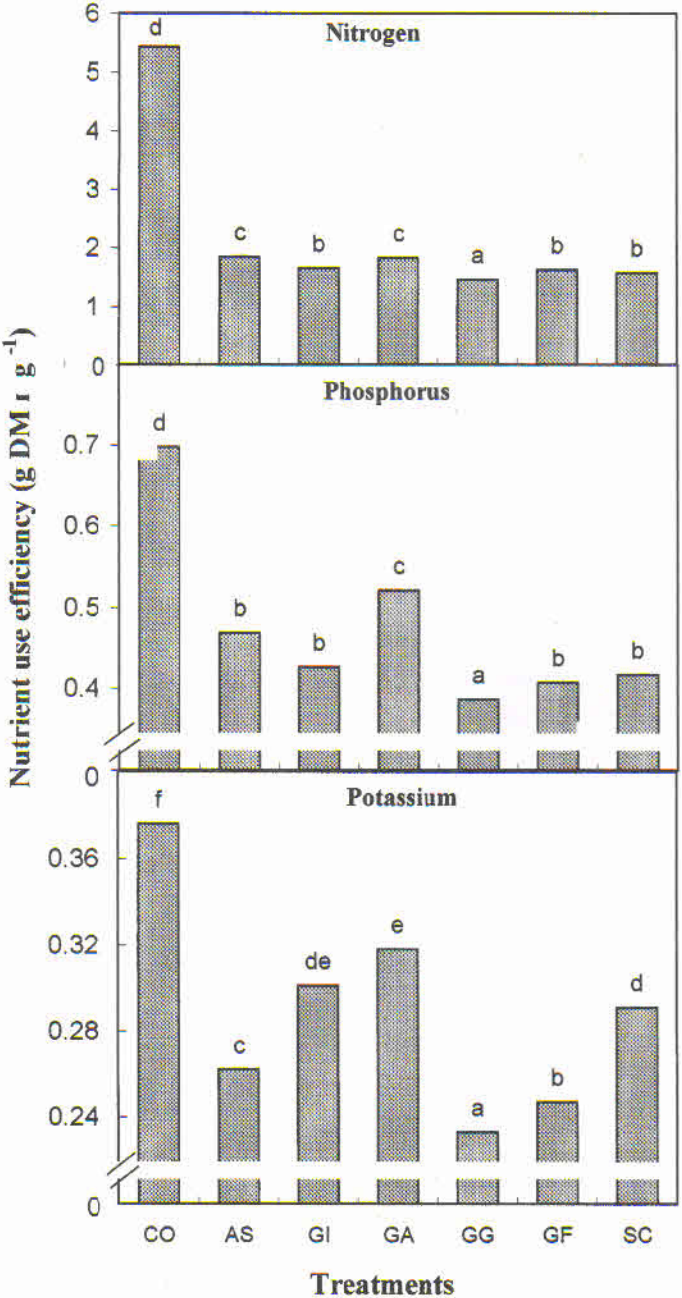

FIGURE 4. Influence of arbuscular mycorrhizal colonization on efficiency of nutrient uptake of tea seedlings. Legends are as in Figure 1.

