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Original article

Relations between rhizobial nodulation and root colonization of *Acacia crassicarpa* provenances by an arbuscular mycorrhizal fungus, *Glomus intraradices* Schenk and Smith or an ectomycorrhizal fungus, *Pisolithus tinctorius* Coker & Couch

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Abstract – The present study was initiated to (i) determine the ability of an ectomycorrhizal and an arbuscular mycorrhizal symbiont to colonize three provenances of *Acacia crassicarpa* root systems, (ii) to examine plant growth response to the mycorrhizal inoculation and (iii) to measure their influence on the rhizobial symbiosis with a *Bradyrhizobium* isolate. This study has been performed with 2 fungal symbionts: *Glomus intraradices*, an Arbuscular Mycorrhizal fungus, and an ectomycorrhizal fungus *Pisolithus tinctorius* strain GEMAS. Two experiments have been performed during two different climatic periods, hot season (30 °C day, 20 °C night, June to October) for ectomycorrhizal inoculation and cold season (25 °C day, 15 °C night, November to March) for endomycorrhizal inoculation. Moreover, *Bradyrhizobium* sp. strain Aus13C has been co-inoculated with each of these fungal symbionts. The results showed that ectomycorrhizal and AM fungal symbiosis clearly benefit to the growth of *A. crassicarpa* provenances and these fungal symbioses greatly improve the rhizobial nodulation process. However, some differences of growth were observed between the provenances tested and our results showed that both Papua New Guinea provenances produced more important total biomass than the provenance from Madagascar in both experiments However, no significant differences were observed in terms of nodulation and mycorrhization. Further research must be undertaken to identify the convenient ecological characteristics in which each kind of mycorrhizal symbiosis exerts the best effect on plant growth and nodulation formation and to identify in such environmental conditions the better rhizobial/mycorrhizal symbiosis combination.

***Acacia crassicarpa* / arbuscular mycorrhizas / ectomycorrhizas / rhizobium**

Résumé – Relations entre la nodulation induite par un rhizobium et la colonisation des racines de plusieurs provenances d'*Acacia crassicarpa* par un champignon mycorhizien arbusculaire *Glomus intraradices* Schenk and Smith ou par un champignon ectomycorhizien, *Pisolithus tinctorius* Coker & Couch. La présente étude a été initiée pour (i) déterminer l'aptitude de champignons mycorhiziens (ectomycorhizes et champignons arbusculaires) à coloniser le système racinaire de 3 provenances d'*Acacia crassicarpa*, (ii) d'évaluer la réponse de la plante à l'inoculation avec des champignons mycorhizes en termes de croissance et (iii) de mesurer leur influence sur la symbiose fixatrice d'azote avec une souche de *Bradyrhizobium*. Cette étude a été réalisée avec 2 symbiotes fongiques: *Glomus intraradices*, un champignon mycorhizien arbusculaire, et un champignon ectomycorhizien, *Pisolithus tinctorius* souche GEMAS. Deux experiences ont été réaliséees durant 2 periodes climatiques differentes, saison chaude (30 °C jour, 20 °C nuit, juin à octobre) pour l'inoculation avec le champignon ectomycorhizien, et saison froide (25 °C jour, 15 °C nuit, novembre à mars) pour l'inoculation avec le champignon endomycorhizien. La souche de *Bradyrhizobium* sp. strain Aus13C a été co-inoculée avec chacun de ces symbiontes fongiques. Les résultats ont montré que les symbioses ecto and endomycorhiziennes améliorent clairement la croissance des plantes des 3 provenances d'*Acacia crassicarpa* et qu'elles ont un effet très positif sur la nodulation des plantes par la souche de rhizobium. Cependant, des différences de croissance ont été observées entre les différentes provenances testées et nos résultats ont montré que dans les deux experiences, les deux provenances originaires de Papouasie-Nouvelle Guinée produisent plus de biomasse que la provenance originaire de Madagascar. Cependant, aucune différence significative n'a été observée en termes de nodulation et de mycorrhization. Des recherches ultérieures devront être entreprises pour identifier les conditions écologiques les plus appropriées dans lesquelles chaque type de symbioses mycorhiziennes exercera son meilleur effet sur la croissance de la plante ainsi que sur sa nodulation, et pour identifier les conditions environnementales les plus favorables à une symbiose mixte mycorhizes/rhizobium.

