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Full Length Research Paper

Contribution of arbuscular mycorrhizal fungi to growth and nutrient uptake by jujube and tamarind seedlings in a phosphate (P)-deficient soil

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A pot experiment was carried out in a nursery to determine the influence of an arbuscular mycorrhizal (AM) fungus on plant growth and nutrient uptake in tamarind (*Tamarindus indica*) and jujube (*Ziziphus mauritiana*), both of which are multipurpose fruit trees in Sahelian agroforestry systems. Plants were inoculated or not with *Glomus aggregatum* and grown for four months in a sterilized phosphorus (P)-deficient sandy soil (2.18 ppm P). Tamarind and jujube seedlings grew very poorly in the absence of *G. aggregatum,* whereas inoculated ones had greater shoot height and total biomass production. The total dry weight of the fruit tree species was increased 3 and 4 fold by *G. aggregatum*, respectively, in tamarind and jujube compared to the controls. Jujube with the highest AM root colonization had a higher P concentration (8.33 fold) in shoots than in tamarind (1.62 fold), indicating greater mycorrhizal dependency in jujube seedlings. The P concentration in shoots of AM plants contributed probably to enhance significantly plant growth and development more than N, K and Mg, particularly in jujube seedlings. Results suggested that fruit trees inoculated with AM fungi absorbed nutrients more efficiently from the soil, which resulted in improved plant growth and biomass production. These beneficial effects of AM inoculation may be useful for the production of these fruit trees in fields. Further investigations are therefore necessary to elucidate the effectiveness and competitiveness of *G. aggregatum* in real nursery conditions.

Key words: Arbuscular mycorrhizal fungi, fruit tree, mycorrhizal root colonization, mycorrhizal dependency, nutrient uptake.

INTRODUCTION

In the rhizosphere, some fungi colonize by infecting plant roots to form symbiotic relationships, called mycorrhizas. Arbuscular mycorrhizal (AM) fungi are the most wide-spread type and ecologically important root fungal sym-bionts with more than 90% of higher plant species, including crop and fruit tree species, and are essential to the survival of many tropical plants (Plenchette, 1991; Chang, 1994; Schreiner et al., 2003; Strack et al., 2003). These microsymbionts occur widely under various envi-ronmental conditions with beneficial effects on soil struc-ture improvement (Miller and Jastrow, 2000; Stutz et al., 2000) and have great importance due to their higher capacity to increase growth and yield through efficient nutrient uptake in infertile soils, water uptake and drought resistance in plants (Marulanda et al., 2003; Nowak, 2004; Chen et al*.,* 2005; Khalafallah and Abo-Ghalia*,* 2008). Furthermore, prophylactic effects have been often reported, proving in many situations that AM fungi can act as biological control agents by lessening proliferation and damage caused by pests and soil-borne diseases (Habte et al., 1999; St-Arnaud and Elsen, 2005.

Among many woody species well adapted and com-monly used in Sahelian and Sudanian areas in West Africa, jujube (*Ziziphus mauritiana* Lam.) and tamarind (*Tamarindus indica* L.) are multipurpose fruit trees inten-sively exploited for fruits, seeds, fodder, wood and tradi-tional medicine (Okafor, 1991). Moreover, fruit tree spe-cies contribute to overcome nutritional problems and are important sources of income for rural communities (Ambé, 2001; Akinnifesi et al., 2004) during the critical food insecure season. Consequently, programmes of improvement and domestication of these fruit trees were initiated in West Africa, particularly in Burkina Faso, to optimise their use in agroforestry systems and to safely meet the needs of a growing human population and agro-food industries in spite of severe edapho-climatic con-straints. However, low available nutrients, particularly P-deficiency, as well as drought and water stress in most tropical soils is one of the important environmental limit-ing factors for plant growth (Atayese et al., 1993; Querejeta et al., 2003). In these conditions, plant growth is largely reliant upon AM symbiosis for nutrients and water uptake (Rajan et al., 2000; Querejeta et al., 2003), which could significantly reduce the use of conventional fertilizers in soils (Raja, 2006).