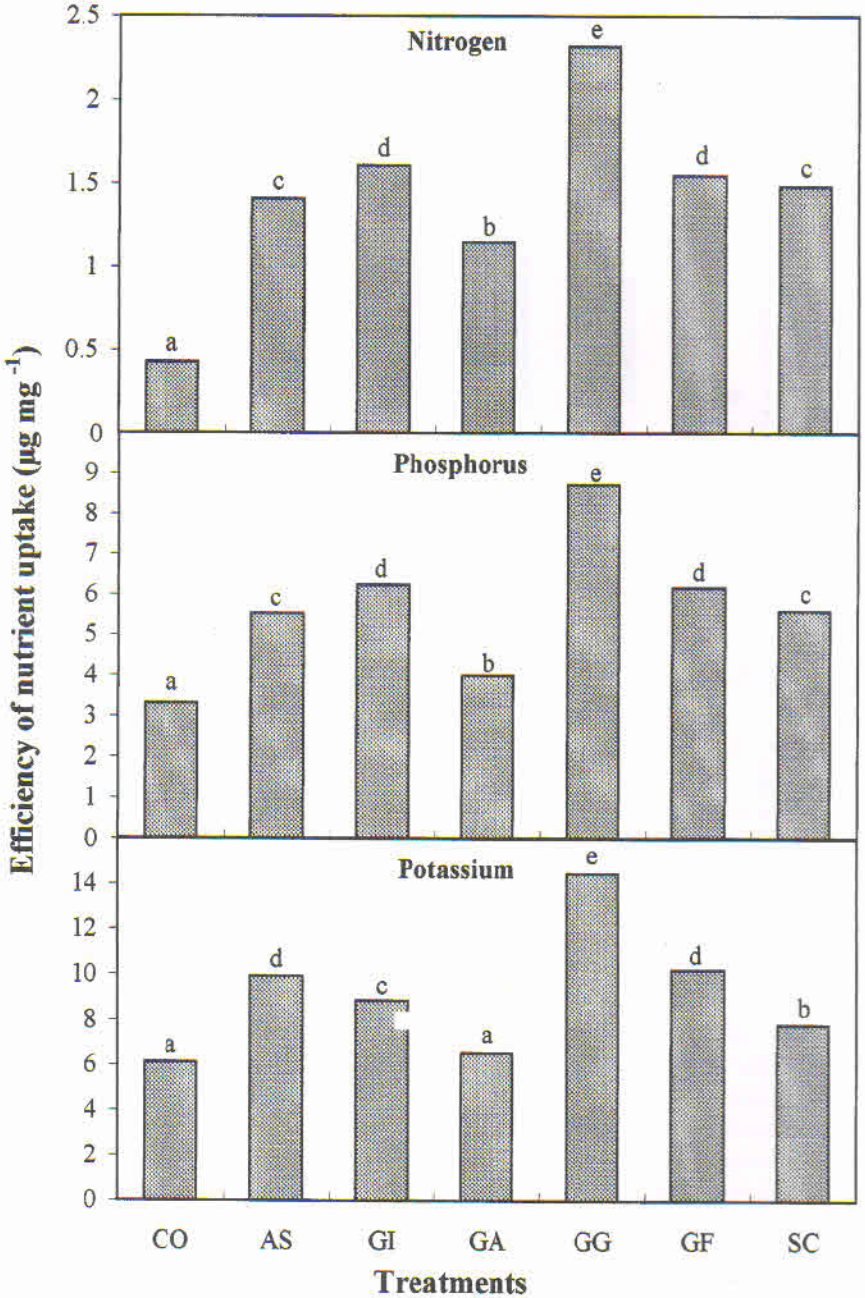

FIGURE 5. Influence of arbuscular mycorrhizal colonization on nutrient use efficiency of tea seedlings. Legends are as in Figure 1.

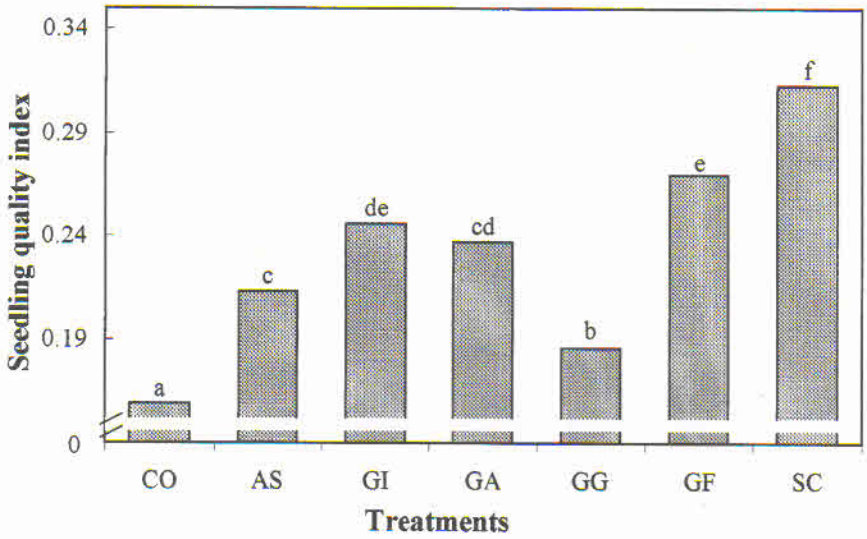

FIGURE 6. Influence of arbuscular mycorrhizal colonization on seedling quality index of tea seedlings. Legends are as in Figure 1.

