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Differences in nitrogen metabolism of *Faidherbia albida* and other N_2-fixing tropical woody acacias reflect habitat water availability

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SUMMARY

The activities of nitrate reductase and glutamine synthetase were evaluated in young plants of *Faidherbia albida*, a tropical woody legume, fed with different N sources under hydroponic conditions. Results showed that assimilation of both NO_3^- and NH_4^+ preferentially took place in shoots. A basal amount of nitrate reductase activity was detected in shoots of plants grown with an NO_3^--free solution or placed under N_2-fixing conditions, and also in nodules of N_2-fixing plants. This strongly suggests that constitutive nitrate reductase activity is present in these organs. Analyses of the soluble nitrogenous content showed that the major form of N in the different organs was α-amino acids (particularly amides), irrespective of the N status of the culture conditions. The same result was obtained for nodulated plants grown in local sandy soil. In this case, amide-N generally accounted for more than 40% of the total soluble N. This was especially true in nodules. Ureide-N never exceeded 9% of the total soluble N and did not appear to increase with increasing nodule nitrogenase activity. Amides were also predominant in three N_2-fixing Sahelian acacias (*Acacia seyal*, *A. nilotica* and *A. tortilis*), showing that *F. albida* does not differ from Sahelian *Acacia* in terms of the metabolism of fixed N. However, like another Sahelian acacia growing preferentially near water (*A. nilotica*), *F. albida* can be distinguished from acacias growing strictly in arid zones (*A. seyal* and *A. tortilis*) in terms of initial growth, water and nitrate management.

Key words: *Acacia*, amides, *Faidherbia albida*, glutamine synthetase, nitrate reductase, N fixation, Sahel, ureides.

INTRODUCTION

Faidherbia albida is currently integrated into the agroforestry systems of arid and semi-arid zones of Africa. Like *Acacia* trees, *F. albida* is of considerable interest not only for wood, firewood and forage production, but also for its potential contribution to the restoration of soil fertility through litter and biological N_2 fixation (Giller & Wilson, 1991). This tree was formerly assigned to the genus *Acacia* (*Acacia albida* Del., Acaciaeae tribe). In view of some of its botanical and biochemical characteristics (Chevalier, 1934; Evans *et al.*, 1977), Vassal (1981) suggested creating in the tribe the new genus *Faidherbia*. However, chloroplast DNA studies recently indicated that *Faidherbia* is more closely related to the subgenus *Aculeiferum* from the genus *Acacia* (Bukhari *et al.*, 1999). *Faidherbia albida* has an inverted phenological cycle, bearing leaves during the dry season, and acquires its potential to fix N_2 generally by association with *Bradyrhizobium* rather than *Rhizobium* strains (Dreyfus & Dommergues, 1981; Dupuy & Dreyfus, 1992; Assefa & Kleiner, 1998). This association confers a low N_2-fixing potential (<30 g N_2 fixed per tree per year) on *F. albida* (Sanginga *et al.*, 1990; Ndoye *et al.*, 1995; Gueye *et al.*, 1997), particularly when growing in arid zones (Dommergues, 1995). The potential to fix N_2 has also been described for other Sahelian acacias