***Acacia crassicarpa* / champignons mycorhiziens arbusculaires / ectomycorhizes / rhizobium**

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1. INTRODUCTION

During recent decades, dramatic deforestation has occurred in West Africa following excessive industrial exploitation, clearing for agricultural purposes and collection of firewood. In order to rehabilitate these degraded areas, a lot of reforestation programs have been attempted to restore sustainable ecosystems [20]. Among the most useful plants in the Soudano Sahelian areas, *Acacia* is the largest mimosoid genus and includes nearly 1200 species [28]. Their positive impacts on the soil, erosion and local economies are well documented [7, 19]. Inside the genus *Acacia*, fast-growing trees, brought from Australia and introduced in West Africa, have been evaluated by the plant growth in field trials [5, 7, 8, 24]. Their associated rhizobial symbioses allow these tree species to grow in nitrogen-deficient soils frequently encountered in tropical areas and the continuous replacement of their leaves and roots ensures transfer of nitrogen to the soil [29]. As with many N_2-fixing trees and shrubs, *Acacia* is very dependent on mycorrhizas for the absorption of nutrients (in particular P assimilation) required for their growth and efficient N_2 fixation [2, 4, 13, 33]. As most of Australian native plants (*Eucalyptus*, *Casuarina*, etc.), *Acacia* may form mycorrhizal associations with arbuscular mycorrhizas (AM) and ectomycorrhizas (EM) [1, 6, 12]. More recently, it has been established that each morphological types of mycorrhizas could greatly interact within the same root system [15]. All these studies have indicated the functional compatibility of both partners and a lot of informations are available on the mycorrhizal requirements of *Acacia* and their growth responses to inoculation with ecto and/or endomycorrhizal fungi in experimental conditions [8, 10, 12, 14]. However, as these forms of mycorrhizal symbiosis are differently efficient at using soil nutrient contents such as organic nitrogen [27], it is important to evaluate the host plant dependencies for ecto or endomycorrhizal symbiosis in order to predict the impact of controlled mycorrhization on the plant growth and on nutrient soil fertility.

A. crassicarpa is one of the fastest-growing of the tropical acacias. A total above-ground biomass of 207 dry t/ha at 3 years was recorded for a Papua New Guinea provenance in Thailand [36]. In Sabah/Malaysia, *A. crassicarpa* trees have attained a mean height outperforming *Acacia mangium* and *Acacia auriculiformis* [34]. *A. crassicarpa* tolerates a wide range of infertiles soils, salt winds, fire and grass competition and is described has having a potential for industrial plantations [9]. In 2000, over 40 000 ha of plantation of this specie have been established in the Island of Sumatra in Indonesia [26]. In 2004, we estimated around 100 000 ha the surface planted with *A. crassicarpa* in Indonesia and it should reach more than 500 000 ha in the five next years (Jean-Pierre Bouillet, personal communication). This species is found in warm to hot, humid and sub-humid climates in the lowland tropics (mean max of hottest month is 32–34 °C and mean minimum of the coldest is 12–21 °C). Regarding wood characteristics, it is dense suitable for burning and strong too. Pulp properties also are promising [9]. Significant provenance variation is reported. Papua New Guinea provenances have consistently grown faster than Queensland sources in trials in Australia, China and Thailand [21]. Few informations are available on the symbiotic requirements of *A. crassicarpa*. Dommergues et al. [8] indicated that *A. crassicarpa* is usually described as being always nodulated in soils from their origin area but also in the countries where it was introduced successfully. However, Doran and Turnbull [9] recommend the inoculation of nursery seedlings with a selected strain of rhizobia. Concerning the functional compatibility between provenances of *A. crassicarpa* and arbuscular mycorrhizal fungi or ectomycorrhizal, as well as the interactions between rhizobial and mycorrhizal symbiosis, there are still largely unknown.

The present study was initiated to (i) determine the ability of an ectomycorrhizal and an arbuscular mycorrhizal symbiont to colonize three provenances of *Acacia crassicarpa* root systems, (ii) to examine the three provenances response to the mycorrhizal inoculation and (iii) to measure their influence on the rhizobial symbiosis with a *Bradyrhizobium* isolate.