DISCUSSION

As expected, the tea cultivar examined in this study responded very differently to colonization by different AM fungi. This is consistent with results pertaining to growth responses of different perennial plant species to different AM fungal species (Siqueira *et al.*, 1998; Bhattacharya & Bagyaraj, 2002; Mamatha *et al.*, 2002). The extent of AM colonization varied between different fungal species, but tea appeared to be moderately mycorrhizal (36–42%) as observed by Kumaran & Santhanakrishnan (1995) in different tea plantations in Tamil Nadu, India. The moderate mycortrophy of tea seedlings is further supported by the low mycorrhizal dependency (MD) values. The MD values obtained in this study with tea, while never exceeding 50%, appear moderate or low compared with values reported for highly mycorrhizal dependent plant species (Plenchette *et al.*, 1983; Siqueira & Saggin-Júnior, 2001). Further, the MD values varied depending on the AM fungal species. Such variation in the MD values for a plant species inoculated with different AM fungi has been reported (Adjoud *et al.*, 1996; Guissou *et al.*, 1998). However, comparisons must be made cautiously as experimental conditions are not identical in these studies.

It appears that the main effect of AM colonization on tea seedling growth was largely attributed to a mycorrhiza-mediated increase in nutrient uptake. This is supported by the existence of significant correlations between AM colonization

levels with plant N ($r = 0.916$; $p < 0.001$; $n = 7$), p ($r = 0.921$; $p < 0.001$; $n = 6$) and K ($r = 0.817$; $p < 0.001$; $n = 6$). However, differences occurred between the fungal species in their relative nutrient uptake because of inherent differences in the ability of different AM fungal isolates to produce the extramatrical hyphae. Interspecific studies on AM fungi have highlighted that species may differ in their formation of extramatrical hyphae (Jakobsen *et al.*, 1992a, 1992b; Boddington & Dodd, 1998). This would enable differences in the exploration of soil volumes, acquisition and transport of nutrients to roots by different AM fungal isolates.

The large increase in nutrient content of tea seedlings caused by AM colonization was not matched by a similar large increase in dry weight. This was because nutrient concentrations were higher in AM tea seedlings, resulting in a large decrease in nutrient use efficiencies. Generally, plants experiencing a greater nutrient deficiency frequently have lower concentrations of the limiting nutrients than those experiencing less deficiency (Chapin, 1980; Haynes *et al.*, 1991). The present results for AM tea seedlings, therefore, are not surprising. They are similar to results of Stribley *et al.* (1980), Koide (1991) and Koide *et al.* (2000) who showed that for a given nutrient concentration, AM plants have significantly lower dry weight than non-mycorrhizal plants. There are at least two possibilities by which AM colonization could decrease nutrient utilization efficiencies. First, it could be due to the loss of carbon from the host to the fungus as explained by Stribley *et al.* (1980). An alternative explanation is that nutrients other than those studied became limiting to AM seedlings (Koide *et al.*, 2000). Although it is generally believed that AM fungi are not host specific, studies have shown that the extent of host benefit may vary with AM fungal species as observed in this study and in other reported studies (Vasanthakrishna *et al.*, 1994).

Seedling quality reflects the integration of a multitude of physiological and morphological characteristics of the seedlings (Ritchie, 1984). Although the concept of seedling quality is widely used in forestry, it could also be used for plantation crops as the field survival and productivity of the nursery raised plantation seedlings are related to the quality of the seedlings. Seedlings inoculated with *S. calospora* had a greater seedling quality index value, indicating a sturdier stem and a proportional top dry weight which is desirable for nursery seedlings. Hence, it can be concluded that even native AM fungal species can be used for inoculating plant species. Though tea seedlings exhibited a varied response to different AM fungal species, *S. calospora* conferred greater growth benefit than all other fungi used in this study. This study clearly indicated that the indigenous AM fungal isolates could significantly enhance the growth and the quality of tea seedlings raised in nurseries. A simple technology for inoculating tea seedlings with selective AM fungi could be adopted in nurseries to improve the quality of seedlings produced.

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