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RESEARCH ARTICLE

Native arbuscular mycorrhizal fungal isolates (*Funneliformis mosseae and Glomus microcarpum*) improve plant height and nutritional status of banana plants

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Abstract

An experiment was carried out to assess the benefits of native arbuscular mycorrhizal fungi (AMF) for banana plants cv. 'Nendran'. The AMF species applied were *Funneliformis mosseae* and *Glomus microcarpum*, which were identified in a previous survey as the most common root associates of *Musa* spp. in traditional monoculture banana fields. Spores of both the AMF species isolated from the natural banana fields were mono-cultured and used in the experiment, individually and in combination, at two inoculum spore levels (2500 or 5000 spores). We evaluated the root colonising potential of AMFs and their effects on plant height, chlorophyll content and leaf N, P and K concentrations at regular intervals up to 90 days after inoculation. All the inoculated plants showed more than 80% root colonisation. Increase in chlorophyll content in the leaves was found significant in all treatments, with the exception of the combination of 5000 spores of *F. mosseae and* 5000 spores of *G. microcarpum* each. Increases in leaf N, P and K were found in all the inoculated plants as compared to control (sterile soil without any AMF). While a significant reduction in soil available nitrogen and soil pH was observed in all treatments with inoculation, the soil available phosphorus and soil total organic carbon were increased by inoculation. Overall data revealed positive effects of AMF species in banana, especially during its early growth. As AMF species were isolated from fields differing in relation to banana variety and soil type and have positive effects in banana nutrition, an integrated soil fertility management using AMF appears promising.

Keywords: *Funneliformis mosseae*; *Glomus microcarpum*; Native AM fungi; Nendran; South Indian Banana

Introduction

Banana, one of the most consumed fruits worldwide, is a common crop in tropics, where it is cultivated in monoculture traditional farms and requires high amount of nutrients as well as chemical control of insects and diseases. However, environment contamination by overusing chemical fertilisers and pesticides is common in banana production systems all over the world (Mahecha-Vásquez *et al.*, 2017). Excessive chemical application in fields due to the intensive crop production strategies is usual in India. In order to overcome the widespread environment pollution quite common from such 'chemicalised' green revolution fields, an alternative farming strategy with minimum environment impact is desirable.

Arbuscular mycorrhizal fungi (AMF) are well-known root symbionts capable of increasing the effective absorbing area of plant roots. AMF are also capable of bringing about biochemical changes in nutrient-deficient soils and to enhance the availability of nutrients to associated plants (Balakrishnan and Subramanian, 2016). Beneficial effects of different species of AMF towards

sustainable nutritional management in diverse varieties of banana have been reported. AMF characteristics such as the extent of extra-radical hyphae can benefit plants and root colonisation by AMF is also positively correlated to plant growth (Saxena *et al.*, 2013), improving leaf chlorophyll content and amount of N, P and K in plant tissues (Abdel-Fattah and Asrar, 2012). It has already been demonstrated that *Glomus mosseae* in *Musa accuminata* can reduce the use of phosphate fertiliser by up to 50% (Shashikala *et al.*, 1999). In general, *Glomus* spp. are known as effective AMF to be used in banana (Jaizme-Vega and Azcon, 1995). However, diverse species of AMF are found as general root colonisers in banana fields, showing low level of host specificity (Gaidashova *et al.*, 2012).

Identification of host-specific AMF strains adapted to local environmental conditions is important towards its successful use in crop fields (Mahecha-Vásquez *et al.*, 2017). This is because application of exotic AMF may affect the local AMF diversity (Schwartz *et al.*, 2006), which can have negative consequences for crops. Experiments performed in banana using native strain and commercial AMF strains also reiterate the fact that native strains induce higher shoot and root dry weight in banana as compared to exotic ones (Usuga *et al.*, 2008).