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but, as in *F. albida*, the pathway by which fixed N is assimilated has not yet been studied. Some tropical legumes, generally members of the Phaseoleae tribe, export fixed N from their nodules in the form of ureides such as allantoin and allantoic acid, and have a drought-sensitive N_2 fixation (Sinclair & Serraj, 1995). The others, like temperate legumes (Sprent, 1980), transport fixed N in the form of amides such as asparagine and glutamine (Yoneyama & Kondo, 1990; Peoples *et al.*, 1991). Some tropical woody legumes have been examined for their soluble N content. Among these, Australian *Acacia* spp. generally appeared to be amide-exporters (Hansen & Pate, 1987; Van Kessel *et al.*, 1988). Similarly, mineral N assimilation, and more especially the partitioning of NO_3^- reduction and NH_4^+ assimilation between shoots and roots, have not yet been defined for *F. albida* and Sahelian acacias. In woody plants, glutamine synthetase (GS, EC 1.6.3.2) is considered the key enzyme of NH_4^+ assimilation (Stewart *et al.*, 1989), as is nitrate reductase (NR, EC 1.6.6.1) for NO_3^- assimilation (Miflin & Lea, 1980). It has been demonstrated that the glutamate synthase cycle (GS/GOGAT) accounted for most NH_4^+ assimilation even if substantial glutamate dehydrogenase (GDH) activity is observed in, for example, roots and tissues under stress conditions (Smirnoff & Stewart, 1987; Srivastava & Singh, 1987). The present paper reports the activities of NR and GS in *F. albida*. It also describes the accumulation of soluble nitrogenous compounds in this plant, with emphasis on the partitioning of mineral N assimilation and the characterization of the pathway of fixed N assimilation in comparison with three other Sahelian acacias.

MATERIALS AND METHODS

Seeds of *Faidherbia albida* (Del.) A. Chev. (synonym *Acacia albida* Del.); *Acacia nilotica* (L.) Willd. ex Del. ssp. *tomentosa* (Benth); *Acacia seyal* (Del.) var. *seyal*; and *Acacia tortilis* (Forssk.) ssp. *raddiana* (Savi) were collected from natural parklands throughout semi-arid and arid zones of Senegal.

Bradyrhizobium strain ORS 188 (Lab. de Microbiologie des Sols, IRD, Dakar, Senegal), isolated from *F. albida*, was selected for its high N_2-fixing efficiency (Dupuy & Dreyfus, 1992) and used to inoculate *F. albida*. *Rhizobium* strain ORS 1073, isolated from *A. senegal*, was used to inoculate the other *Acacia* species, with which it is usually associated. Cultures in liquid medium (Vincent, 1970) were obtained for inoculation.

Growth conditions

Acacia seeds were surface-sterilized (36 M H_2SO_4, 30 min) and then washed in sterilized H_2O and germinated for 72 h at 30°C in the dark prior to transplanting in a glasshouse under natural daylight conditions.

A hydroponic system was used in the first study with *F. albida* (Table 1; Fig. 1). Pre-germinated seeds (24 for each assay) were transferred to 80-l culture vessels containing N-free Hoagland solution (Hoagland & Arnon, 1950). For plants fed with mineral N, the culture medium was primarily and regularly enriched with KNO_3 or NH_4NO_3 solution to maintain a constant concentration of 3 mM. For inoculated plants, 50 ml of a liquid culture (10^8 cells) of *Bradyrhizobium* strain ORS 188 were added to the N-free medium immediately after seed transplanting. Ten plants were collected for each assay following 60 d of culture.

For the other experiments, plants were grown in local sandy soil. Pre-germinated seeds were planted in 30 pots (three seedlings per pot) filled with 2 kg of a 1/3 (v/v) mixture of polystyrene balls and sterilized local soil (1.9% total C and 0.025% total N, d. wt basis) containing 1.3 ± 0.4 mM NO_3^- and 0.11 ± 0.03 mM NH_4^+. Inoculation was performed (1 ml, 10^8 cells) on two occasions, immediately and 7 d after planting. Pots received 100 ml of sterilized water daily. In order to follow soluble N accumulation during growth, *F. albida* was maintained under these conditions for 8 months. However, growth for 2–3 months sufficed for studies on the effects of different concentrations or for comparisons of different *Acacia* species. For supplementation with N, 10 pots received daily 100 ml of 3 mM KNO_3 whilst another 10 received 3 mM NH_4NO_3. Controls were inoculated as described previously and received daily 100 ml of sterilized water.

Analytical procedure

In experiments involving different concentrations of N, nine plants were collected every month. In other experiments, nine plants were collected after 2 months of culture. Five of these plants were then used for soluble-N analyses and the others for evaluation of N_2 fixation using the acetylene reduction assay (ARA).

