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# Arbuscular mycorrhizal colonization and nodulation improve flooding tolerance in *Pterocarpus officinalis* Jacq. seedlings

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**Abstract** *Pterocarpus officinalis* (Jacq.) seedlings inoculated with the arbuscular mycorrhizal fungus, *Glomus intraradices*, and the strain of *Bradyrhizobium* sp. (UAG 11A) were grown under stem-flooded or nonflooded conditions for 13 weeks after 4 weeks of nonflooded pretreatment under greenhouse conditions. Flooding of *P. officinalis* seedlings induced several morphological and physiological adaptive mechanisms, including formation of hypertrophied lenticels and aerenchyma tissue and production of adventitious roots on submerged portions of the stem. Flooding also resulted in an increase in collar diameter and leaf, stem, root, and total dry weights, regardless of inoculation. Under flooding, arbuscular mycorrhizas were well developed on root systems and adventitious roots compared with inoculated root systems under nonflooding condition. Arbuscular mycorrhizas made noteworthy contributions to the flood tolerance of *P. officinalis* seedlings by improving plant growth and P acquisition in leaves. We report in this study the novel occurrence of nodules connected vascularly to the stem and nodule and arbuscular mycorrhizas on adventitious roots of *P. officinalis* seedlings. Root nodules appeared more efficient fixing $N_2$ than stem nodules were. Beneficial effect of nodulation in terms of total dry weight and N acquisition in leaves was particularly noted in seedlings growing under flooding conditions. There was no additive effect of arbuscular mycorrhizas and nodulation on plant growth and nutrition in either flooding treatment. The results suggest that the development of adventitious roots, aerenchyma tissue, and hypertrophied lenticels may play a major role in flooded tolerance of *P. officinalis* symbiosis by increasing oxygen diffusion to the submerged part of the stem and root zone, and therefore contribute to plant growth and nutrition.

**Keywords** Arbuscular mycorrhizas · *Glomus intraradices* ·
Mycorrhizal adventitious roots · *Bradyrhizobium* ·
Stem nodules

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## Introduction

Much of the wetlands is subject to flooding due to the presence of shallow water tables and a decrease in surface water infiltration (Barrett-Lennard 2003). Flooding causes hypoxia or anoxia in soils because of the low solubility and diffusivity of oxygen in water and the rapid use of dissolved oxygen by microorganisms and roots. Therefore, there is a decrease in the mineralization of organic matter and an increase in the denitrification (Barrios and Herrera 1993). Moreover, heavy leaching of soils brought about by seasonal flooding contributes to the shortage of available N and P. A number of wetland plants are nodulated legumes (Walter and Bien 1989; Moreira et al. 1992; Loureiro et al. 1995; Saur et al. 1998; James et al. 2001; Koponen et al. 2003) that also associates with arbuscular mycorrhizal (AM) fungi (Sanchez-Diaz et al. 1990; Carvalho et al. 2003; Bâ et al. 2004). While the occurrence of AM fungi in wetland is well documented (Bohrer et al. 2004; Carvalho et al. 2004; Saint-Etienne et al. 2006), their importance and function are limited and often contradictory. It is not clear whether flooding modifies AM effects, as some results show an improvement in growth and P nutrition over noncolonized plants (Wigand and Stevenson 1997; Osundina 1998; Miller and Sharitz 2000; Muok and Ishii 2006; Neto et al. 2006), some showed a decrease (Stevens et al. 2002), and others did not detect a clear relationship (Hartmond et al. 1987). The lack of external input of N in ecosystems increases the demand for biological nitrogen fixation (Dommergues et al. 1999). Nodulated legumes can contribute significantly to the N balance of tropical wetlands and rainforests (Roggy et al. 1999a,b; Koponen et al. 2003; Diabaté et al. 2004). Legumes generally require P from mycorrhizas for their nodule formation, nitrogen fixation, and growth (Dommergues et al. 1999; Vance 2001). However, little is known about the interactions between AM fungi and nitrogen-fixing bacteria in legumes growing in wetland ecosystems.

