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Growth response of *Afzelia africana* Sm. seedlings to ectomycorrhizal inoculation in a nutrient-deficient soil

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Abstract The growth and mineral nutrition responses of seedlings of two provenances of *Afzelia africana* Sm. from Senegal and Burkina Faso, inoculated with four ectomycorrhizal (ECM) fungi (*Scleroderma* spp. and an unidentified isolate) from the same regions were assessed in a pot experiment in a savanna ECM-propagule-free soil deficient in NPK. There was little variation in the ability of the different fungal species to colonize roots of either provenance of *A. africana* or to produce external hyphal in soil. Root colonization by ECM fungi and their hyphal development were not related to mineral nutrition or ECM dependency. Differences in P, N, Mg and Ca concentrations in the leaves of inoculated and non-inoculated *Afzelia* seedlings were not always associated with production of biomass. Only leaf K concentration increased in both provenances after ECM inoculation. However, the Burkina Faso provenance responded better to inoculation with the two fungal isolates than the Senegal provenance in terms of biomass production. This was due to stimulation of root dry weight of the Burkina Faso provenance. Therefore, the hypothesis arises that non-nutritional rather than nutritional effects explain the contribution of ECM inoculation to the growth of *A. africana* seedlings.

Key words *Afzelia africana* · Provenances · *Scleroderma* spp. · Plant growth · Mineral nutrition

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Introduction

In tropical Africa, ectomycorrhizal (ECM) trees are mainly distributed in the Guineo-Congolian, Zambezian and Sudanian regions according to the phytochoria defined by White (1983) and data from other literature (e.g. Alexander 1989; Buyck et al. 1995). These ECM tree species include many cesalpinoid legumes (12 genera in the Amherstieae and *Afzelia* in the Detarieae) and some members of the Dipterocarpaceae (*Marquesia* and *Monotes*), Euphorbiaceae (*Uapaca*), Papilionoideae (*Pericopsis*) and Proteaceae (*Faurea*) which occur in certain well-defined areas in rain forests and dry woodlands in Africa (Högberg and Nylund 1981; Högberg 1982; Newbery et al. 1988; Thoen and Bâ 1989; Thoen and Ducousso 1989; Sanon et al. 1997). Among the ECM African trees growing in poor soils of these areas, *Afzelia africana* Sm. is the more well-known species with respect to its economic importance and data on diversity, ecology and physiology of fungi involved in this symbiosis (Bâ 1990; Sanon 1999).

Afzelia africana is a caesalpinoid legume producing timber of high commercial value and fodder in West Africa (Thiès 1995). Field observations indicate that it is associated with at least 37 ECM fungi species collected in the lowland forests of southern Senegal and Burkina Faso, and in the forests of the Fouta Djalon plateau in Guinea (Thoen and Bâ 1989; Thoen and Ducousso 1989; Sanon et al. 1997). However, only five *Scleroderma* species and one unidentified fungus are shown to form ectomycorrhizas on *A. africana* seed-

lings in glasshouse and laboratory trials (Bâ 1990; Bâ and Thoen 1990; Sanon et al. 1997). Some of these fungi colonize the roots soon after germination of the seed and before the first leaf is formed, while other fungi colonize later (Bâ et al. 1991, 1994).

Until now, the role of the fungi involved in ECM symbioses with most tropical trees, specially those in western Africa, was unknown. However, Bâ (1990) reported positive effects of some ECM fungi on a provenance of *A. africana* seedlings growing in Bezange forest sandy soil (Meurthe et Moselle, France) in a glasshouse at INRA, Nancy, France. This result needs to be confirmed in a tropical area where ectomycorrhizas may be of a particular importance for tree growth because P is often the main limiting factor (Smith and Read 1997).

The objectives of this present investigation were to evaluate the effects of four ECM fungi on growth and mineral nutrition of two provenances of *A. africana* growing in a savanna ECM propagule-free soil deficient in NPK, and to compare external hyphal length of these fungi.

Materials and methods

Fungal inocula and inoculation

Four ECM fungi were used. One culture of *Scleroderma dictyosporum* Pat. (IR.109) was isolated from sporocarps collected from under an *A. africana* stand in the Sindou forest in southern Burkina Faso (Sanon et al. 1997). Two *Scleroderma* species (IR.406 and IR.408) were also isolated from sporocarps collected from under a *Uapaca guineensis* Müll.Arg. stand in the Dinderesso forest in southern Burkina Faso (Sanon et al. 1997). One unidentified fungal culture (ORS.XM002) belonging to the Basidiomycetes was isolated from dark brown ectomycorrhizas of *A. africana* seedlings growing in Bayottes forest soil in southern Senegal (Bâ and Thoen 1990). Fungal cultures were kept at 30 °C on MMN medium (Marx 1969). Glass jars (1 l) were half filled with a mixture of vermiculite and peat moss (4:1; v/v) and autoclaved (120 °C, 20 min). This substrate was moistened with 300 ml liquid MMN medium and autoclaved again. Then, 10 plugs were inoculated per jar after cooling. The substrate was colonized by ECM fungi after 2–3 months of growth at 30 °C.