2. MATERIALS AND METHODS

2.1. Host plant

The geographical origins of the *A. crassicarpa* provenances are indicated in Table I. Seeds of *A. crassicarpa* were surface-sterilized with concentrated sulphuric acid (36 N) for 60 min. The acid solution was then decanted off and the seeds rinsed and imbibed for 12 h in 4 changes of sterile distilled water. Seeds were then transferred aseptically in Petri dishes filled with 1% (w:v) water agar medium. These plates were incubated for 4 days at 25 °C in the dark. The germinating seeds were used when rootlets were 1–2 cm long.

2.2. Rhizobial and fungal inocula

The isolate of *Bradyrhizobium* Aust 13C has been isolated from nodules collected in a forest plantation of *A. mangium* in Australia (Queensland, 16° 15' S, 145° 20' E) [18]. It is very effective with *Acacia mangium* into several field trials set up all around the world [16–18], and it was identified as forming an efficient nitrogen-fixing symbiosis with *A. crassicarpa* in vitro and under nursery conditions (Antoine Galiana, personal communication). It was cultured in glass flasks containing liquid yeast extract – mannitol medium [35] at 37 °C for 5 days on an orbital shaker. The bacterial suspensions were approximately 10^9 cells mL^{-1}.

Table I. Geographical origins of *A. crassicarpa* provenances.

Provenance	Seeddlot	Region	Seed collection site	Altitude (m)
P1	89/08809N	Papua New Guinea	141° 55' E, 8° 36' S	25
P2	89/08829N	Papua New Guinea	141° 55' E, 8° 36' S	25
P3	99/10485N	Madagascar	48° 55' E, 18° 57' S	30

The arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith (DAOM 181602, Ottawa Agricultural Herbarium) was propagated on leek (*Allium porrum* L.) for 12 weeks in greenhouse on Terragreen™ substrate. This calcined clay (particule size average 5 mm), Oil-Dri US-special Ty/IIIR (Oil-Dri Company, Chicago, USA) is an attapulgite from Georgia used as substrate for propagation of mycorrhizal fungi [31]. This fungal isolate is very effective with another fast-growing Australian acacia, *Acacia holosericeae* [12]. Before inoculation, the leek plants were uprooted, gently washed with tap water and roots cut into segments 0.5 cm long bearing around 250 vesicles cm^{-1}. Non-mycorrhizal leek roots, prepared as above, were used for the control treatment without endomycorrhizal inoculation.

The ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker & Couch strain GEMAS was isolated from a sporocarp collected under a monospecific forest plantation of *A. mangium* in Malaysia. It was routinely maintained in Petri dishes on MMN agar medium at 25 °C [25]. This fungal strain significantly increased the growth of an Australian *Acacia* species, *A. holosericea*, in a previous experiment [12].

Fungal inoculum was prepared in 1.6 L glass jars containing 1.3 L vermiculite-peat mixture (4/1; v/v) and autoclaved for 20 min at 120 °C. The substrate was then moistened to field capacity with 300 mL liquid MMN medium. The jars were sealed and autoclaved at 120 °C for 20 min. After cooling, the substrate was then inoculated aseptically with fungal plugs taken from the margin of the fungal colonies and incubated for 6 weeks at 28 °C in the dark [11].

2.3. Glasshouse experiment

Seedlings of *A. crassicarpa* provenances were grown in 1 dm^3 pots filled with an autoclaved sandy soil (140 °C, 40 min) sampled from a 17-year-old plantation of *A. holosericea* at Sangalkam (50 km from Dakar). Its physicochemical characteristics were as follows: 3.6% clay, 1.6% silt, 2.9% unrefined silt, 51% sand, 40.9% unrefined sand, 0.43% organic matter, 0.25%, organic carbon 5.15 ppm of phosphate, 0.21‰ total nitrogen, C/N of 11.9, pH_{H2O} 5.7). For ectomycorrhizal inoculation, the soil was mixed with 10% (v/v) fungal inoculum or 10% moistened (MMN medium) vermiculite-peat mixture (4/1; v/v) for the treatment without fungus. For endomycorrhizal inoculation, one hole (1 cm × 5 cm) was made in each pot and filled with 1 g fresh leek root (mycorrhizal or not for the control without fungus). The holes were then covered with the same autoclaved soil. These experiments have been performed during two different climatic periods, hot season (30 °C day, 20 °C night, June to October) for ectomycorrhizal inoculation and cold season (25 °C day, 15 °C night, November to March) for endomycorrhizal inoculation.