It may be noted that most of the currently available experimental trials revealing beneficial impacts of AMF in banana were carried out in micro-propagated banana plantlets. So far no experiment has been conducted on benefits of any AMF in a native banana variety of South India, which is one of its centres of origin. *Funneliformis mosseae* and *Glomus microcarpum* are commonly found in soils and have a worldwide distribution. Recently, we found *F. mosseae* and *G. microcarpum* as the two dominant AMF in fields where many varieties of banana are cultivated over widely different soil types (Nidheesh *et al.*, 2017). Since many species of AMF were found in native banana fields, and trials usually investigate interaction of diverse AMF species (Pal and Pandey, 2017), AMF must be studied individually and in combination. Herein, it was hypothesised that both *F. mosseae and G. microcarpum* are beneficial to banana and there would be some positive interaction between them. We aimed to assess the role of the native AMF on leaf nutrition and plant height, evaluating root colonisation efficacy, soil fertility and spore production in soil.

Materials and Methods

Plant material and AMF preparation

Tissue cultured and hardened banana plantlets of the cultivated variety (cv) Nendran (*Musa paradisiaca* L., AAB genome) were obtained from nearby nursery. Healthy plants (10 cm height) with five leaves were selected.

Cultures of *F. mosseae* and *G. microcarpum* were developed separately in *Eleusine coracana* (finger millet) as monospecific inoculum. Inoculum cultures were developed and multiplied in sterile plastic pots containing double sterilised soil (soil pH 6.86 ± 0.06, soil available nitrogen 13.53 ± 0.23 mg kg^{-1}, soil available phosphorus 2.50 ± 0.01 mg kg^{-1}, soil available potassium 18.96 ± 0.29 mg kg^{-1} and total organic carbon 21.70 ± 0.35 g kg^{-1}) under sterile condition. After flowering of the host plant, cultures were examined for spore density. Mean spore density of *F. mosseae* and *G. microcarpum* were 122.0 ± 2.45 and 97.0 ± 2.24 spores per gram soil, respectively.

Soil preparation

Soil used in the experiment was clayey-skeletal (total sand 22.1%, silt 18% and clay 59.1%), Isohyperthermic, Ustic Haplohumult belonging to Kottappuram soil series and order Ultisol (Soil Survey Organization, 2007). Top (0–15 cm) soil collected from the field was analysed for its fertility, showing: pH 7.86 ± 0.03, 28.23 ± 0.23 mg N kg^{-1}, 2.27 ± 0.13 mg P kg^{-1}, 17.61 ±

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0.10 mg K kg^{-1} and soil total organic carbon 22.10 ± 0.34 g kg^{-1}. The soil was then sterilised by double autoclaving (Equitron) at 121 °C, 15 lbs for 20 min in a steel container. After sterilisation the soil was mixed well and about 10 kg of sterilised soil was used to fill in each grow bag (UV sterilised, 150 microns, 40 × 24 × 24 cm). No fertiliser addition or amendment was done in the soil.

Experimental design

Completely randomised design was used with four treatments and a control, and five replications. Treatment combinations and corresponding spore density of each AMF used in the experiment were (1) control, sterile soil without any AMF; (2) Fm5000, 5000 spores of *F. mosseae*; (3) Gm5000, 5000 spores of *G. microcarpum*; (4) Fm+Gm5000, combination of 5000 spores of *F. mosseae and* 5000 spores of *G. microcarpum* each and (5) Fm+Gm2500, combination of 2500 spores of *F. mosseae and* 2500 spores of *G. microcarpum* each.

Inoculation process

For inoculation, the AMF culture was weighed in such a way that inoculum contains 5000 and 2500 spores for each AMF species: 40.9 g and 20.45 g inoculum for *F. mosseae*, respectively; 51.54 g and 25.77 g for *G. microcarpum* inoculum, respectively. Half of the inoculum was placed in a 10-cm deep pit made in the soil using a sterile glass rod and banana plantlet was planted at the same position. The rest of the inoculum was placed on the top of roots of banana plantlets and the pit was filled with surrounding soil and watered. Bags were arranged in such a way that each row consisted of five bags of each treatment and kept 1.5 m apart to avoid cross-contamination.