The soil used in this investigation was collected in a savanna ecosystem (Sangalkam, Senegal), an area where *A. africana* was absent. It was known to be free of ECM propagules (unpublished data) with 88.8% sand, 5.8% silt, 5.4% clay, 0.6% organic matter, 0.3% total C, 0.02% total N, C/N ratio 14, 333.5 ppm total K, 41.4 ppm total P, 2.1 ppm P-Bray 1, Ca 1.03, Mg 0.30, K 0.05 meq 100 g^{-1} soil, pH (of a soil/water mixture, ratio 1:2) 6.0 and pH (of a soil/KCl mixture, ratio 1:2) 4.6. It was an NPK-deficient soil according to regional standards for growing plants. It was crushed, passed through a 2-mm sieve and mixed with fungal inoculum (10:1; v/v). The uninoculated control received a mixture of moistened vermiculite-peat moss with nutrient solution and without fungal culture.

Plant culture

Seeds of two provenances of *A. africana* from Nazinga (AaN) and Diatock (AaD), were obtained from Centre National des Semences Forestières in Burkina Faso and Centre National des Recherches Forestières in Senegal, respectively. They were scarified in 95% sulfuric acid for 2 h, rinsed with tap water and transferred into plastic bags containing 2 kg of soil mixed with fungal inoculum. Plants were watered daily with tap water. The experiment was set up as a 2×5 factorial design consisting of the two provenances of *A. africana* and four ECM fungi plus a non-inoculated control, which were arranged in a randomized design with 10 replicates per treatment combination. The plants were grown under natural light (daylength approximately 12 h, mean daytime temperature 30 °C).

Seedling harvest and measurement

Plants were harvested after 4 months. Height and dry weight (6 days at 80 °C) of shoots and roots were measured. ECM dependency was calculated as follows: (biomass of ECM plants–biomass of non-ECM plants/biomass of ECM plants) × 100 (Plenchette et al. 1983). For each inoculated treatment, a sample of lateral roots was examined under a stereomicroscope at × 160 magnification to determine the percentage of ectomycorrhizal colonization (number of mycorrhizal roots/total number of roots × 100).

Length of external hyphae was also evaluated according to the method of Thomson et al. (1994). For each treatment, five plants were randomly selected and for each plant five cores of soil were taken at random, mixed and stored in plastic bags at –20 °C until use. A sub-sample of 10 g of soil was suspended in 500 ml of tap water using a Waring blendor for 1 min. Ten ml of the suspension was passed through a 0.45-μm cellulose membrane and the membrane was then floated on 0.05% trypan blue in lactic acid, glycerin and distilled water overnight at 4 °C. The membrane was then rinsed with distilled water, cut and mounted on a microscope slide with 50% glycerol for observation. The total length of hyphae per gram of soil sample was estimated by the gridline-intersect method at × 300 magnification using a compound microscope fitted with an eyepiece scale (Newman 1966).

Total P and N contents of leaves were determined by the molybdate blue (Murphy and Riley 1962) and Kjeldahl methods, respectively, using a Technicon Autoanalyser (Laboratory of IFDC/ICRISAT Sahelian Centre, Niger). Total K, Mg and Ca contents were determined by means of an atomic absorption spectrophotometer (Laboratory of IFDC/ICRISAT Sahelian Centre, Niger).

Statistical analysis

All data were subjected to two-way analysis of variance, and mean values were compared using Bonferroni's multiple range test (SAS Institute 1987).

Results

Root colonization and external hyphal length

There were no mycorrhizas in the non-inoculated control, confirming that the savanna soil in Sangalkam lacks ECM propagules. ECM colonization ranged from 22% to 69%, depending on the fungal species and provenances of *A. africana* (Table 1). For example, *Scleroderma* sp. 2 (IR.408) colonized AaN well, but did not appear to colonize roots of AaD extensively. In contrast, there was little difference in the ability of fungal species to colonize roots of either provenance of *A. africana* or to produce external hyphae in soil (Table 1).