Since the majority of vegetable crops and fruit trees that are important in human and animal nutrition (Lamien et al., 1996; Bonkoungou et al., 1998; Nair, 1998; Ambé, 2001) form symbiotic associations with AM fungi, it is important to use these microsymbionts in agriculture and forestry by selecting best plant-AM fungi combinations to improve plant growth and soil fertility. It becomes thus possible to encourage healthy cultural systems by re-ducing the use of chemical inputs (pesticides and other fertilizers), and to sustain a better productivity and eco-system preservation.

Utilization of AM fungi to stimulate and improve juvenile plant growth in nurseries prior transplanting is not yet well developed in West Africa, particularly in Burkina Faso. Moreover, the fundamental importance of the mycorrhizal associations in restoration and to improve revegetation is well recognized (Reynolds et al., 2006), but the use of inoculation techniques in plantations in agroforestry systems is not extensive in Burkina Faso. The aim of this study was to investigate the effects of an AM fungus on the growth of tamarind and jujube seedlings in a P-defi-cient soil in nursery conditions. We evaluated, in semi-controlled conditions, the influence of an AM fungus on juvenile growth and nutrient uptake in these two fruit trees with the hope to reduce transplant shock and improve fruit production in field conditions.

MATERIALS AND METHODS

The soil used in this experiment was a sandy top soil (0-20 cm), collec-ted in a stand of *Afzelia africana* Sm. at Dinderesso (South Sudanean zone of Burkina Faso). The soil was sieved (2 mm) and sterilized by autoclaving (120°C for 1 h) to eliminate native microflora. After sterili-zation, the soil contained 6.7% clay, 6.5% silt, 86.6% sand, 0.6% organic matter, 0.3% total carbon (C), 0.05%, nitrogen (N), C/N ratio of 7, 98 ppm total P, 2.18 ppm P-Bray 1, pH (1:2, soil-to-water ratio) 7.4, and pH (1:2, soil-to-KCl ratio) 6.8, as described by Guissou et al. (2001). A 10-kg portion of air-dry soil was then transferred into alumi-nium pots (51 cm height, 17 cm diameter) for plant growth.

Spores of an AM fungus, *Glomus aggregatum* Schenck and Smith emend. Koske, isolated from a rhizosphere of *Acacia mangium* at Dinderesso (Bâ et al., 1996) was used in this experiment. This AM fungus was efficient on plant growth and nutrient uptake in wild fruit trees and crops (Dianou and Bâ, 1999; Bâ et al., 2000). To obtain the AM inoculum, spores of *G. aggregatum* were multiplied in pot culture on maize for four months (Bâ et al., 1996). The AM inoculum consisted of sand, spores, pieces of hyphae and infected maize root fragments. For inoculated plants, a 20 g portion of the inoculum was distributed into a planting hole in each aluminium pot at 5-10 cm just below the soil surface before inserting seedlings during the transplantation. Non-inoculated plants received the same amount of autoclaved inoculum (120°C for 20 min) with a 10 ml water extract of the inoculum by filtra-tion (Whatman No. 1 filter paper) as controls to balance composition of the microbial community between inoculated and non-inoculated plants (Jansa et al., 2005). The inoculum density was calibrated by the Most Probable Number method as approximately 10^3 infective propagules per 20 g of inoculum (Guissou et al., 1998).