Leaves, stems, roots and nodules were separated, weighed and stored at -80°C until analysis. For assays of NR and GS, soluble proteins were extracted at 4°C by a modified version of the technique of O'Neal & Joy (1973). Samples (0.5 g for roots and leaves) were ground in a frozen mortar with 2 ml of 50 mM Tris–HCl (pH 8.4) containing 1 μM Na_2MoO_4, 10 μM FAD, 1 mM Na_2EDTA, 5 mM cysteine, 1 μM leupeptin, 11 mM mercaptoethanol and 1 mM dithiothreitol (DTT) in the presence of polyvinylpyrrolidone (PVP 10, 1% w/v). After centrifugation (15 000 ***g***, 20 min, 4°C), nitrate reductase (NR, EC 1.6.6.1) and glutamine synthetase (GS, EC 6.3.1.2) activities were determined as

Table 1. *Nitrogen content and activities of nitrate reductase (NR) or glutamine synthetase (GS) in crude extracts of* Faidherbia albida *cultivated in hydroponic conditions with N-free medium, with medium containing 3 mM KNO_3 or 3 mM NH_4NO_3, or with N-free medium but inoculated with* Bradyrhizobium *strain ORS 188*

Organ	Treatment	F. wt (g)	N content (mg g^{-1} f. wt)		Enzyme activity (μmol h^{-1} g^{-1} f. wt)	
			Total	Soluble	NR	GS
Shoot	N-free	2.66a	4.50b	0.21b	0.48b	208.5a
	KNO_3	4.14a	10.05a	0.34a	2.18a	225.1a
	NH_4NO_3	4.37a	10.62a	0.33a	1.71a	235.2a
	Inoculated	3.47ab	8.70a	0.27ab	0.69b	238.3a
Root	N-free	2.55a	3.69b	0.20	—	7.1
	KNO_3	2.87a	5.47a	0.43a	0.37a	13.4a
	NH_4NO_3	3.09a	5.87a	0.41a	0.27a	17.4a
	Inoculated	2.77a	5.42a	0.28ab	—	9.9
Nodule	Inoculated	1.12	=	0.19	0.09	73.4

Plants were harvested 2 months after planting. For each organ, values of the same column (means of two replicates, each of 10 plants) followed by the same letter do not differ significantly at $P \leqslant 0.05$ according to one-way ANOVA.
—, not detected (< 0.01 μmol NO_2^- h^{-1} f. wt); =, not measured.

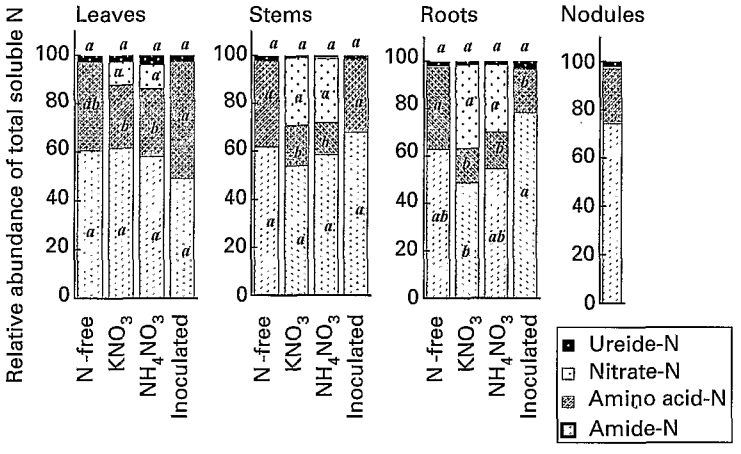

Fig. 1. Soluble nitrogenous compounds in different organs of *Faidherbia albida* maintained hydroponically for 2 months with different sources of N. The data are expressed as relative abundance (percentage of total soluble N) on a molar basis, and compared between treatments: plants incubated without any source of N; plants inoculated with *Bradyrhizobium* ORS 188; or plants fertilized with 3 mM KNO_3 or NH_4NO_3. For the same organ, values (means of two replicates of 10 plants each) followed by the same letter do not differ significantly at $P \leqslant 0.05$ according to one-way ANOVA.

described by Wallace (1986) and O'Neal & Joy (1973), respectively. Extraction was performed in duplicate and measurements were made in triplicate.