*Pterocarpus officinalis* (Jacq.) (Fabaceae) is the dominant wetland tree species of the seasonally flooded swamp forests in the Caribbean basin (Eusse and Aide 1999; Imbert et al. 2000; Muller et al. 2006). It covers large areas of the coastal floodplain, and individual trees occur along rivers and in the mountains. The establishment and population maintenance of *P. officinalis* are affected by the variations in salinity and hydrology and differences in soil microtopography in swamp forests (Alvarez-Lopez 1990; Eusse and Aide 1999). This tropical wetland tree species forms bradyrhizobial nodules and arbuscular mycorrhizas on lateral roots of buttresses both above and below the water table (Saur et al. 1998; Bâ et al. 2004; Saint-Etienne et al. 2006). Alvarez-Lopez (1990) suggested that *P. officinalis* cannot establish seedlings under flooding conditions because rooting of germinated fruit did not occur in water over 3–4 cm deep. If flooding persists, only germinated seeds transported by water movement to higher elevations may survive (Alvarez-Lopez 1990). We hypothesized that nodulation and arbuscular mycorrhizas could improve the performance of *P. officinalis* seedlings under flooding. Two questions are addressed in the present study: (1) Are *P. officinalis* seedlings adapted to flooding? and (2) Do arbuscular mycorrhizas and $N_2$-fixing nodules increase the performance of *P. officinalis* seedlings under flooding?

## Materials and methods

### Fungal and bacterial inocula

The AM fungus, *Glomus intraradices* Schenck & Smith (DAOM 181602, Ottawa, Agricultural Herbarium, Canada), was propagated on leeks (*Allium porrum* L.) growing on TerraGreen™ substrate for 12 weeks on a calcined clay (Oil-Dri US-special Ty/IIIR, Oil-Dri Company, Chicago, USA) (Plenchette et al. 1996) under greenhouse conditions. The leek plants were uprooted, their roots gently washed, and cut into 0.5-cm-long pieces. Non-AM leek roots, prepared as above, were used for the control treatment.

*Bradyrhizobium* sp. (UAG 11 A) strain was isolated from a root nodule collected during the dry season from a mature *P. officinalis* tree growing in the swamp forest of Port Louis in Guadeloupe, Lesser Antilles (Bâ et al. 2004). The bacterial inoculant consisted of a 10-day-old pure culture grown on liquid yeast extract mannitol medium (Vincent 1970).

### Seed germination

Pods of *P. officinalis* were collected along the river in the swamp forest of Grande Ravine in Guadeloupe. Pods were shelled and seeds were surface-sterilized with 3% sodium hypochlorite (w/w) for 10 min. They were then rinsed several times in sterile water and germinated in sterile vermiculite at 25°C in the dark. The germinated seeds were moved into pots when tap roots were 2–3 cm long.

### Experimental design

The potting substrate was a mixture of heat-sterilized pouzzolane (crushed volcanic rock with particle size averaging 2 mm) and vermiculite (4:1, v/v). The nutrient contents (in parts per million) of the heat-sterilized crushed volcanic rock were as follows: 4.28 K, 15.67 Na, 6.36 Ca, 4.99 Mg, 1.26 $NH_4^+$, 2.75 $NO_3^-$O-extractable), and 0.12 Olsen-P [pH=8.41 ($H_2O$) and pH=7.2 (KCl), 0.11 g/l total salt and electrical conductivity 0.036 mS/cm]. Black pots

(22 cm deep, 9 cm diameter) were filled with the substrate to within 4 cm below the rim. Soil leakage was prevented by placing a wad of polyester fiber at the bottom of each pot.