Plant height, AMF association and soil analysis

The experiment was done between December 2016 and March 2017. About 2 L of sterile distilled water was given to each plant every morning and evening. Air temperature varied from 34 °C (day) to 23 °C (night) and humidity from 93% (night) to 46% (day).

Measurements of the pseudostem height, number of functional leaves and leaf sampling were done on the 30th, 60th and 90th days after inoculation (DAI). The pseudostem height was measured from its base to the crossing point of the first two leaves from the top. On the 90th day, all plants were harvested and fresh weight of the shoot after removing the corm was assessed using a weighing balance. Only the wet weight of the total shoot biomass was evaluated at 90 DAI.

The fully expanded third leaf from the top of the plant was taken for estimating total chlorophyll and tissue N, P and K. One gram of fresh leaf tissue near the middle portion of the midrib was taken (three replications) from each plant for the estimation of leaf chlorophyll content (Arnon, 1949). Sample preparation (500 mg) for analysing tissue N, P and K was done as per AOAC (1978) and the leaf tissues were dried in an oven at 60 °C until the weight became constant. The estimation of tissue nitrogen was carried out by micro-Kjeldahl method (AOAC, 1978). Tissue phosphorus and potassium were measured after triple acid digestion, with phosphorus content being estimated by Vanado-molybdate yellow-colour method and potassium by Flame photometric (Systronics Flame Photometer 128) method, following Jackson (1973).

Root and soil samples were collected from each treatment at 90 DAI. Estimation of mycorrhizal colonisation of the root system was done after clearing and staining the roots (Philips and Hayman, 1970). The fine, healthy, lateral roots were cut into pieces of 1 cm length, boiled in KOH 10% and washed with distilled water. After washing, the root segments were neutralised with HCl 10% for one minute. The cleared root segments were stained with trypan blue in lactophenol 0.05% and left overnight. One hundred root segments were examined for AMF colonisation under the light microscope

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Motic BA 310 (Speed Fair Co., Ltd, Hong Kong, China) with N-WF 10×/20 eyepiece and 10× objective. Presence of mycelium, arbuscule or vesicle was taken as the indication of colonisation. The result was expressed as per cent colonisation, following Valsalakumar *et al.* (2007).

The percentage of root length containing linear hyphae (RLH), arbuscules (RLA), vesicles (RLV) and the percentage total root length (RLTC) colonised by AMF were analysed separately.

Spores were isolated from soil sample (50 g) following wet sieving and decanting procedure (Gerdmann and Nicolson, 1963). The spores extracted through sieves were filtered using a Whatman No. 1 filter paper. The filter paper was washed with distilled water and the spore extract was poured into a measuring cylinder. It was diluted up to 50 mL so that 1 mL of the extract represented 1 g soil. 1 mL of the extract was pipetted out in parallel lines on to a filter paper (7.5 × 2.5 cm) placed on a glass slide. The filter paper in wet condition was observed under the microscope (Motic BA 310) with N-WF 10×/20 eyepiece and 10× objective, and soil samples were assessed for spore density (Smith and Skipper, 1979). Spore density was represented as number of spores per g soil. The spore count was carried out by the plate method (Smith and Skipper, 1979).

Soil pH was measured from a mixture of air dried soil mixed with distilled water in the ratio of 1:2.5 using a pH meter (PCSTestr 35 Multi-parameter; Eutech, Thermo Fisher Scientific Inc, New Delhi, India). Microdiffusion method (Sparks, 1996) was employed for quantifying soil available nitrogen. Spectrophotometry (Hitachi U5100, Hitachi-Hightechnologies Corporation, Tokyo, Japan) was used to estimate soil available phosphorus and flame photometry (Systronics Flame photometer 128, Systronics Ltd Co, Ahmedabad, India) to estimate available potassium in acid-digested soil samples (Jackson, 1973). Total organic carbon was estimated as per wet digestion method (Walkley and Black, 1934).