Soluble nitrogenous compounds were extracted according to the method of Yoneyama & Kondo (1990) modified by adding PVP 10 (1% w/v) during grinding (Ultra-Turrax, IKA, Staufen, Germany) in hot distilled water (5 ml g^{-1} for roots, leaves or stems and 1 ml g^{-1} for nodules). The extracts were filtered (Miracloth, CalBiochem Inc., La Jolla, USA) and centrifuged for 20 min at 15000 ***g***. Samples of xylem sap were obtained by introducing shoot cuttings into a pressure chamber (PMS Instrument Co, Corvallis, USA). Exudation was obtained by exerting a pressure of 3.5 MPa for 1 min and xylem sap was collected in microtubes, kept cold and then deep-frozen at −80°C. The ureide content (allantoin plus allantoic acid) was measured colorimetrically (Young & Conway, 1942). The total content of α-amino acids was determined by the ninhydrin method (Rosen, 1957) and amides (glutamine plus asparagine) were assayed using the method of Mitchell (1972), as modified by Boddey *et al.* (1987). Non-amide α-amino acid content was calculated as the difference between amide and α-amino acid content. Nitrate was estimated by the sulfanilamide technique (Snell & Snell, 1949). The corresponding total soluble N was expressed as μmol N per g f. wt or per plant, and calculated assuming that nitrate, ureides, amides and non-amide α-amino acids contain 1, 4, 2 and 1 atom N per molecule, respectively. The relative abundance of N in each compound was expressed as a

percentage (100 × (N in each compound/total soluble N)) as described previously (Van Kessel *et al.*, 1988). Nitrogenase (EC. 1.18.6.1) activity was measured by ARA in triplicate (Hardy *et al.*, 1973) as described by Dupuy and Dreyfus (1992). Total N content (including NO_3^-) was determined by a modified Kjeldahl method using oven-dried (70°C for 3 d) samples harvested at the beginning and end of each experiment (Eastin, 1978). Water content was expressed as a percentage and calculated as 100 × ((f. wt − d. wt)/f. wt). Data were statistically analysed by one-way ANOVA and individual means were compared using Fisher's test ($P \leqslant 0.05$).

RESULTS

Nitrogen assimilation and soluble nitrogen forms in different culture conditions

Nitrogen content was evaluated in *F. albida* after 2 months of culture with different sources of N in hydroponic systems. Statistically meaningful differences in growth and N content were seen only in plants completely starved of N (Table 1). Nitrogen deprivation also impaired NR activity and, except in shoots of inoculated plants, GS activity. Nitrate reductase activity was undetectable in roots of plants deprived of combined N whether or not they were fixing N_2. However, a basal level of NR activity was detected in the shoots and nodules of the same plants. Glutamine synthetase and NR activities were always lower in roots than in shoots, regardless of N source. Analysis of the relative abundance of N within soluble-N compounds showed that the majority of N was in the form of amide, whatever the organ and regardless of culture conditions (Fig. 1). Only 1–4% of N was in the form of ureides. The highest percentages of ureide-N were observed in roots of inoculated plants, but in leaves of the other plants. Plants fed mineral N contained high concentrations of NO_3^-, particularly in roots and stems, where NO_3^- accounted for more than 25% of the total soluble N.