The seedlings were transplanted into pots and inoculated with *Bradyrhizobium* sp. alone, *G. intraradices* alone, or with both microbial partners. A crude inoculum of 0.35 g of fresh leek roots colonized by *G. intraradices* (with about 250 vesicules $cm^{-1}$) was placed in a hole of the substrate close to the seedlings root system, or 0.35 g of non-AM leek roots for the controls. Bradyrhizobial inoculation was then performed on seedlings by spreading 5 ml ($10^9$ bacterial cells $ml^{-1}$) of a suspension of *Bradyrhizobium* sp. on tap root or 5 ml of the culture medium without bacteria for the controls. All the plants were grown under well-watered conditions without nutrients for 4 weeks in a shaded greenhouse receiving approximately 280 W $m^{-2}$ between February and June 2003, at 24–34°C with a day-length of about 12 h. Four weeks after transplanting, half of the pots went through a process of flooding. Seedlings were flooded every other day with tap water to maintain the water level 3 cm above the soil surface and to replace evaporated and/or transpired water in pots without drainage holes. Nonflooded seedlings were watered every other day to reach the field capacity in pots with drainage holes. The pots were arranged in a completely random 4×2 factorial design comprising eight treatments: (1) flooded *G. intraradices*, (2) flooded *Bradyrhizobium* sp., (3) flooded *G. intraradices* plus *Bradyrhizobium* sp., (4) flooded non-inoculated, (5) nonflooded *G. intraradices*, (6) nonflooded *Bradyrhizobium* sp., (7) nonflooded *G. intraradices* plus *Bradyrhizobium* sp., and (8) nonflooded, noninoculated. Each treatment consisted of ten replicates. The plants were supplied each month with 50 ml of Long Ashton's nutrient solution (Hewitt 1966) without P and N. Treatments were maintained for 13 weeks after flooding was performed.

## Seedling measurements and nutrient analysis

Thirteen weeks after flooding, the number of adventitious roots was assessed by counting the roots emerging from the epicotyl. Nodules were collected separately from root and stem (including adventitious root nodules) and counted.

An acetylene reduction assay (ARA) was performed on ten freshly detached nodules randomly collected on roots or stems 13 weeks after flooding was performed. Nodules were immediately sealed in serum-capped vials, filled with 10% acetylene ($C_2H_2$) in air, and incubated at laboratory temperature for 1 h. Then, gas aliquots of 10 ml were removed from the vial and injected into a "Vacutainer" for storage. Ethylene ($C_2H_4$) and acetylene concentrations of the sample were analyzed using flame ionization in a Hewlett-Packard 5890 II gas chromatography. The ARA results were considered as positive when the average $C_2H_4$ concentration after incubation with nodule was more than 1 μmol $g^{-1}$ dry nodule $h^{-1}$. Control root samples without nodules did not show concentrations above this value. Nodules were dried at 80°C for 7 days and weighed after each assay.

Height, collar diameter, and dry weight (7 days at 80°C) of leaves, shoots, roots, adventitious roots, and the remaining stem and root nodules were evaluated. AM fungal colonization of roots and adventitious roots was also measured. The roots were randomly collected for each seedling, gently washed, cleared, and stained (Phillips and Hayman 1970). Roots were then cut into 1-cm pieces, mixed, and placed on slides for microscopic observations at ×250 magnification (Brundrett et al. 1985). A hundred root pieces were observed per plant. The extent of AM colonization was expressed as a percentage of the number of mycorrhizal root pieces per number of nonmycorrhizal root pieces.

After drying, ground leaf samples were mineralized through heating at 500°C and digested in hydrochloric acid for determination of N and P. The total N contents of leaves were assessed using a Technicon AutoAnalyzer. P was determined by colorimetry with chain in continuous flow (Technicon) according to Novozamsky et al. (1983).

## Microscopy

Nodule-bearing stem portions were cut from plants, fixed overnight in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C, and rinsed in the same buffer. Thick sections (100 μm thick) of whole nodules plus the bearing stem were obtained using a Vibratome (Leica, France), cleared in sodium hypochloride and observed under an Olympus SZH stereomicroscope.

For thin sections, samples were postfixed for 1 h in 2% osmium tetroxide and rinsed in distilled water. They were dehydrated through an ethanol series followed by three washes in pure ethanol, infiltrated by a resin series of ethanol–Spurr and then embedded in 100% Spurr resin. Polymerization took place at 70°C for 48 h. Approximately 0.5- to 1-mm-thick sections were stained with 0.05% Toluidine Blue O in 1% borax and examined using a Leitz Ortholux light microscope.