Data analysis

Data analysis was performed with IBM SPSS software version 19 (IBM India, Bangalore, India). Shoot height, number of functional leaves, chlorophyll content in leaves, wet weight of shoot, leaf N, P and K concentrations, AMF colonisation in roots, AMF spore density in soils, and comparison of soil indices before and after experiment were analysed by one-way ANOVA after ensuring data normality (Shapiro–Wilks test). To identify significant differences ($P < 0.05$) between the treatments, Tukey's Honestly Significant Difference (HSD) was employed.

Results

Plant development

Shoot length, number of functional leaves (30, 60 and 90 DAI) and wet weight of pseudostem (90 DAI) were measured. Shoot length in Fm5000 and Fm+Gm5000 was significantly higher than in control treatment at 30 DAI, as also found for Gm5000 at 60 DAI. At 90 DAI, shoot length was significantly higher in both Fm5000 and Gm5000. The maximum shoot length at 90 DAI was 61.2 cm and was noticed in plants under Fm5000 (Figure 1). Average number of functional leaves in Gm5000 and Fm5000 treatments at 30 DAI was higher than in control, and no significant differences among treatments for this trait were found at 60 and 90 DAI. At 90 DAI, wet weight of pseudostem (weight of pseudostem and leaves) in control plants (469.2 ± 26.7 g) was lower ($P < 0.05$) than that of plants under Gm5000 (626.0 ± 18.6 g), Fm5000 (731.6 ± 27.9 g), Gm+Fm5000 (637.2 ± 15.0 g) and Gm+Fm2500 (666.6 ± 16.3 g). Fm5000 caused the highest shoot weight among the treatments.

Leaf chlorophyll and N, P and K concentrations

All the treatments caused significantly higher chlorophyll content as compared to control, with the exception of Fm+Gm5000 at 30 DAI. The highest leaf content of chlorophyll was measured in

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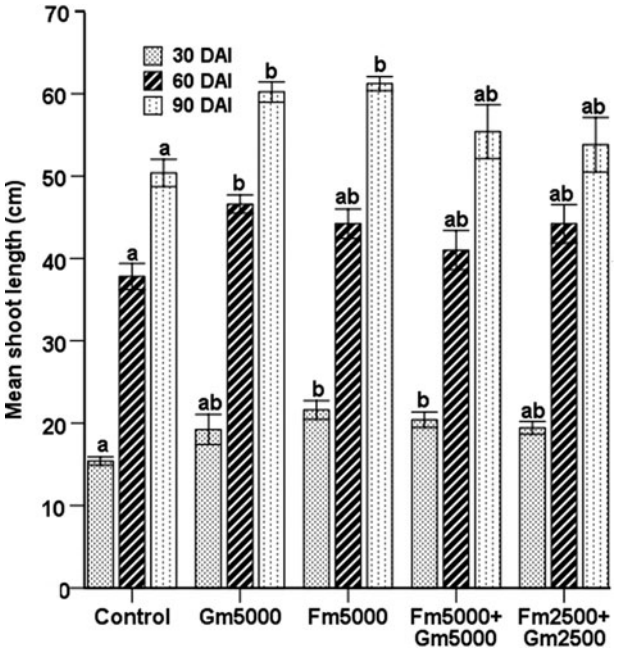

Figure 1. Shoot length of banana plants (cv. Nendran) as affected by *Funneliformis mosseae and Glomus microcarpum*. Measurements were taken at 30, 60 and 90 DAI. Bars represent mean ± se. In each evaluation time, different letters represent significant differences among treatments (Tukey's HSD at $P < 0.05$).

banana plants under Fm+Gm2500 at 30 DAI. In general, there was a decrease in the total chlorophyll content in the leaves after 30 DAI (Figure 3A). As compared to control, all treatments increased leaf N concentration, with the exception of Fm+Gm2500 at 90 DAI (Figure 3B). The highest N concentration was observed under Gm5000 at 30 DAI and there was a decrease after 30 DAI. Leaf P concentration was higher in all the AMF-inoculated banana plants at 30, 60 and 90 DAI (Figure 3C). The highest leaf P concentration was found in Fm+Gm2500 at 90 DAI and there was an increasing trend from 30 to 90 DAI (Figure 3C). Leaf K concentration in the AMF-inoculated plants was also higher than in control treatment, regardless of evaluation time (Figure 3D). At 30 DAI, Gm5000 caused the highest leaf K concentration among treatments. At 60 DAI, both Gm5000 and Fm5000 treatments caused higher leaf K concentration as compared to the other treatments. However, no significant difference ($P > 0.05$) in leaf K concentration among the AMF-inoculated banana plantlets was observed at 90 DAI.