The plants were placed in a glasshouse and watered twice a week without fertiliser addition. After 1-week culture, the young seedlings were inoculated by 5 mL of the *Bradyrhizobium* sp. suspension (10^9 bacterial cells) or 5 mL of the culture medium without bacteria for the control treatments as it was suggested by Diouf et al. (2003) [6] for the inoculation of *Acacia mangium*. The pots were arranged in a randomised complete block design with nine replicates per treatment.

After 5 months culture, *Acacia* plants were uprooted and the root systems gently washed. The oven-dried (1 week at 65 °C) weight of the shoots was measured. After drying, a sub-sample of leaf tissue was ground, ashed (500 °C), digested in 2 mL HCl 6N and 10 mL HNO_3 and then analysed by colorimetry for P [22] and by flame emission for K. Another sub-sample of leaf tissue was ground and digested in 15 mL H_2SO_4 36N containing 50 g L^{-1} salicylic acid for N (Kjeldhal) determination. Root nodules were counted and weighed (1 week at 65 °C). Then entire root systems of each plant were cut into 1 cm root pieces and mixed. For the ectomycorrhizal treatments, roots with a fungal sheath, randomly chosen in each root samples, were free-hand sectioned, stained with 0.25% Congo red and observed under a light microscope. Then the ectomycorrhizal colonization (number of ectomycorrhizal roots/total number of roots × 100) was determined under a stereomicroscope at ×40 magnification on a random sample of at least 100 short roots per root systems. For the internal colonization of arbuscular mycorrhizal fungus, roots fragments were cleared with a KOH solution (10%, v/v) and stained according to the method of Phillips and Hayman [30]. The roots were placed on a slide for microscopic observation (×250 magnification) [3]. The endomycorrhizal colonization (length of root fragments with visible mycorrhizal structures/total length of root fragments) was determined on a random sample of at least fifty 1-cm root pieces per plant. Then these root fragments used to estimate mycorrhizal colonization were oven dried (60 °C, 1 week) and weighed.

Then all data were subjected to one-way analysis of variance and mean values were compared using Newman-Keuls multiple range test ($P < 0.05$). Mycorrhizal rates were Arc sinsqrt (x) transformed before statistical analysis.

3. RESULTS

3.1. Ectomycorrhizal inoculation

The fungal strain *P. tinctorius* GEMAS was able to form typical ectomycorrhizae with all *A. crassicarpa* provenances after 5 months of growth. The color of ectomycorrhizae was yellow and their morphology varied from simple to branched as it has been previously observed with the ectomycorrhizal symbiosis between *P. albus* COI007 and *A. mangium* [10]. Cross section showed a Hartig net whose penetration did not exceed the epidermal cells.

Inoculation with *Bradyrhizobium* Aust 13C significantly increased shoot growth of all provenances but this positive effect was lower than that recorded with *P. tinctorius* for P1 and P2 (Tab. II). When both were co-inoculated, shoot dry weight was significantly increased over the single inoculation treatments (Tab. II). For the root biomass, *Bradyrhizobium* Aust 13C and *P. tinctorius* exerted a significant positive effect for all the provenances without significant differences between each single microbial treatment. The dually inoculation did not exert a higher stimulation than that of the singly inoculations, except for P2 provenance (Tab. II).

For all the provenances, ectomycorrhizal colonization ranged from 40.4% (P1 provenance) to 49.4% (P2 provenance). It was significantly lower when *P. tinctorius* was inoculated alone compared to that recorded in the dually treatments (Tab. III).

Even in the treatments without *Bradyrhizobium* Aust 13C and despite of the soil autoclaved and the disinfection of seed surface, some nodules were observed along the root systems of *A. crassicarpa* seedlings for all the provenances. For Aust 13C treatments, the number and the total dry weight of nodules per plant were significantly higher in the treatments with *P. tinctorius* than those found in the treatments without fungal inoculation (Tab. III).

Bradyrhizobial inoculation enhanced leaf nitrogen content for P2 and P3 provenances, leaf phosphorus and potassium contents for P3 provenance (Tab. IV). These leaf mineral contents were increased for all provenances after ectomycorrhizal inoculation. When both symbionts were inoculated, highest values were recorded except for leaf K content with P1 provenance (Tab. IV).

Table I. Effect of the rhizobial and/or ectomycorrhizal inoculations on plant growth of *A. crassicarpa* provenances after 5-month culture.