Changes in the distribution of N among soluble compounds were studied using young seedlings of *F. albida* cultivated for 8 months in local sandy soil and inoculated at days 0 and 7 with *Bradyrhizobium* ORS 188. In these culture conditions, roots and aerial parts (expressed as f. wt) showed nearly identical growth curves (Fig. 2). Nodules rapidly appeared on the root collar and increased in number and weight from the second week to the sixth month after inoculation. From the first month after inoculation, N_2 fixation was detectable by ARA and increased sharply after 6 months of growth. Except for leaves after 2 months' culture, more than 80% of soluble N was in the form of amino acids (Fig. 3). Among these, amide-N dominated. In all extracts, ureides were present at low concentrations. The highest

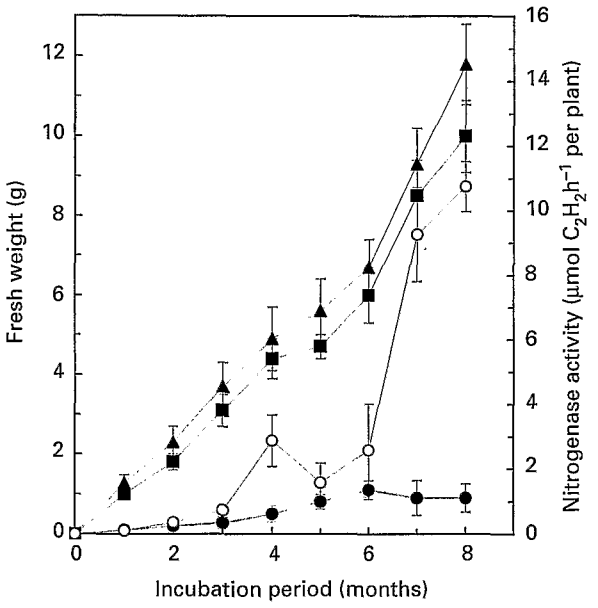

Fig. 2. Growth and nodule nitrogenase activity (acetylene reduction: ARA) of *Faidherbia albida* seedlings inoculated with *Bradyrhizobium* ORS 188. Filled circles, nodule; filled squares, root; filled triangles, shoot; open circles, ARA. Values are means ± SD of two replicates.

concentrations of ureide were noted in leaves, but even in these organs the relative abundance of N in ureides never exceeded 9%. During the first months of culture, NO_3^- accounted for a relatively high percentage of the soluble N, particularly in nodules and leaves of 2-month-old plants. At this time, NR activity was detectable in roots but, like root GS activity, represented less than 10% of the enzyme activity in the entire plant (Table 2).

Comparison with other Sahelian acacias

The content of soluble N in 3-month-old Sahelian *Acacia* species (*A. nilotica*, *A. seyal* and *A. tortilis*) inoculated with a *Rhizobium* strain (ORS 1073) was analysed and compared with that of *F. albida* at the same stage, cultivated in the same conditions and inoculated with *Bradyrhizobium* strain ORS 188. Although no significant difference was observed in nodule growth between these species, evaluation of nodule nitrogenase activity by ARA (Table 3) and measurement of total N (Fig. 4) showed that the effectiveness of N_2 fixation was significantly lower in *F. albida* and *A. nilotica* than in the other species. These two species also differed from the others in their low total dry matter weights, high NO_3^- contents and slightly higher water content, particularly in leaves and stems. However, when these parameters were evaluated in the entire plant, *A. nilotica* and *F. albida* generally appeared similar to *A. seyal* and *A. tortilis*. Similarly, no distinction between *F. albida* and the other species could be made in terms of total soluble N or in the contents of amino acids and ureides. Ureide content never