AMF root colonisation and spore density

All the AMF-inoculated plants showed root colonisation and the highest RLTC was observed in Fm+Gm2500 (Figure 4). More than 80% root colonisation was observed in all the inoculated plants. The highest RLH, RLA and RLV were found in Fm+Gm5000-, Gm5000- and Fm5000-inoculated plants, respectively (Figure 4). Soils inoculated with *G. microcarpum* and *F. mosseae* showed higher spore density than soils that received combined application of both the species (Figure 5). The highest spore density was observed in soils inoculated with only *G. microcarpum* (34 spores g^{-1} soil).

Soil fertility before and after inoculation

Significant reduction of soil available N and pH was observed at the end of the experiment, regardless the treatments (Figure 6). An increase in the available P was found in all the

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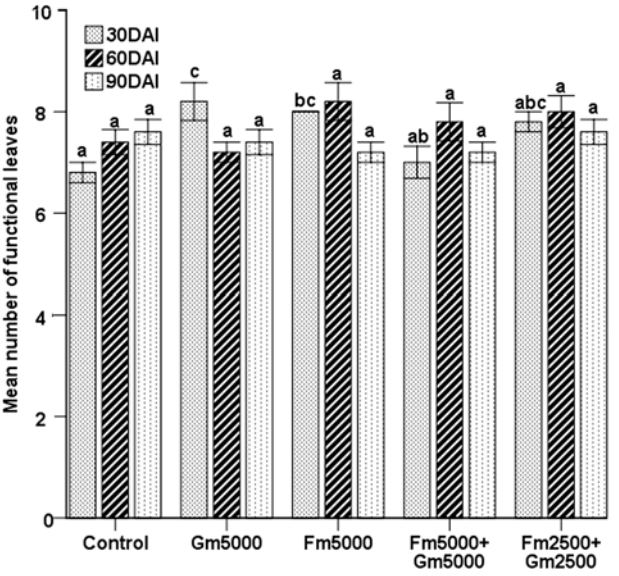

Figure 2. Number of functional leaves of banana plants (cv. Nendran) as affected by *Funneliformis mosseae and Glomus microcarpum*. Measurements were taken at 30, 60 and 90 DAI. Bars represent mean ± se. In each evaluation time, different letters represent significant differences among treatments (Tukey's HSD at $P < 0.05$).

AMF-inoculated soils, as compared to control soil. No significant difference ($P > 0.05$) in soil available K content was noticed before and after the experiment. However, soil total organic carbon in all the treatments was increased at the end of experimental period.

Discussion

This is the first report showing the effects of *F. mosseae* and *G. microcarpum* in banana *M. paradisiaca* cv. Nendran, the most widely cultivated banana variety in South India. Higher shoot height in the AMF-inoculated plants implied in a growth promotion effect (Figure 1), highlighting the beneficial effects of AMF on plant. In fact, higher shoot height in *M. accuminata* inoculated with *G. mosseae* (Shashikala *et al.*, 1999) and in *M. paradisiaca* cv. Robusta inoculated with an AM fungal consortium (Thaker and Jasrai, 2002) was already known. Significant increase in the number of functional leaves was observed in plants growing in the *F. mosseae* and *G. microcarpum* inoculated soils, but not in the soils with combined inoculation of both the species (Figure 2). In a similar mycorrhizal treatment with *M. acuminata*, Shashikala *et al.* (1999) observed higher number of functional leaves at 90 DAI, which was not found herein. As Nendran is well known for its high nutrition demand during its early stage of growth, a possible explanation for the similar number of leaves among treatments at the end of experimental period may be a limitation in available N in pots with limited amount (5 kg) of soil.