Provenances	Treatments	Shoot Biomass (mg dry weight)	Root biomass (mg dry weight)	Total biomass (mg dry weight)
P1	Not inoculated	1074 a[1]	221 a	1296 a
	Aust 13C	1776 b	407 b	2184 b
	P. tinctorius	2724 c	470 b	3194 c
	P. tinctorius + Aust 13C	3884 d	490 b	4374 d
P2	Not inoculated	508 a	142 a	650 a
	Aust 13C	1396 b	269 b	1664 b
	P. tinctorius	2211 c	298 b	2509 c
	P. tinctorius + Aust 13C	3884 d	490 c	4374 d
P3	Not inoculated	844 a	191 a	1035 a
	Aust 13C	1979 b	367 b	2346 b
	P. tinctorius	2370 b	385 b	2798 b
	P. tinctorius + Aust 13C	3187 c	428 b	3572 c

[1] For each provenance, data in the same column followed by the same letter are not significantly different according to the one way analysis of variance ($P < 0.05$).

Table II. Effect of microbial inoculation on ectomycorrhizal establishment and on rhizobial symbiosis after 5-month culture.

Provenances	Treatments	Ectomycorrhizal colonization (%)	Number of nodules per plant	Nodule dry weight (mg/plant)
P1	Not inoculated	0 a[1]	6 a	14 a
	Aust 13C	0 a	31.1 a	34 a
	P. tinctorius	40.4 b	36.7 a	77 b
	P. tinctorius + Aust 13C	61.2 c	90.6 b	85 b
P2	Not inoculated	0 a	0 a	0 a
	Aust 13C	0 a	19.7 ab	16 a
	P. tinctorius	49.4 b	24.9 b	69 b
	P. tinctorius + Aust 13C	74.6 c	87 c	77 b
P3	Not inoculated	0 a	8.6 a	15 a
	Aust 13C	0 a	35.3 b	43 b
	P. tinctorius	46.4 b	42.9 b	71 bc
	P. tinctorius + Aust 13C	66.8 c	78.8 c	92 c

[1] For each provenance, data in the same column followed by the same letter are not significantly different according to the one way analysis of variance ($P < 0.05$).

3.2. Arbuscular mycorrhizal fungal inoculation

In this experiment, *Bradyrhizobium* Aust 13C inoculation stimulated greatly the P3 provenance shoot and root growth (Tab. V) such as in the previous experiment. In contrast, the AM fungus, *G. intraradices*, had a positive effect on the root and shoot biomass with all the provenances. The dually inoculation did not modified the fungal effect on the plant growth except with P1 provenance where plant growth was decreased compared to that recorded in the *G. intraradices* treatment and with P2 provenance for the root growth (Tab. V).

The endomycorrhizal colonization ranged from 34.3% (P1 provenance) to 61.7% (P3 provenance). It was not affected by the Aust 13C inoculation for all the provenances (Tab. VI). No nodules were observed in the control or when Aust 13C was inoculated alone. On the contrary, the formation of nodules was recorded along root systems of endomycorrhizal seedlings (Tab. VI). The number of nodules was significantly higher with P3 provenance when Aust 13C was co-inoculated with *G. intraradices* whereas the same bacterial effect was recorded with nodule biomass for P2 provenance (Tab. VI).

Aust 13C inoculant only increased leaf mineral contents for P3 provenance whereas *G. intraradices* inoculation significantly enhanced leaf P, N and K contents for all the provenances (Tab. VII). Compared to the *G. intraradices* treatment, the dual inoculation involved a depletion of P and K contents for P1 provenance seedlings and an increase of leaf nitrogen content for P2 and P3 provenance seedlings (Tab. VII).

Table III. Effect of ectomycorrhizal inoculation and/or *Bradyrhizobium* Aust 13C on leaf mineral content after 5-month culture.