## Statistical analysis

All data were subjected to a two-way analysis of variance and mean values were compared using Newman–Keuls's multiple range test (Gagnon et al. 1989). The mean number of nodules and the percentages of mycorrhizal colonization were calculated from arcsine (square root) transformed data.

## Results

After 13 weeks of flooding, hypertrophied lenticels and aerenchyma (data not shown) tissue were observed on the submerged portion of the stem above the soil line (Fig. 1a). A few thick, white, elongated, and branched adventitious roots had grown from stems (Fig. 1a).

Serial thick sections of stem nodules (Fig. 1b) clearly reveal the direct link of the peripheral nodular vascularization to the stem vascular bundles. Numerous fixation zones are visible in the nodule with cells densely filled with bacteroids (Fig. 1c).

Inoculated treatments produced significant effects on nearly all growth and mineral acquisition traits (Table 1). Flooding treatments had no significant effect on root and root nodule dry matter, number of root nodules, nitrogen fixation in root nodules, and N and P in leaves. Interactions between inoculation and flooding were significant for height, stem dry matter, number of root and stem nodules, stem nodule dry matter, AM colonization, and nitrogen fixation in stem nodules.

Inoculated treatments did not increase number and dry weight of adventitious roots (Tables 1 and 2). Collar diameter and leaf, stem, and total dry weights were generally higher under flooding than nonflooding (Table 2). *Bradyrhizobium* alone enhanced total biomass only under nonflooding. By contrast, *G. intraradices* alone stimulated total biomass under both flooding and nonflooding. There was no additive effect of *Bradyrhizobium* and *G. intraradices* on plant growth. However, *G. intraradices* alone or with *Bradyrhizobium* appeared to be more effective in increasing total biomass of *Pterocarpus* seedlings, particularly under flooding.

A few nodules were observed on roots of control plants (Table 3). However, they did not fix nitrogen efficiently when compared with inoculated treatments (Table 4). The number, dry weight, and nitrogen fixation of root nodules were nearly the same in plants inoculated by *Bradyrhizobium* sp. under both flooding and nonflooding (Tables 3 and 4).

No AM fungal colonization was noted in roots and adventitious roots of control plants (Table 3). AM structures (vesicles and hyphal coils) were found both flooded and nonflooded. *Pterocarpus* plants grown under flooding had relatively high AM fungal colonization compared to inoculated plants under nonflooding. Elongated adventitious roots also were well colonized by *G. intraradices* as they penetrated into the flooded soil (Fig. 1d and Table 3).

Nodules formed on submerged stems and adventitious roots of flooded seedlings and were able to fix $N_2$ (3–4 $\mu$mol $C_2H_4$ $h^{-1}$ $g^{-1}$ nodule dry weight) (Table 4). Root nodules also formed on both nonflooded and flooded seedlings and were capable of fixing $N_2$ (7–9 $\mu$mol $C_2H_4$ $h^{-1}$ $g^{-1}$ nodule dry weight). Nitrogenase activity was lower in stem nodules than in root nodules of seedlings