Leaf chlorophyll content is often related to leaf nitrogen content (Arantes *et al.*, 2016) and higher chlorophyll content was found in the leaf tissues of AMF-inoculated banana regardless the combination of AMF in the inoculum (Figure 3A). At 90 DAI, the leaf chlorophyll content in all the AMF-inoculated banana plants became similar, probably because of the limitation in N availability for potted-grown banana plants. However, a steady increase in the leaf chlorophyll content of *M. paradisiaca* cv. Robusta from 20 to 100 days after AMF inoculation was reported (Thaker and Jasrai, 2002). As compared to the control treatment, higher leaf N, P and K concentrations were very evident in all the AMF-inoculated plants. But, at 90 DAI, leaf N, P and K

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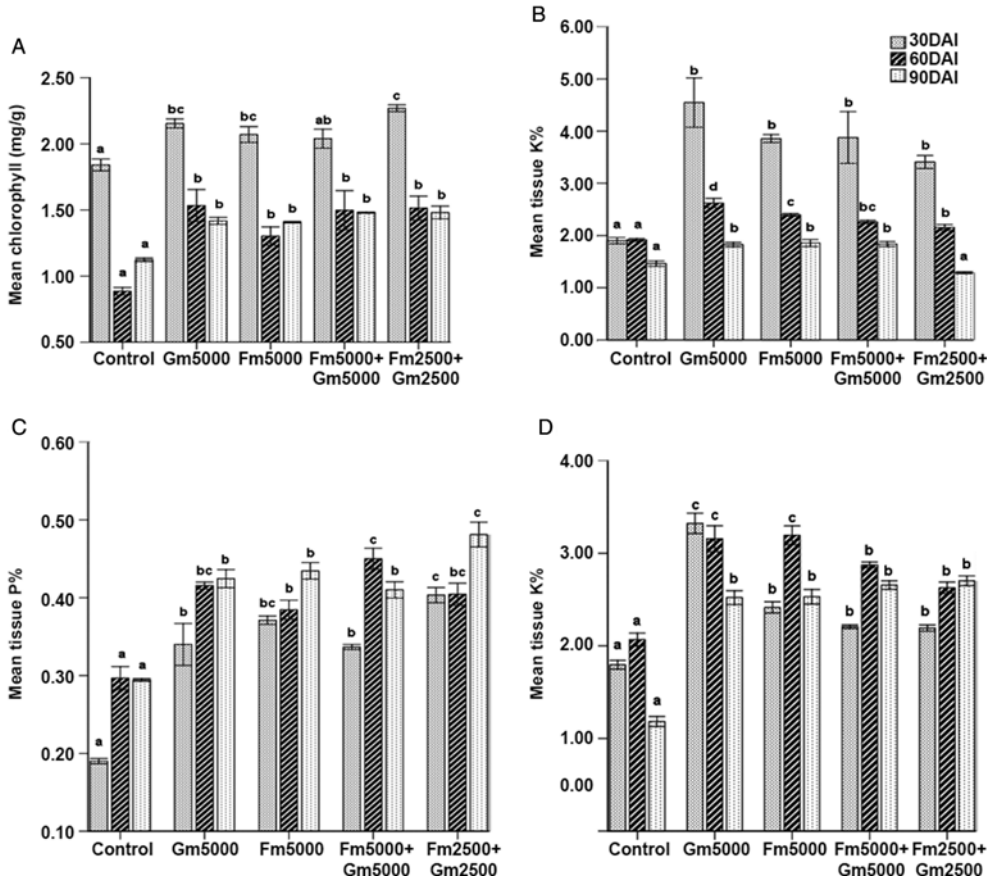

Figure 3. Total chlorophyll content (A), and Leaf nitrogen (B), Leaf phosphorus (C) and Leaf potassium (D) content of banana plants (cv. Nendran) as affected by *Funneliformis mosseae* and *Glomus microcarpum*. Measurements were taken at 30, 60 and 90 DAI. Bars represent mean ± se. In each evaluation time, different letters represent significant differences among treatments (Tukey's HSD at $P < 0.05$).