Provenances	Treatments	N (mg/plant)	P (µg/plant)	K (µg/plant)
P1	Not inoculated	18.7 a[1]	569.5 a	9809.7 a
	Aust 13C	27.7 a	710.5 a	13908.1 a
	P. tinctorius	53.6 b	1743.6 b	38196.7 b
	P. tinctorius + Aust 13C	66.6 c	2214.1 c	45603.4 b
P2	Not inoculated	9.0 a	213.2 a	5631.2 a
	Aust 13C	21.1 b	419.3 a	12741.1 a
	P. tinctorius	39.2 c	2034.4 b	33167.3 b
	P. tinctorius + Aust 13C	81.3 d	3108.1 c	55742.1 c
P3	Not inoculated	12.9 a	388.1 a	8252.1 a
	Aust 13C	34.6 b	930.0 b	17414.2 b
	P. tinctorius	42.9 b	1564.3 c	34768.3 c
	P. tinctorius + Aust 13C	60.5 c	2263.4 d	42606.2 d

[1] For each provenance, data in the same column followed by the same letter are not significantly different according to the one way analysis of variance ($P < 0.05$).

Table IV. Effect of the rhizobial and/or endomycorrhizal inoculations on plant growth of *A. crassicarpa* provenances after 5-month culture.

Provenances	Treatments	Shoot Biomass (mg dry weight)	Root biomass (mg dry weight)	Total biomass (mg dry weight)
P1	Not inoculated	167 a[1]	10 a	177 a
	Aust 13C	300 a	30 a	330 a
	G. intraradices	3056 c	778 c	3833 c
	G. intraradices + Aust 13C	2067 b	411 b	2478 b
P2	Not inoculated	189 a	23 a	212 a
	Aust 13C	356 a	67 a	422 a
	G. intraradices	2411 b	578 c	2989 b
	G. intraradices + Aust 13C	2333 b	422 b	2756 b
P3	Not inoculated	100 a	44 a	144 a
	Aust 13C	1100 b	256 b	1356 b
	G. intraradices	1989 c	411 c	2400 c
	G. intraradices + Aust 13C	1844 c	289 bc	2133 c

[1] For each provenance, data in the same column followed by the same letter are not significantly different according to the one way analysis of variance ($P < 0.05$).

4. DISCUSSION

From this research work, two main conclusions can be done: (i) ectomycorrhizal and AM fungal symbiosis clearly benefit to the growth of *A. crassicarpa* provenances, and (ii) these fungal symbioses greatly improve rhizobial nodulation process.

Our results demonstrated the effectiveness of ectomycorrhizal and endomycorrhizal symbiosis in improving the growth of all *A. crassicarpa* provenances. It is in accordance with results obtained with *A. holosericea* and *A. mangium* inoculated with *Pisolithus* isolates (*P. albus* and *P. tinctorius* strains) or *Scleroderma* isolates (*S. dictyosporum* and *S. verrucosum* strains) [12–14]. The results of the present study suggest clearly that *A. crassicarpa* is also highly dependent to the mycorrhizal symbiosis and that this mycorrhizal dependency is not related to their provenance. As *A. crassicarpa* is usually considered to be suitable for planting on degraded areas in the tropics and on a wide range of infertile soils, the inoculation of selected mycorrhizal symbionts should be useful in rehabilitation programs to restore sustainable ecosystems and for private companies who want to set up new industrial plantations of *A. crassicarpa*.

The symbiotic efficiency of the *Bradyrhizobium* strain Aust13C with *A. mangium* has been largely demonstrated through several studies carried out in the field [16–18]. This strain was also able to form nodules on the root system of *A. crassicarpa* and to improve its growth (Antoine Galiana, personal communication). According to these results, we decided to use it for inoculating *A. crassicarpa* in both experiments presented

Table V. Effect of microbial inoculation on endomycorrhizal establishment and on rhizobial symbiosis after 5-month culture.

Provenances	Treatments	Endomycorrhizal colonization (%)	Number of nodules per plant	Nodule dry weight (mg/plant)
P1	Not inoculated	0 a[1]	0 a	0 a
	Aust 13C	0 a	0 a	0 a
	G. intraradices	34.3 b	13 b	41 b
	G. intraradices + Aust 13C	45.2 b	17.8 b	47 b
P2	Not inoculated	0 a	0 a	0 a
	Aust 13C	0 a	0 a	0 a
	G. intraradices	60.8 b	17 b	35 b
	G. intraradices + Aust 13C	55.2 b	25.6 b	89 c
P3	Not inoculated	0 a	0 a	0 a
	Aust 13C	0 a	0 a	0 a
	G. intraradices	61.7 b	9.9 a	46 b
	G. intraradices + Aust 13C	57.2 b	25.4 b	55 b

[1] For each provenance, data in the same column followed by the same letter are not significantly different according to the one way analysis of variance ($P < 0.05$).