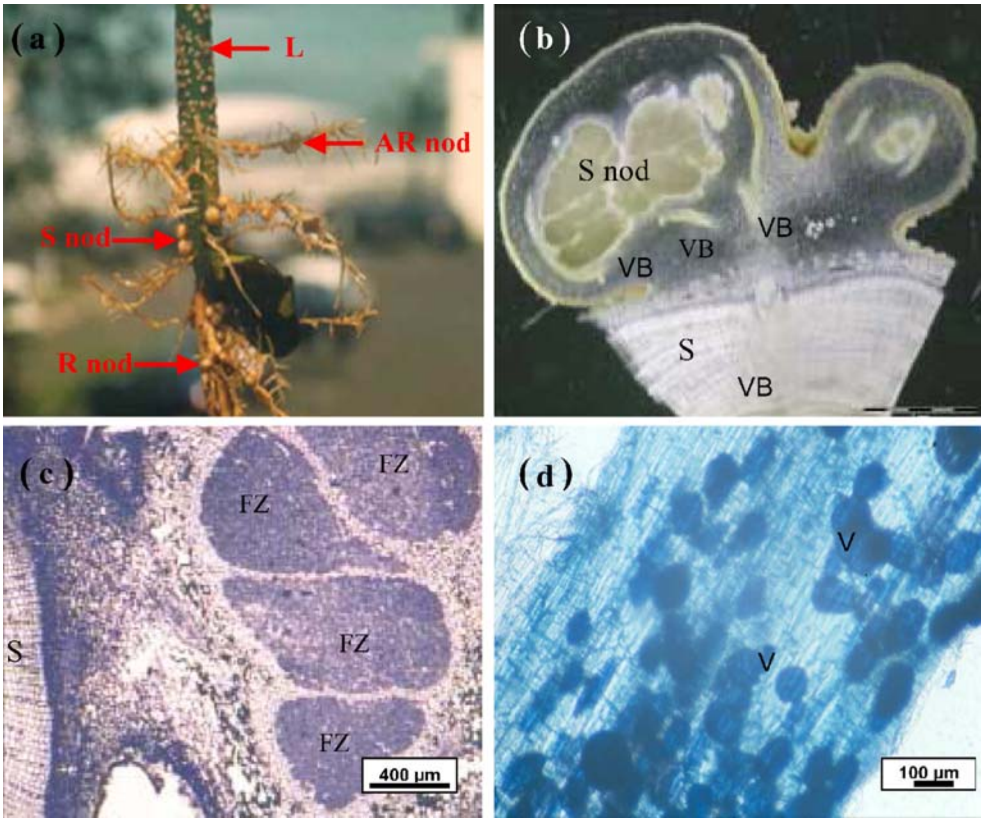

**Fig. 1** **a** Partial view of the submerged part of stem in a *P. officinalis* seedling. *L* Lenticels; *AR* nod, adventitious root nodules; *S* nod, stem nodules; *R* nod, root nodules. **b** Longitudinal sections through stem nodules of *P. officinalis*showing the direct link of the peripheral nodular vascular bundles (*VB*) to the stem (*S*) vascularization. **c** Thin sections through nodular stem nodules illustrating cells are filled with densely packed bacteroids (*FZ*). **d** Vesicles (*V*) within adventitious root

**Table 1** Significance levels for growth and nutritional parameters in *P. officinalis* seedlings at two water levels and inoculated or not with *G. intraradices* and *Bradyrhizobium* sp. alone or together

| Trait | Flooding | Inoculation | Flooding × inoculation |
|---|---|---|---|
| Height | S | NS | S |
| Collar diameter | S | S | S |
| Leaf dry weight | S | S | NS |
| Stem dry weight | S | S | S |
| Root dry weight | NS | S | NS |
| Number of adventitious roots | S | NS | NS |
| Adventitious root dry weight | S | NS | NS |
| Total dry weight | S | S | NS |
| Adventitious root colonization | S | NS | NS |
| Root colonization | S | S | S |
| Number of root nodules | NS | S | S |
| Root nodule dry weight | NS | S | NS |
| Number of stem nodules | S | S | S |
| Stem nodule dry weight | S | S | S |
| Nitrogen fixation in root nodules | NS | S | NS |
| Nitrogen fixation in stem nodules | S | S | S |
| N in leaves | NS | S | NS |
| P in leaves | NS | S | NS |

*S* Significant at $p<5\%$, *NS* not significant

inoculated by *Bradyrhizobium* under flooding. There was no difference in nitrogen-fixing root nodules of inoculated *P. officinalis* seedlings by *Bradyrhizobium* both under flooding and nonflooding (Table 4). The concentrations of N in leaves of plants inoculated by *Bradyrhizobium* compared with noninoculated plants showed that nitrogen-fixing root nodules were similarly efficient both under flooding and nonflooding. Moreover, *G. intraradices* significantly contributed to P acquisition in seedlings both under flooding and nonflooding. Nevertheless, P acquisition did not improve nitrogen-fixing stem and root nodules of *Pterocarpus* seedlings (Table 4).