concentrations were reduced as compared to previous evaluation dates (Figure 3B–D). This may be because of the dilution effect (Pinochet *et al.*, 1997) or the excessive AMF-assisted uptake of nutrients by the plant in the early DAI. Afterwards, soil nutrient content may become insufficient due to the limited soil volume for each plant. According to Pinochet *et al.* (1997), the fast growth rate is the reason for lower leaf P concentration in 'Grand Naine' variety of banana inoculated with *Glomus intraradices*. A survey of high and low yielding Nendran cultivated areas in South India (Jeyabaskaran *et al.*, 2005) compared with leaf N, P and K concentration observed herein, the average leaf N concentration at 90 DAI (1.29% to 1.86%) was quite lower than the value (2.24%) observed in Nendran variety at low yielding areas.

More than 80% AMF root colonisation was observed in all the inoculated plants, but there was no significant variation in the total root length colonisation among the treatments (Figure 4). The high root colonisation of *F. mosseae* and *G. microcarpum*, their sporulation and increased plant height (Figures 1, 4 and 5) confirmed the receptiveness of *F. mosseae* and *G. microcarpum* by Nendran variety of banana in experimental conditions.

Non-significant changes in soil available K content before and after experimentation were found in all treatments (Figure 6). However, the soil total organic carbon was significantly increased in all experimental and control treatments at the end of experimental period (Figure 6), a likely consequence of the positive influence of root exudates on general microbial activity in soil.

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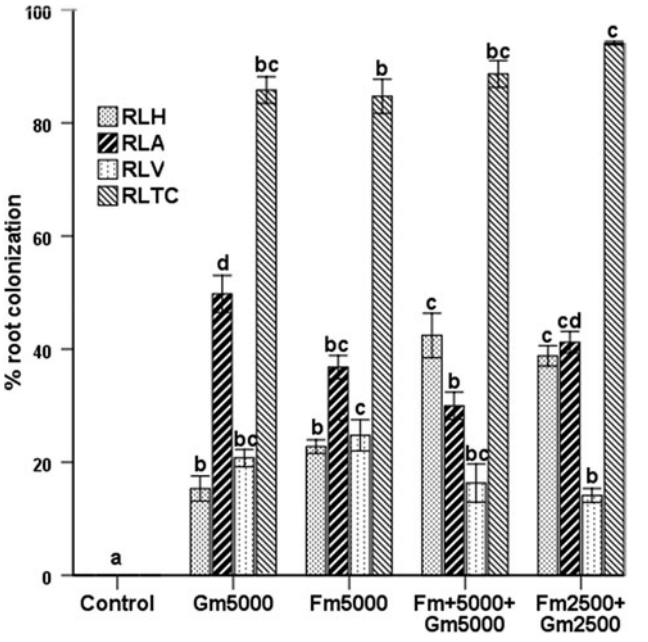

Figure 4. Root length containing linear hyphae (RLH), root length containing arbuscule (RLA), root length containing vesicles (RLV) and total root length colonised by AMF (RLTC) in banana plants (cv. Nendran) as affected by *Funneliformis mosseae and Glomus microcarpum*. For a given trait, different letters represent significant differences among treatments (Tukey's HSD, $P < 0.05$).