Table 1 Effect of inoculation with ectomycorrhizal (ECM) fungi on growth variables, ECM colonization, external hyphal length and ECM dependency (calculated according to Plenchette et al. 1983) of two provenances of *Afzelia africana* seedlings. Different letters within a column indicate significantly different values at $P<5\%$ (*) (*AaN A. africana* from Nazinga, *AaD A. africana* from Diatock)

Provenances of *Afzelia africana*	Height (cm)	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)	Root colonization (%)	External hyphal length (cm/g of soil)	ECM dependency (%)
AaN							
Scleroderma dictyosporum IR.109	41.97 b	4.98 a	2.83 abc	7.81 ab	69.00 a	2.00 a	21.25 ab
Scleroderma sp.1 IR.406	44.96 ab	5.20 a	2.53 bc	7.73 ab	32.00 abc	2.47 a	20.43 ab
Scleroderma sp.2 IR.408	47.78 ab	5.71 a	3.29 ab	9.00 a	64.00 a	1.81 a	31.66 a
Fungal isolate ORS.XM002	42.75 b	5.12 a	3.98 a	9.10 a	46.00 ab	1.66 a	32.24 a
Control	38.98 b	4.39 a	1.76 c	6.15 b	0.00 c	–	–
AaD							
Scleroderma dictyosporum IR.109	46.85 ab	4.27 a	2.07 bc	6.34 b	58.00 ab	2.81 a	0.15 b
Scleroderma sp.1 IR.406	47.80 ab	4.23 a	2.71 abc	6.95 ab	38.00 ab	1.95 a	8.92 ab
Scleroderma sp.2 IR.408	52.40 a	4.86 a	2.17 bc	7.03 ab	22.00 bc	2.62 a	9.95 ab
Fungal isolate ORS.XM002	52.78 a	5.26 a	2.92 abc	8.18 ab	64.00 a	1.95 a	22.61 ab
Control	52.60 a	4.62 a	1.73 c	6.33 b	0.00 c	–	–
ECM fungi	*	*	*	*	*	*	*
Provenances of *A. africana*	*	*	*	*	*	*	*
ECM fungi × Provenances of *A. africana*	*	*	*	*	*	*	*

Height and dry weights

Non-inoculated AaD grew better than AaN in terms of height (Table 1). Nevertheless, shoot dry weights were comparable for both non-inoculated provenances. There was also no significant difference between inoculated provenances for total dry weight. However, AaN responded better to inoculation than AaD when compared to non-inoculated plants. Only two fungal species (isolates IR.408 and ORS.XM002) appeared to be more effective in increasing total dry weight of AaN.

ECM dependency of the two provenances ranged from 0.15% to 32.24% (Table 1). AaN in symbiosis with isolates IR.408 or ORS.XM002 showed the highest ECM dependency value, and AaD associated with fungal isolate IR.109 the lowest.

Nutrient concentrations in leaves

There was a large variation in N, P, K, Mg and Ca concentrations in leaves of both provenances, depending on the fungal isolate used (Table 2). Comparison of the concentrations of mineral nutrient in leaves of ECM plants with non-ECM plants of both AaN and AaD showed that K nutrition is the most enhanced by inoculation. Three different types of response occurred: increase of K concentration in leaves associated with growth stimulation in terms of biomass production (AaN/*Scleroderma* sp.2 IR.408 and AaN/ORS.XM002), increase of K without a growth stimulation (AaN/*S. dictyosporum* IR.109, AaN/*Scleroderma* sp.1 IR.406 and in all AaD/fungus combinations), and no increase in either K concentration in leaves or plant growth (AaN/*S. dictyosporum* IR.109). Of all plant/fungus combinations, increased P concentration in leaves was only associated with the AaN growth stimulation by *Scleroderma* sp.2 IR.408. Increase in N, Mg and Ca concentrations in leaves was not associated with plant growth stimulation.

Discussion

In this study, both provenances of *A. africana* differed in their responses to inoculation with four ECM fungi at a deficient supply of P. AaD growth was not improved by inoculation irrespective of the ECM fungus, whereas AaN growth was improved by inoculation with fungal isolate IR.408 or ORS.XM002. The increased growth of this inoculated provenance could be attributed to increased development of the root system, and this was reflected in the total dry weight of ECM plants. This result confirms the previously identified positive response of a provenance of *A. africana* from Bayottes (AaB) to ECM colonization (Bâ 1990). Our finding agrees with this study, which showed that AaB seedlings varied in their response to inoculation with different ECM fungi. In order to examine this, Bâ (1990) screened four ECM fungi (*Scleroderma dictyosporum* Pat. ORS.7731, *S. verrucosum* Pers. ORS.7732, and two unidentified fungal isolates ORS.XM004 and ORS.XM002) in steamed or unsteamed soil at a non-limiting supply of P (44 ppm P-Olsen). Bâ (1990) finally selected the unidentified fungal isolate ORS.XM002 because it improved biomass production of AaB seedlings due to increased growth of roots.