## Discussion

Flooding induced several physiological and morphological changes in *P. officinalis* seedlings, including formation of hypertrophied lenticels, aerenchyma tissue, and adventitious roots on submerged portions of the stem. Flooded plants grew overall better than nonflooded ones regardless of inoculation. These results suggest that *P. officinalis* seedlings can endure 13 weeks of flooding by developing some adaptive mechanisms. The high flooding tolerance of some trees was mainly attributed to the production of adventitious roots that play a major role in water absorption and stomatal opening (Gomes and Kozlowski 1980; Crawford 1982; Liao and Lin 2001; Entry et al. 2002; Carter et al. 2005). However, we did not measure the activity of adventitious roots in the present study.

Arbuscular mycorrhiza and root nodules were formed during the first 4 weeks when *Pterocarpus* seedlings were watered to near field capacity. This means that we have, in part, assessed the proprieties of mycorrhizas and nodules already established and those of new arbuscular mycorrhizas and nodules formed under flooding. These symbiotic associations appeared to have been maintained after the substrate was flooded. Because AM fungi require oxygen to thrive, stressful regularly flooded environments may be detrimental to their survival and infectivity (Smith and Read 1997). Nevertheless, evidence that the AM fungus remained viable under flooding was provided by the

**Table 2** Effect of inoculation with *G. intraradices* (*Gi*) and *Bradyrhizobium* sp. (*Br*) on growth of *P. officinalis* seedlings under flooded conditions ($p<5\%$) (dry weight, d.w.)

| Treatments | Number of adventitious roots | Height (cm) | Adventive root d.w. (mg) | Collar diameter (mm) | Total d.w. (g) | Leaf d.w. (g) | Stem d.w. (g) | Root d.w. (g) |
|---|---|---|---|---|---|---|---|---|
| Flooded | | | | | | | | |
| Control | 4.1 b | 51.6 c | 20.1 b | 8.5 d | 5.8 bc | 1.9 b | 2.3 b | 1.6 ab |
| *Gi* | 4.6 b | 47.2 abc | 21.3 b | 8.3 d | 7.1 d | 1.9 b | 3.2 c | 1.9 b |
| *Br* | 4.1 b | 48.3 bc | 19.2 b | 7.1 bc | 6.7 cd | 1.8 b | 3.5 cd | 1.4 ab |
| *Gi* + *Br* | 4.8 b | 53.8 c | 23.4 b | 8.0 cd | 7.6 d | 2.2 b | 3.8 d | 1.6 ab |
| Nonflooded | | | | | | | | |
| Control | 0.0 a | 40.6 a | 0.0 a | 6.1 b | 3.6 a | 1.0 a | 1.5 a | 1.1 a |
| *Gi* | 0.0 a | 51.5 c | 0.0 a | 4.9 a | 5.6 bc | 1.7 b | 2.1 b | 1.8 ab |
| *Br* | 0.0 a | 47.5 abc | 0.0 a | 4.6 a | 4.7 b | 1.3 a | 1.8 ab | 1.5 ab |
| *Gi* + *Br* | 0.0 a | 42.8 ab | 0.0 a | 6.6 b | 4.8 b | 1.3 a | 2.1 ab | 1.5 ab |

Those means in a column not sharing a similar letter differ significantly at $p<5\%$ by use of the Newman–Keuls multiple range test

**Table 3** Effect of inoculation with *G. intraradices* (*Gi*) and *Bradyrhizobium* sp. (*Br*) on mycorrhizal colonization and nodulation of *P. officinalis* seedlings under flooded conditions ($p<5\%$) (dry weight, d.w.)