Seeds of jujube (provenance Lery, sample No. 1774) and tamarind (provenance Kongoussi, sample No. 1389) were purchased from the National Tree Seed Centre (Ouagadougou, Burkina Faso). Seeds of jujube and tamarind were surface scarified and sterilized in 95% sul-phuric acid immersion for 10 min and 45 min respectively, and abun-dantly washed in sterile water. Seeds were maintained for 24 h in sterile distilled water and were then aseptically germinated on moist sterilized cotton in Petri dishes at 30°C. Two germinated seedlings were planted into each aluminium pot, and were thinned after emergence to one seedling per pot. The experiment was conducted from March 15, 2007 to July 15, 2007 in nursery conditions and plants were grown under natural light (day length approximately 12 h, mean temperature appro-ximately 35°C day). Plants were watered to a level near field capacity twice a day with tap water. The experiment was arranged in a factorial design with 2 AM treatments (AM plants, non-AM plants as controls) for each fruit tree species. Treatments were set-up in a com-pletely randomized design with 10 replicates for each treatment combi-nation in nursery.

Plants were harvested four months after inoculation in July 15, 2007. Shoot height, and total dry weight (TDW) of plants by treatment were measured. Plant material was dried in an oven at 70°C for 7 days and weighed. Plant growth was recorded as plant shoot height and dry matter production. Mycorrhizal dependency (MD) of plants was deter-mined by expressing the difference between the TDW of AM plants and the TDW of non-AM plants as a percentage of the TDW of AM plants according to Plenchette et al. (1983).

To identify the AM fungal colonization rate, randomly sampled roots were collected from each plant and cut up into at least 1-cm-long fragments, cleared 1 h in 10% KOH at 80°C. Roots were then washed with tap water, and stained with 10% Trypan blue according to the method of Phillips and Hayman (1970). After washing roots with tap water, a total of 100 x 1-cm root pieces per plant were randomly selec-ted, mounted on microscopic slides and examined for colonization pat-tern (X40 magnification) using a compound microscope fitted with an eyepiece scale. The parameter retained for AM colonization rate was the colonization intensity calculated as length of cortical cells colonized (in mm) by the AM fungal for each root fragment, and expressed as a percentage of total root length colonized (Mc Gonigle et al., 1990; Declerck et al., 1996). Total P and N contents of leafed stems were determined by the molybdate blue method (Murphy and Riley, 1962) and Kjeldahl method, respectively. Total K and Mg contents were determined by means of an atomic absorption spectrophotometer (John, 1970).

For each fruit tree species, all data were statistically analysed by the procedure of analysis of variance using SPSS 11.0 for Windows Soft-ware (SPSS Inc., USA). A one-way analysis was performed and differ-rence between treatment means was assessed using Student-Newman-Keuls multiple comparisons test ($P<0.05$).

RESULTS

The importance of shoot growth and aerial biomass pro-duction on tamarind and jujube seedlings inoculated with *G. aggregatum* compared to the non-inoculated control plants are displayed in Figures 1 and 2, respectively. Control plants displayed the lowest height growth, and total biomass production decreased significantly as con-sequence (Figures 1 and 2), in particular with jujube seedlings (Figure 2). Inoculation with *G. aggregatum* sig-nificantly enhanced shoot height by 1.69 and 4.0 times in

Figure 1. Effect of inoculation with *Glomus aggregatum* or not on shoot height and aerial biomass production in nursery-grown tamarind (*T. indica*) seedlings grown for four months after inoculation.

tamarind (Figure 3a) and jujube (Figure 4a), respecttively, than in controls. In these conditions, the total dry weight produced in inoculated plants was 3 and 4 times higher, respectively, in tamarind and jujube compared to the controls (Figures 3b and 4 b).AM root colonization was significantly higher in jujube (91%) than in tamarind (78%), and no mycorrhizal structures were observed in the roots of non-inoculated control plants in both fruit trees (Figures 3c and 4c). Jujube was the most mycorrhizal dependent fruit tree for juvenile growth compared to tamarind (Figure 3d and 4d). Mycorrhizal dependency (MD) values were at about 64 and 77%, respectively, in tamarind (Figure 3d) and in jujube seedlings (Figure 4 d).