Table 2 Changes in phosphorus (P). nitrogen (N), potassium (K), magnesium (Mg) and calcium (Ca) concentrations in leaves of two provenances of *A. africana* seedlings in response to colonization by ECM fungi. Different letters within a column indicate significantly different values at $P<5\%$ (*) (*AaN A. africana* from Nazinga, *AaD A. africana* from Diatock)

Provenances of *Afzelia africana*	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
AaN					
Scleroderma dictyosporum IR.109	1.96 d	0.11 bc	1.41 cde	0.65 ab	2.13 bc
Scleroderma sp. 1 IR.406	2.33 ab	0.09 c	2.12 a	0.65 a	2.65 ab
Scleroderma sp. 2 IR.408	1.94 d	0.14 a	1.66 bc	0.56 abc	1.77 c
Fungal isolate ORS.XM002	1.85 d	0.10 bc	1.45 cd	0.58 abc	1.99 c
Control	2.02 cd	0.10 bc	1.02 e	0.50 c	2.00 c
AaD					
Scleroderma dictyosporum IR.109	2.03 cd	0.13 ab	1.55 c	0.55 abc	1.82 c
Scleroderma sp. 1 IR.406	2.28 abc	0.10 bc	2.05 ab	0.62 abc	2.65 a
Scleroderma sp. 2 IR. 408	2.54 abc	0.11 bc	2.11 a	0.64 ab	3.09 a
Fungal isolate ORS.XM002	2.08 bcd	0.12 abc	1.80 abc	0.57 abc	1.76 c
Control	1.90 d	0.10 bc	1.13 de	0.53 bc	2.20 bc
ECM fungi	*	*	*	*	*
Provenances of *A. africana*	*	*	*	*	*
ECM fungi × Provenances of *A. africana*	*	*	*	*	*

The ECM dependency of AaB ranged from 24% to 34%, which is similar to results obtained for AaN and AaD. The ECM dependency of these three provenances of *A. africana*, which did not exceed 34%, appears moderate to low when compared to values reported for highly dependent tropical tree species introduced into western Africa, such as *Pinus* spp., hybrid eucalypts (*Eucalyptus urophylla* × *E. kirtoniana*) and *Acacia mangium* Willd. (e.g. Marx et al. 1985; Garbaye et al. 1988; Duponnois and Bâ 1999). Nevertheless, meaningful comparisons are difficult as the experimental conditions were not identical and the number of provenances of *A. africana* used in our study was low.

In West African areas, low soil P is one of the limiting factors for plant growth because the slow diffusion of this element through the soil results in a depletion zone around the root (Piéri 1989). One of the major contributions of ECM symbiosis is to improve P content in plants, because ECM fungi colonize roots extensively and the external hyphae take up P from soil by passing through the P depletion zone immediately around the root (Bougher et al. 1990; Thomson et al. 1994). When we compared ECM treatments with the non-ECM control, increases in leaf P, N, Mg and Ca concentrations were not associated with plant growth stimulation in terms of biomass production (except for P in AaN/*Scleroderma* sp.2 IR.408). Only leaf K concentration increased to a higher extent in inoculated than non-inoculated plants but was not always associated with plant growth stimulation. This suggests that these nutrients do not limit the growth of either provenance of *A. africana* investigated. Thus, the high levels of ECM colonization did not foster an increase in nutrient concentrations in leaves of either provenance of *A. africana* because there was no evident relationship between the degree to which plants were colonized by ECM fungi and the potential for the plant to benefit from this. Differences in the effectiveness of ECM fungi which colonized similarly the roots of *A. africana* also could not be related to hyphal development in soil. Therefore, in this case, ECM fungi could not be screened for effectiveness according to their ECM colonization and external hyphal development. However, some studies have been able to discriminate ECM fungi in this way. For example, Thomson et al. (1994) found that *Eucalyptus globulus* Labill. seedlings benefited most from increased uptake with ECM fungi which colonized extensively; differences in growth-effectiveness between ECM fungi could not attributed to their external hyphal development. In some circumstances, variability in fungal efficiency reflects differences in hyphal length densities (Jones et al. 1990).

In conclusion, two fungal isolates, *Scleroderma* sp.2 IR.408 and the early fungal colonizer ORS.XM002, may be the most appropriate species for improving the first phase of *A. africana* growth. However, further work is required to evaluate variability in growth responses of provenances of *A. africana* to ECM inoculation, and to identify non-nutritional characteristics of ECM fungi (early colonizer, water uptake, protection against pathogens, hormonal effects). These can be used for screening and selection of inoculant fungi under nursery and field conditions to develop effective inoculation programmes.

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