Table VI. Effect of endomycorrhizal inoculation and/or *Bradyrhizobium* Aust13C on leaf mineral content after 5-month culture.

Provenances	Treatments	N (mg/plant)	P (µg/plant)	K (µg/plant)
P1	Not inoculated	2.1 a[1]	38.3 a	1201.7 a
	Aust 13C	5.2 a	78.1 a	2282.0 a
	G. intraradices	36.3 b	1619.4 c	25788.9 c
	G. intraradices + Aust 13C	34.1 b	992.1 b	19261.3 b
P2	Not inoculated	2.8 a	37.8 a	1518.7 a
	Aust 13C	5.9 a	106.7 a	3349.3 a
	G. intraradices	32.5 b	1615.4 b	22568.0 b
	G. intraradices + Aust 13C	44.9 c	1703.3 b	24266.7 b
P3	Not inoculated	2.1 a	37.3 a	1114.7 a
	Aust 13C	17.7 b	352.1 b	12035.7 b
	G. intraradices	26.5 c	1113.8 c	19172.9 c
	G. intraradices + Aust 13C	35.1 d	1198.9 c	20344.2 c

[1] For each provenance, data in the same column followed by the same letter are not significantly different according to the one way analysis of variance ($P < 0.05$).

in this work. Results obtained confirmed those already obtained, especially in the first experiment carried out during the hot season. *A. crassicrapa* is described as being originated from warm and hot humid and sub-humid areas. So it means that this species is more adapted to hot than cold temperatures. This characteristic could explain why *A .crassicarpa* seedlings inoculated with Aust 13C and grew during the cold season were poorly nodulated compare to seedlings of the same treatment of the other experiment carried out during the hot season. Regarding the presence of nodules on the root system of non-inoculated plants, we know that usually, autoclaved soils are not totally sterilized. So it means that when the environmental conditions are favourable, we can observe a nodulation of these plants, nodulation usually induced by native rhizobia which persist in the soil. It may be that we observed in the experiment carried out during the hot season. We could assume also that the nodules formed on the non-inoculated plants were preferentially occupied by the strain Aust 13C rather than by the native rhizobia. No nodules analyses were done. It will impossible to conclude on this. But the Aust 13c strain is very competitive for nodulating the compatible host plant. For exemple in Madagascar, Prin et al. [32] demonstrated in an inoculation field

trial that the progressive reduction of the positive effect of Aust13C inoculation on *A. mangium* tree growth after field transplanting was not due to a progressive disappearance of the introduced strain, but on the contrary, was related to the widening spreading of Aust13c in all the plots. These authors concluded on the higher competitiveness of Aust13c over the local strains naturally present on the soil. It was not the case in our first experiment because for the three provenances, seedlings inoculated with Aust13C produced more significant biomass than the not inoculated plants (Tab. II). It suggests that the plants control and those inoculated with *Pisolithus tinctorius* only were essentially nodulated by native rhizobia. This assumption is strengthened by the fact that *A. crassicarpa* is not highly specific for nodulating with rhizobia [8]. It can easily nodulate with native rhizobia. It is interesting to note that the ectomycorrhizal fungus stimulated the nodulation of the not inoculated plants with Aust 13C. It seems to correspond to a synergistic effect. This synergistic effect on the plant growth was also recorded when *P. tinctorius* and the bradyrhizobial strain Aust 13C were co-inoculated. This ectomycorrhizal fungal symbiosis increased root growth and could facilitate rhizobial colonization and infection. Moreover, it is well established that nodule formation and functioning are dependant on mycorrhizal formation through the improvement of P uptake which enhances nodulation and N_2 fixation [7, 32]. It is also well demonstrated that the root exudation is modified both qualitatively and quantitatively by the presence of an active mycorrhizal symbiosis [2, 23, 37]. These changes could increase the rhizobial multiplication in the mycorrhizosphere.

Hardwood et al. [21] demonstrated that the Papua New Guinea provenances grew faster than the other provenances. Our results confirm these data because for both experiments, the more important total biomasses were obtained with the provenances P1 and P2 from Papua New Guinea and not with P3 from Madagascar. However, no significant difference of nodulation and mycorrhization were observed between three provenances.

In conclusion, our results demonstrated that mycorrhizal associated to rhizobial symbiosis could be a great interest in reafforestation programs where *A. crassicarpa* are used to rehabilitate degraded areas.

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