The nutrients concentrations (N, P, K, Mg) in leafed stems of both fruit seedlings were significantly increased by *G. aggregatum* compared to the non-inoculated controls (Figures 3 and 4). In AM plants, N concentrations in shoots were enhanced by 59.50 and 78.08%, respecttively, in tamarind and jujube compared to the controls (Figures 3e and 4e). Compared to the controls, *G. aggregatum* significantly increased P concentrations by approximately 1.62- and 8.33-fold in tamarind and jujube seedlings, respectively (Figures 3f and 4f). The K concentrations were significantly increased by 37.89% and 62.99%, respectively, in tamarind and jujube over the controls (Figures 3g and 4g). In comparison to control plants, *G. aggregatum* increased the Mg concentration by 72.73% in tamarind seedlings (Figure 3 h), whereas the concentration of this element was 2.67 fold higher in jujube seedlings (Figure 4h).

DISCUSSION

It was clearly seen from the parameters measured in the present study that *Glomus aggregatum* had positive effects on tamarind and jujube growth in P-deficient soil. Non-inoculated fruit trees showed very weak shoot growth and produced little total biomass compared to the inoculated plants. With jujube in particularly, the absence of the AM inoculation is a factor that could limit seriously the growth and the development of plants in nursery. Practically in these conditions, the lack of height growth with little aerial biomass production in control plants could lead to a higher rate of mortality of plants (Chen et al., 2008), particularly with jujube seedlings in nursery.

This study shows the beneficial effects of AM fungi for growth and nutrient absorption in both fruit trees, and confirms previously identified positive responses on tamarind (Reena and Bagyaraj, 1990) and jujube (Mathur and Vyas, 2000). Jujube is the fruit tree that benefits more from mycorrhization, probably because of its high P uptake in the soil. Plants inoculated with *G. aggregatum* resulted in a significant stimulation of P concentration in shoots of both fruit trees over the controls. In AM plants, the P concentration was greatly increased in jujube (8.33-fold) than in tamarind (1.62-fold). Concentrations of N, K, and Mg were significantly increased when seedlings of both fruit trees were inoculated with *G. aggregatum*; this led to increased shoot height and biomass and corroborates previous research (Clark and Zeto, 2000; Smith et al., 2000; Sailo and Bagyaraj, 2005; Copetta et al.,

Figure 2. Effect of inoculation with *Glomus aggregatum* or not on shoot height and aerial biomass production in nursery-grown jujube (*Z. mauritiana*) seedlings grown for four months after inoculation.

2006; Kumar et al., 2007; Fan et al., 2008; Manoharan et al., 2008). However, results obtained under our experimental conditions are in contrast with other studies showing a stimulation of nutrients uptake, particularly the P by AM fungi with negative or no significant effect on growth response (Klironomos, 2003; Smith et al., 2004; Reynolds et al., 2006; Sudova and Vosatka, 2008).

Phosphorus is essential for plant nutrition and beneficial effects of AM infection on plant growth were often assigned in particular to a better uptake of this element (Chen et al., 2005; Sailo and Bagyaraj, 2005; Fan et al., 2008). The capacity of a plant to absorb P in P-deficient soils is one of factors determining the variability observed in mycorrhizal dependency (MD) of plants (Singh, 2001; Zhu et al., 2001; Janos, 2007). In comparing the two fruit trees, it clearly appears that the jujube showed the highest value of MD, confirming the greater mycorrhizal dependency in this fruit tree for juvenile growth (Mathur and Vyas, 2000; Guissou et al., 2001). Compared to the controls, the higher P uptake in both fruit trees inoculated with *G. aggregatum* showed in fact that the P deficiency in the soil used (2.18 ppm P) is a limiting factor for plant growth. These results corroborate previous reports, which showed that in nutrient deficient soils, plant growth strongly rely upon on AM symbiosis (Zhu et al., 2001; Sailo and Bagyaraj, 2005; Janos, 2007; Reynolds et al., 2006).