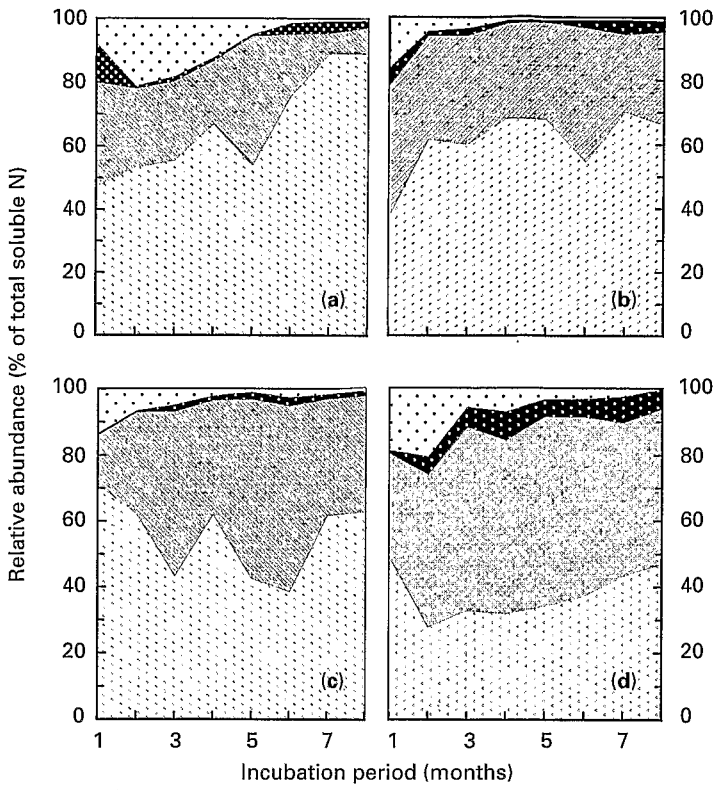

Fig. 3. Variation in soluble nitrogenous compounds in different organs of young *Faidherbia albida* inoculated (at zero time and again at 0.25 months) with *Bradyrhizobium* ORS 188. Relative abundance of N in amides, other amino acids, ureides and nitrate in (a) nodule, (b) root, (c) stem and (d) leaf extracts. Values represent means of two replicates, each of five plants.

Table 2. *Activities of nitrate reductase (NR) and glutamine synthetase (GS) in crude extracts from* Faidherbia albida *inoculated with* Bradyrhizobium *strains ORS 188 and cultured in sandy soil*

		Enzyme activity (μmol h^{-1} g^{-1} f.wt)	
Organ	F. wt (g)	NR	GS
Shoots	2.45±0.48	1.16±0.04	207.3±35.9
Roots	2.04±0.35	0.08±0.05	11.5±3.9
Nodules	0.20±0.09	0.10±0.07	43.6±15.4

Values are means±SD of two replicates of five plants harvested 2 months after planting.

Table 3. *Nodule fresh weight and nitrogenase activity of 3-month-old Sahelian* Acacias

Species	Nodule f. wt (g)	ARA (μmol h^{-1} g^{-1} f. wt)
Faidherbia albida	0.28±0.05	2.82±0.75
A. nilotica	0.27±0.05	2.58±0.92
A. seyal	0.43±0.15	6.30±1.13
A. tortilis	0.32±0.07	5.63±1.61

Faidherbia albida was inoculated with *Bradyrhizobium* strain ORS 188, the other acacias with *Rhizobium* strain ORS 1073. Nitrogenase activity was evaluated by the acetylene reduction assay (ARA) and values represent means±SD of three replicates each of four plants.

exceeded 1.4 μmol g^{-1} d. wt matter, and accounted for less than 5% of total soluble N in all species studied. Like *F. albida*, Sahelian *Acacia* contained high concentrations of amino acids and very low concentrations of ureides.

DISCUSSION

In young trees of *F. albida* grown either hydroponically or in sandy soil, assimilation of mineral N (NO_3^- reduction and NH_4^+ assimilation) preferentially occurred in shoots, as indicated by comparing the activities of NR and GS in roots and shoots. Nitrate reductase activities were lower in the leaves and roots of *F. albida* inoculated with the N_2-fixing *Bradyrhizobium* strain ORS 188 than in plants fed mineral N. However, in both cases the shoot : root NR ratio was always >5. This type of NR partitioning is often observed in tropical and subtropical species including legumes such as *Glycine max* and *Phaseolus vulgaris* (Andrews, 1986) and woody species such as *Ficus exasperata* or *Guierra senegalensis* (Stewart *et al.*, 1989). Wallace (1986) showed that herbaceous Phaseoleae of tropical origin carried out only 10% of their NO_3^- reduction in roots and preferentially accumulated NO_3^- in shoots. The distribution of NR activity in roots and shoots has previously been compared in 46 legume species (Woodall & Forde, 1996). Greater activity of NR