| Treatments | Adventitious root colonization (%) | Number of root nodules | Root colonization (%) | Root nodule d. w. (mg) | Number of stem nodules | Stem nodule d.w. (mg) |
|---|---|---|---|---|---|---|
| Flooded | | | | | | |
| Control | 0.0 a | 5.3 a | 0.0 a | 22.0 a | 2.3 b | 0.4 a |
| *Gi* | 56.2 b | 3.3 a | 66.1 d | 41.0 ac | 1.6 ab | 0.4 a |
| *Br* | 0.0 a | 44.6 bc | 0.0 a | 131.0 c | 12.1 c | 7.0 c |
| *Gi + Br* | 65.8 b | 60.3 c | 69.6 d | 162.0 c | 10.5 c | 4.0 b |
| Nonflooded | | | | | | |
| Control | – | 1.3 a | 0.0 a | 8.0 a | 0.0 a | – |
| *Gi* | – | 8.5 a | 28.6 b | 34.0 ab | 0.0 a | – |
| *Br* | – | 50.5 c | 0.0 a | 181.0 c | 0.0 a | – |
| *Gi + Br* | – | 31.8 b | 44.7 c | 112.0 bc | 0.0 a | – |

Those means in a column not sharing a similar letter differ significantly at $p<5\%$ by use of the Newman–Keuls multiple range test

increase in the proportion of colonized root and P acquisition in leaves of *P. officinalis* seedlings. The increase in AM colonization with flooding in seedlings suggests a direct relation between flooding and AM colonization. This can be explained by the development of lenticels, aerenchymatous tissue, and adventitious roots on the submerged part of the stem that could facilitate oxygen transport to support newly colonized roots. This is congruent with reports for salt marsh plants colonized by AM fungi (Brown and Bledsoe 1996; Carter et al. 2005; Neto et al. 2006). Mycorrhizal *Casuarina equisetifolia* seedlings were better adapted to flooding than noninoculated seedlings because the greater development of adventitious roots and lenticels increased oxygen availability and therefore AM colonization of plants (Osundina 1998). Accumulation of acetaldehyde, a highly toxic intermediate in ethanol formation, in roots was suggested as the main factor responsible for flooding injury in flood-sensitive species (Osundina 1998; Rutto et al. 2002). Adventitious roots and lenticels may contribute to root aeration and ethanol removal in flood-tolerant species (Crawford 1982; Liao and Lin 2001). AM colonization also contributed to suppress ethanol accumulation in the roots of peach seedlings (Rutto et al. 2002). Some studies have also suggested that once AM colonization has taken place, the AM association can endure prolonged exposure to flooding (Miller and Sharitz 2000; Neto et al. 2006). The AM symbiosis seem to have, like flooding, a stressful effect on *Aster trifolium* at an early stage of plant development (Neto et al. 2006). Once the AM symbiosis was established, the better tolerance of AM *A. trifolium* plants to flooding was mediated through an improvement of the osmotic adjustment by accumulating soluble sugars and proline in plant tissues (Neto et al. 2006).

Flooding induced nodules both on adventitious roots arising from stems and on the stems themselves. Stem nodules formed only on submerged parts of flooded seedlings to within 1 cm of the soil surface, and flooding appeared essential for their formation. It is clear that, in flooding condition, *P. officinalis* seedlings formed true stem nodules, i.e., connected vascularly to the stem and not to adventitious roots arising from the stem (James et al. 1992). The histological organization of stem nodules is similar to that of aeschynomenoid type of nodules according to Corby

**Table 4** Effect of inoculation with *Glomus intraradices* (*Gi*) and *Bradyrhizobium* sp. (*Br*) on nitrogen fixation, N and P concentrations in leaves of *Pterocarpus officinalis* seedlings under flooded conditions ($p<5\%$) (dry weight, d.w.)

| Treatments | Nitrogen-fixing root nodules (μmol $C_2H_4$/h/g nodule d.w.) | Nitrogen-fixing stem nodules (μmol $C_2H_4$/h/g nodule d.w.) | N leaves % | P leaves % |
|---|---|---|---|---|
| Flooded | | | | |
| Control | 1.75 a | 0.00 a | 1.52 a | 0.05 a |
| *Gi* | 1.83 a | 0.00 a | 1.45 a | 0.10 c |
| *Br* | 9.03 b | 3.73 b | 2.10 b | 0.06 ab |
| *Gi + Br* | 8.40 b | 4.21 b | 2.16 b | 0.11 c |
| Non-flooded | | | | |
| Control | 0.00 a | – | 1.56 a | 0.07 b |
| *Gi* | 1.88 a | – | 1.43 a | 0.11 c |
| *Br* | 8.30 b | – | 2.21 b | 0.06 ab |
| *Gi + Br* | 6.97 b | – | 2.23 b | 0.13 c |