The intensity of root mycorrhizal colonization, which quantify the biomass of AM fungi in plant roots (Declerck et al., 1996), significantly differ between jujube and tamarind seedlings, confirming previous work on other plant species (Ingleby et al., 1997; Lesueur and Duponnois, 2005). The high rate of root colonization observed in fruit seedlings, particularly with jujube plants, foster a signifycant increase in shoot growth, total dry weight and nutrient uptake. These results indicate that there is an evident relationship between the degree to which a root system of a plant is colonized by AM fungi and the potential for the plant to benefit significantly from the symbiosis (Koide and Mosse, 2004; Fan et al., 2008).

The morphological as well as physiological properties of root system can influence the P absorption of plants in soil, and therefore the beneficial effects observed on mycorrhizal plants. Research has shown that the length and the density of root hairs are indicators of the mycorrhizal dependency (MD) of plant species or cultivars (Schweiger et al., 1995; Tawaraya et al., 1999; Collier et al., 2003; Sorensen et al., 2005; Janos, 2007). This hypo-

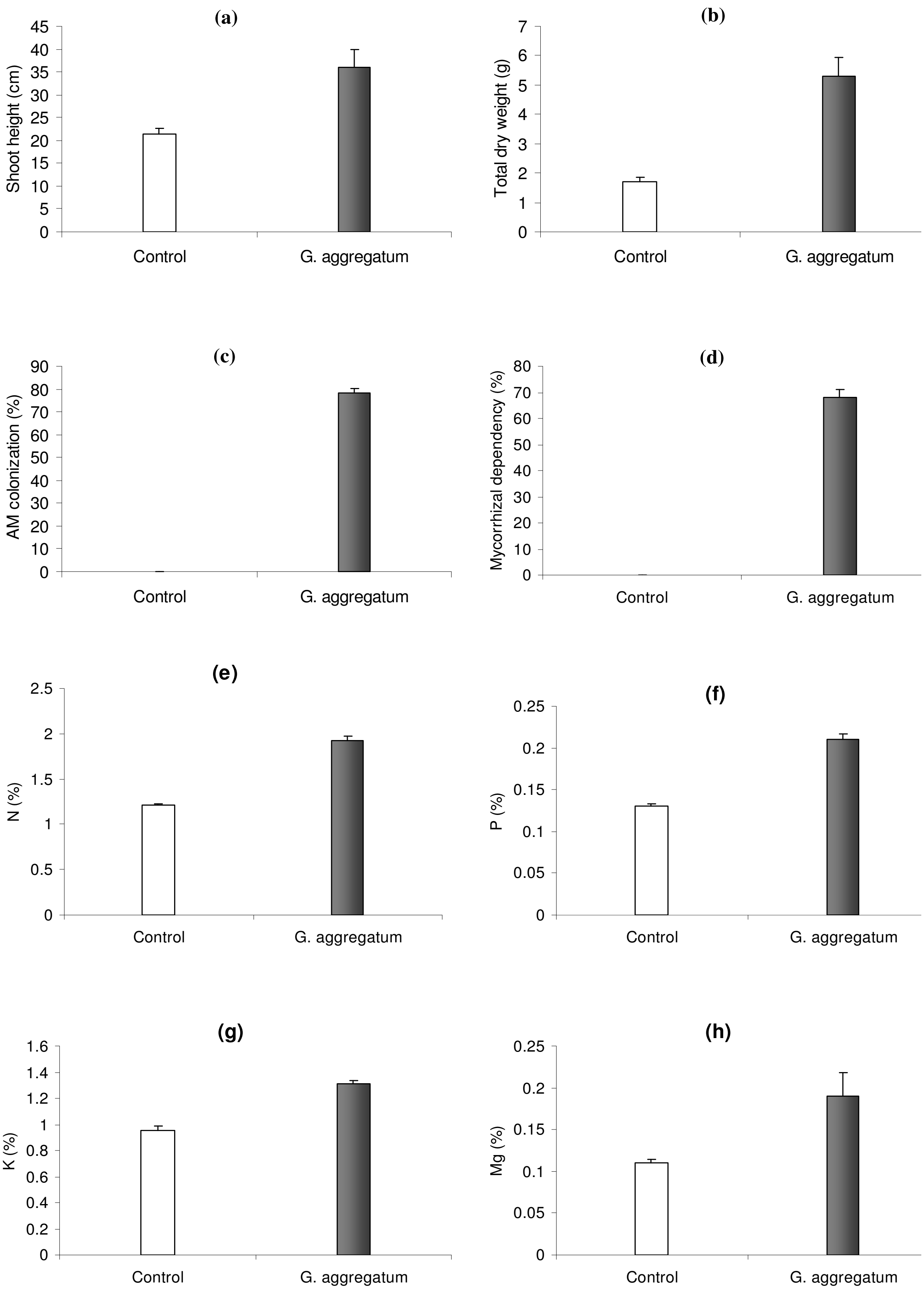

Figure 3. Shoot height, total dry weight, AM root colonization, mycorrhizal dependency, and mineral concentrations (N, P, K, Mg) on four month-old tamarind (*T. indica*) seedlings inoculated or not (control) with *G. aggregatum* in nursery. Bars represent the standard errors of means.