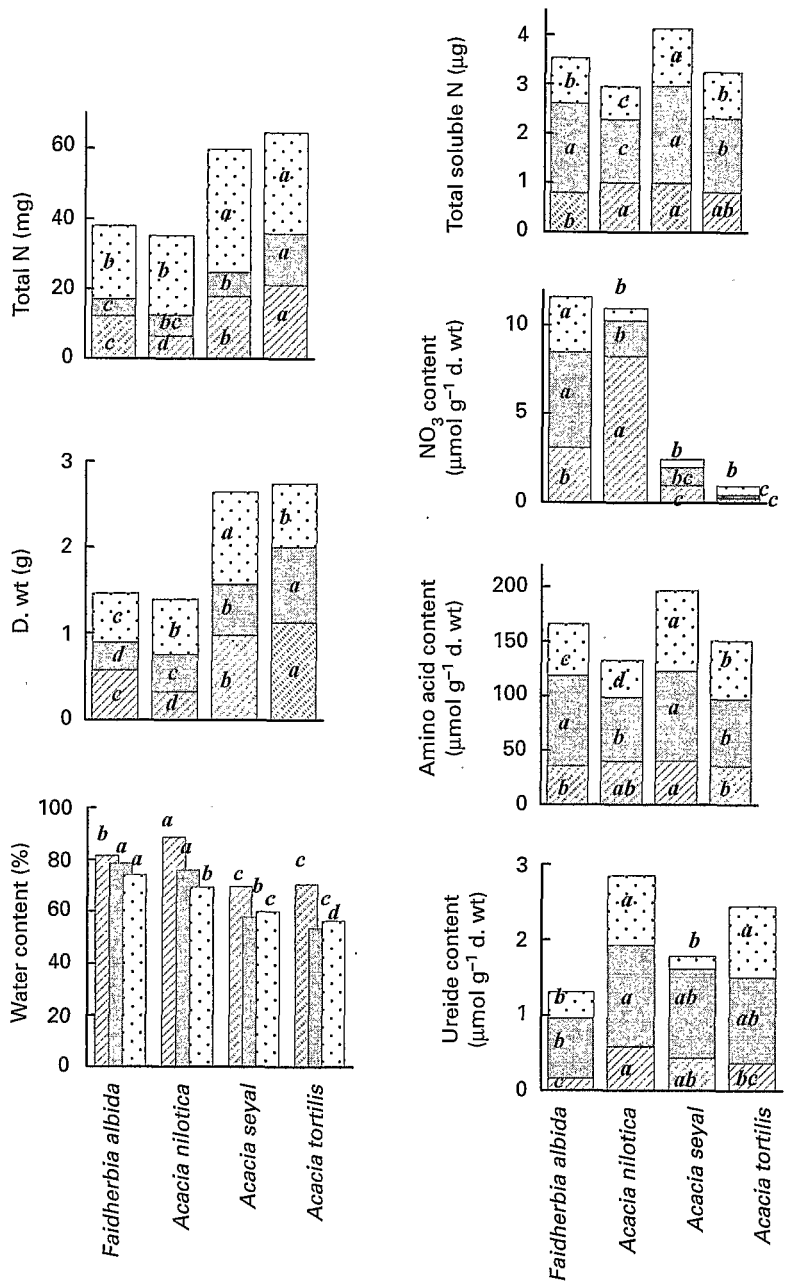

Fig. 4. Comparison of d. wt, water content and total and soluble N in organs of N_2-fixing acacias after 3 months growth . *Acacia* spp. were inoculated with *Rhizobium* ORS 1073 and *Faidherbia albida* with *Bradyrhizobium* ORS 188. Leaves, dotted bars; stems, shaded bars; roots, hatched bars. For the same organ, values (means of two replicates, each with five plants) followed by the same letter do not differ significantly between species at $P \leqslant 0.05$ according to one-way ANOVA.