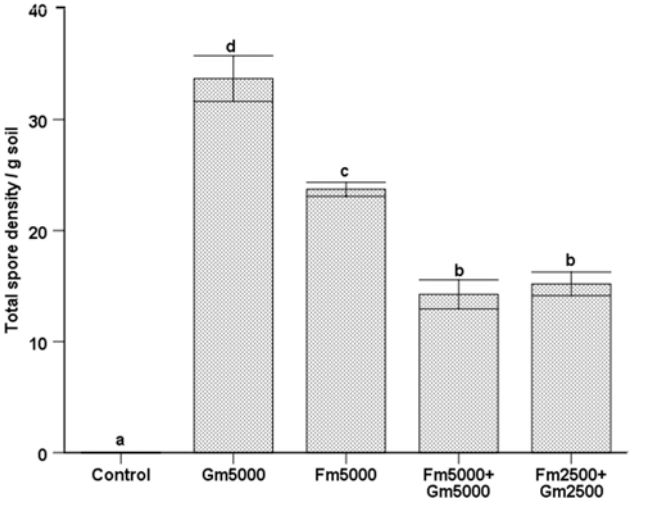

Figure 5. Total spore density of AMF in soils (number of spores per gram soil) after 90 days of inoculation. Soil received *Funneliformis mosseae* and *Glomus microcarpum*. Bars represent mean ± se. Different letters represent significant differences among treatments (Tukey's HSD, $P < 0.05$).

Irrespective of the AMF combination in the inoculum, all the inoculated banana plants showed significantly higher plant height than the control. Overall, this study strengthens the earlier report of mycorrhizal dependency of banana plants for an optimum growth (Usuga *et al.*, 2008) and proved that the two native strains of *F. mosseae* and *G. microcarpum* are competent in

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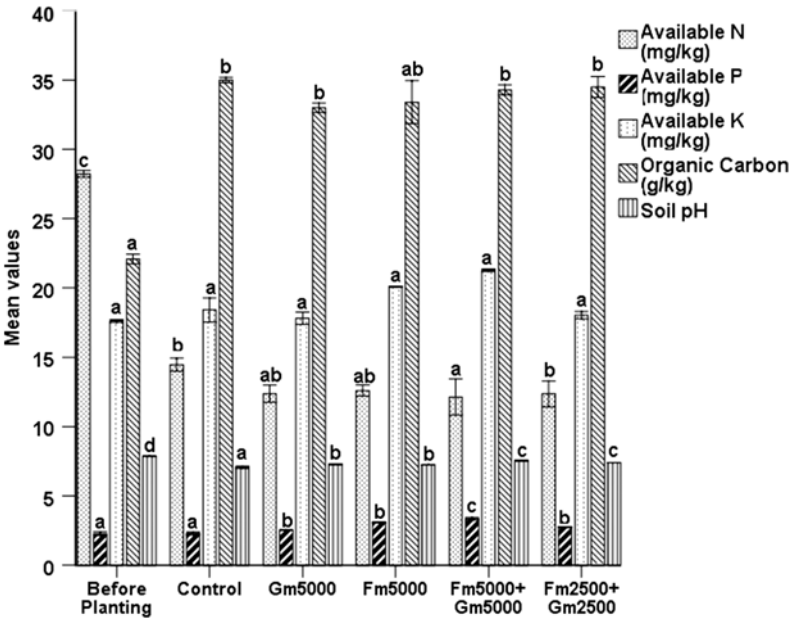

Figure 6. Soil nitrogen, phosphorus, potassium, organic carbon and pH as affected by banana plants and *Funneliformis mosseae* and *Glomus microcarpum*. Measurements were taken before planting and 90 days after treatments. Bars represent mean ± se. Different letters represent significant differences among treatments, including the initial condition (Tukey's HSD, $P < 0.05$).

accomplishing successful root association and are beneficial to the early growth of the Nendran variety. These findings enable further field trial of both the species as promising biofertiliser candidates common to banana crops, especially in integrated fertility management in sustainable agricultural systems as both strains are isolated from commercially cultivated fields.

Conclusions

Identification and proper use of native strains of AMF from crop fields and the development of biofertilisers may represent a sustainable alternative for managing banana fields. Herein, we found that AMF inoculum containing *F. mosseae* and *G. microcarpum* alone or in combination enhanced nutritional status of banana plants and could be further explored as biofertilisers. Moreover, the positive impact of AMF on soil phosphorus availability using spores isolated from commercial fields suggests that these species are useful in integrated soil fertility management systems as well. However, further field studies are essential to find the optimum amount of AMF inoculum required and timing of application and also to evaluate the exact beneficial effect of the AMF on banana yield.

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