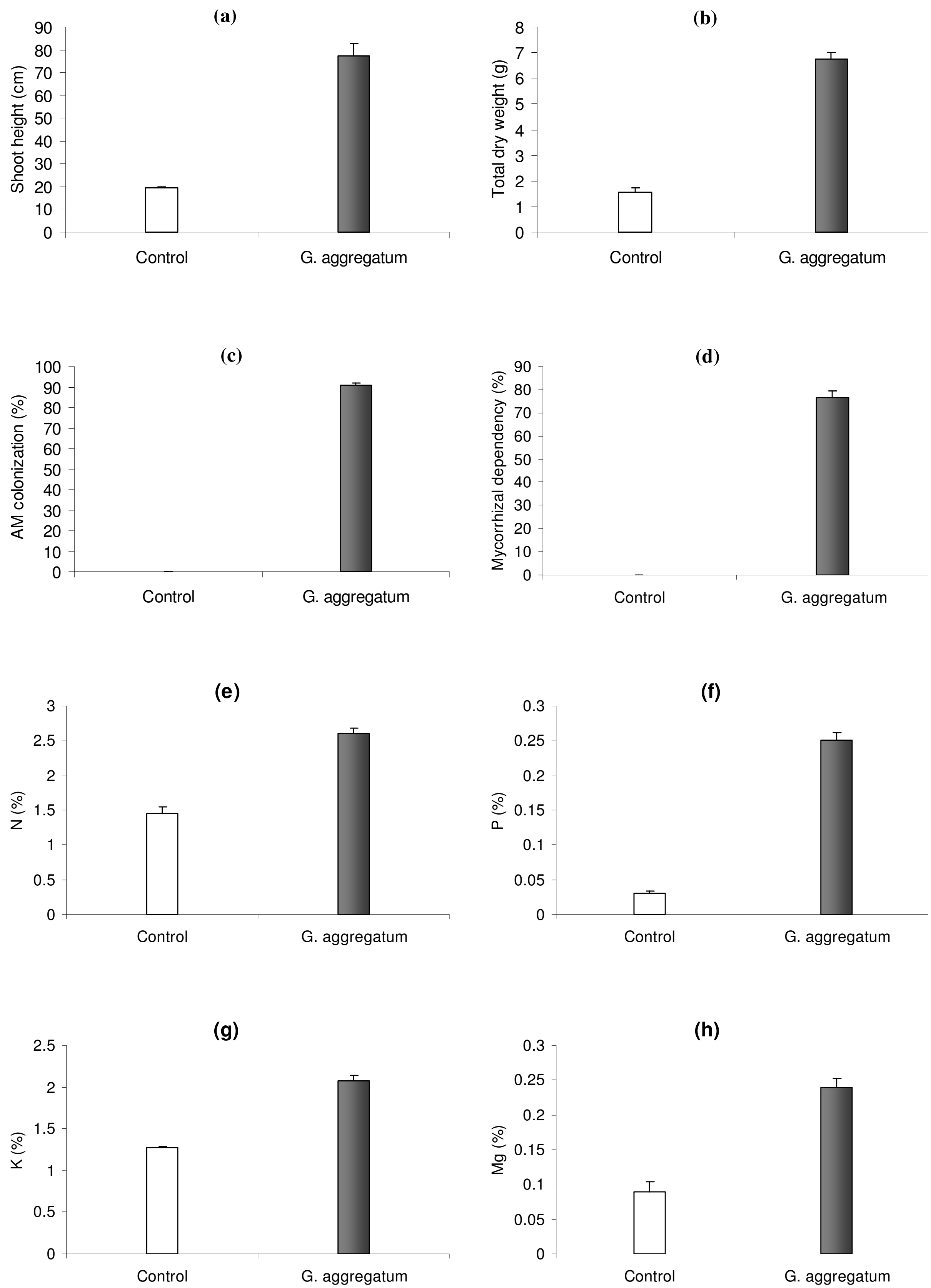

Figure 4. Shoot height, total dry weight, AM root colonization, mycorrhizal dependency, and mineral concentrations (N, P, K, Mg) on four month-old jujube (*Z. mauritiana*) seedlings inoculated or not (control) with *G. aggregatum* in nursery. Bars represent the standard errors of means.

thesis has not been examined in this study because our previous results indicated that the morphology of the root system was not an indicator of the MD of these fruit trees studied (Guissou et al., 1998). The length and root hair density were more important in jujube seedlings than in tamarind. However, tamarind was less mycorrhizal dependent than jujube (Guissou et al., 1998). Such positive correlations between the MD and the length and root hair density in plants were also observed by Declerck et al. (1995). It is well established that AM stimulates the biomass of the root system, which leads to higher nutrient uptake in soils for plants (Copetta et al., 2006; Raja, 2006; Khan et al., 2007; Fan et al., 2008). The rate of P absorption by growing roots is much higher than the rate of soil P diffusion, which results in the formation of a P depletion zone at the root level and consequently, limits the supply of P to the plant (Smith et al., 2004). In this situation the extra-radical mycelium network formed by AM fungi grows and explores far beyond the depletion zone around actively-absorbing roots and microporosities of soil, reaching a new pool of soluble P comparatively to non-AM plant roots (Zhu et al., 2001; Smith et al., 2004; Boureima et al., 2007; Schnepf et al., 2008). The development of an external hyphal network in soil in terms of density and surface explored confers to AM fungi the ability to take up P and other nutrients such as N, K, and Mg out of the depletion root zone of plants. This system allows the plant to reach a great quantity of water and nutrients from the soil necessary for plant growth and development (Rajan et al., 2000; Marulanda et al., 2003; Smith et al., 2004; Schnepf et al., 2008). Such high accumulation of nutrients, particularly P in AM plants, could be a strategy for species growing in degraded and marginal fertility soils. It is well known that AM fungi efficiency depends on several physiological and ecological factors which deserve further studies.

In conclusion, our results clearly showed that AM inoculation with *Glomus aggregatum* is highly beneficial for these two fruit trees, and indicated that the AM colonization promote plant growth by enhancing significantly nutrient uptake, particularly the P. The absence of AM inoculation in these two fruit trees could lead to a higher mortality of plants, particularly with jujube which is highly dependent of AM fungi for its juvenile growth and development in P-deficient soils. Additional research is needed under field conditions to elucidate the effectiveness and competitiveness of the AM fungus, *Glomus aggregatum*, on other soil microorganisms to improve plant development and fruit production of jujube and tamarind seedlings in fields.

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