was detected in shoots of all the tropical trees and shrubs examined, such as *Mimosa pudica* (tribe Mimoseae) and *Albizia julibrissin* (tribe Ingeae), two members of the Mimosoideae family. By contrast, Andrews *et al.* (1990) found that in two other Mimosoideae, *Acacia raddiana* (syn. *tortilis*) and *Mimosa acanthocarpa*, NR activity was greater in roots. Under our experimental conditions, *F. albida* exhibited greater NR activity in shoots than in roots. In addition, a basal rate of NR activity was detected in shoots of plants grown either in NO_3^--free solution or under N_2-fixing conditions. This result strongly suggests that constitutive NR activity is present in shoots of *F. albida*. The discrepancy between our results and those of Andrews *et al.* (1990) can be explained either by different growth conditions or by species-specific characteristics in terms of NO_3^- reduction. Interestingly, constitutive NR activity was also detected in nodules of *F. albida*, as already reported for other legume species (Deroche & Babalar, 1987).

With regard to NO_3^- content and its partitioning between roots and shoots, *F. albida* is different from one subspecies of *A. nilotica*, which accumulates

NO_3^- only in roots (Van Kessel *et al.*, 1988). *Faidherbia albida* also differs from *A. seyal* and *A. tortilis*, which contain very low amounts of NO_3^- in all organs.

It is well known that NO_3^- influx depends upon a transport system induced by NO_3^- (Siddiqi *et al.*, 1990). Following uptake, NO_3^- can either be translocated via the xylem to the shoots, or stored in the vacuole. NO_3^- can be reduced to nitrite (NO_2^-) or released to the external medium through an efflux system (Redinbaugh & Campbell, 1991; Crawford, 1995). In shoots of young *F. albida*, accumulation of NO_3^- and the presence of constitutive NR activity might both represent an adaptation of the plant to a shortage of mineral N at the surface of the soil in its natural habitat. This strategy might allow more efficient assimilation of N when availability of NO_3^- is limited. In addition, the high NO_3^- content in roots of *F. albida* is correlated to an inhibition of nodulation (Diouf *et al.*, 1998), thus explaining the low capacity of the species for fixation of atmospheric N_2. Then, as already suggested (Dommergues, 1995), the soil improvement observed under the canopy of *F. albida* (Vandenbeldt, 1992) might be linked more to its ability to restore soil fertility through its litter composition than to its capacity for N_2 fixation.

Despite the characteristics of its N_2-fixation system (a symbiotic association with *Bradyrhizobium* rather than *Rhizobium* and a low capacity for N_2 fixation), we demonstrate for the first time that *F. albida* is an amide-exporter like most of the tropical leguminous trees from the Aeschynomeneae, Sesbanieae, Crotalarieae or Acacieae tribes (Van Kessel *et al.*, 1988; Yoneyama & Kondo, 1990). We have also shown that amides were predominant in other Sahelian acacias such as *A. nilotica*, *A. seyal* and *A. tortilis*, suggesting that in these three closely related species, these metabolites are preferentially used for N export.

It is also worth stressing that both *F. albida* and *A. nilotica* contain more water and more NO_3^- than the two other Sahelian species in this study. This can be explained by the ability of the latter to grow in semi-arid or arid conditions, whereas *F. albida* and *A. nilotica* are adapted to more humid areas. However, *F. albida* is more extensively distributed than *A. nilotica*, because of the great diversity in its water-use efficiency (Roupsard *et al.*, 1998) and because of its capacity to form deep roots that allow efficient groundwater absorption (Dupuy & Dreyfus, 1992).

It is well known that amide-transporters require less water than ureide-transporters to transport combined N, and that amides are more water-soluble than ureides (Schubert & Boland, 1990). It would therefore be an advantage for amide-transporting species to colonize arid regions, ureide-transporting species being more adapted to areas of greater water availability (Schubert & Boland, 1990). The fact that species transporting ureides are more susceptible to drought also supports this idea (Sinclair & Serraj, 1995). The finding that Sahelian acacias adapted to arid or semi-arid conditions are amide-transporters fits well with these previous observations, suggesting that these characteristics are adaptations to adverse environmental conditions such as water deficit.

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