Those means in a column not sharing a similar letter differ significantly at $p<5\%$ by use of the Newman–Keuls multiple range test

(1988), where an intercellular mode of entry takes place at the base of lateral or adventitious roots (Goormachtig et al. 2004). These observations, combined with observations made on seedlings in the swamp forests of Guadeloupe (A. M. Bâ, unpublished data), suggest that stem nodules are formed and grow on *P. officinalis* seedlings only under flooding. Hence, we report in this study the novel occurrence of stem nodules in *P. officinalis* seedlings. Stem nodulation in legumes is a relatively rare structure that was confirmed only in some nodulated hydrophytes such as *Aeschynomene*, *Sesbania*, *Discolobium*, and *Vigna* growing on seasonally or permanently flooded wetlands in Africa and South America (Dreyfus and Dommergues 1981; Alazard 1985; Loureiro et al. 1995; James et al. 2001). In our study, nitrogenase activity of stem nodules was lower than that of corresponding root nodules on *P. officinalis* seedlings. Root nodules developed and fixed $N_2$ similarly under both flooding and nonflooding conditions. Therefore, flooding did not affect root nodule formation and $N_2$ fixation as shown on *Discolobium pulchellum* (Loureiro et al. 1994). Furthermore, there was also a significant increase in N within leaves of both flooded and nonflooded inoculated plants by *Bradyrhizobium*, resulting from an increase in the $N_2$-fixing of root nodules. Nevertheless, under flooded conditions, N acquisition did not enhance total dry weight of seedlings when compared with non-inoculated controls. One possible explanation for this may be the nitrogenase activity that we detected in root nodules of controls. However, $N_2$ fixation appeared to be similar to that in swamp forests (Saur et al. 1998), but is very low compared to that of hydrophytes such as *Aeschynomene*, *Sesbania*, and *Discolobium* (Dreyfus and Dommergues 1981; Alazard 1985; Loureiro et al. 1994).

It is surprising to note that AM colonization did not increase root nodulation in *Pterocarpus* seedlings under both flooding and nonflooding. We also found that plants double inoculated with *G. intraradices* and *Bradyrhizobium* displayed a reduction of root nodulation compared with plants singly inoculated with *Bradyrhizobium* in nonflooded conditions. However, colonization of plants by *G. intraradices* did not increase ARA activity compared with plants singly inoculated with *Bradyrhizobium*. These results suggest a competitive interaction between both endophytes. *G. intraradices* was described as an aggressive AM fungus in terms of intensity of root colonization, production of vesicles and spores, and requirement for photosynthetic products (Graham et al. 1996; Ruiz-Lozano et al. 2001). This could have lead to a competitive interaction between *G. intraradices* and *Bradyrhizobium*, which resulted in a less effective combination of endophytes for root nodulation in nonflooded conditions. In this respect, Ruiz-Lozano et al. (2001) described detrimental effects on root nodulation in soybean plants after dual inoculation with *G. intraradices* and *Bradyrhizobium*.

To conclude, this study showed that infectivity and activity of the nodulated and AM plants seem to be dependent on soil flooding. Furthermore, it provides supporting evidence that AM colonization by *G. intraradices* contributed substantially to the flooded tolerance of *P. officinalis* seedlings. This could be due to the increasing $O_2$ diffusion through the greater development of adventitious roots, aerenchymatous tissue, and hypertrophied lenticels on the root zone and submerged part of the stem. It is therefore possible that AM colonization and nodulation may contribute to the establishment of *P. officinalis* along a wider range of soil flooding levels in swamp forests. However, further experimental investigations should be done to understand the mechanisms by which AMF increases AM colonization of *P. officinalis* seedlings in flooding and its adaptive significance.

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