# Active $N_2$ Fixation in Several *Faidherbia albida* Provenances

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*For tree legumes,* Faidherbia albida *is among those with low symbiotic capability, and for which significant improvement is needed. An experiment was conducted in the greenhouse, using the* $^{15}N$ *isotope dilution technique to measure differences in the proportion (%Ndfa) and total N fixed in seven* F. albida *provenances grown in an Arenosol soil in Senegal. Total dry matter and N yield values were not significantly different among the seven provenances. In contrast, significant variability was mea-*

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*sured in $N_2$ fixation, with %Ndfa ranging from 0% in two provenances to 38% in the Kabrousse provenance. Also, total N fixed was highest in Kabrousse, 0.44 g N $plant^{-1}$, compared with the 0 to 0.26 g $plant^{-1}$ fixed in the other provenances. Based on these results, Kabrouse was selected for further examination in the field, where still higher %Ndfa values were obtained, 54, 58, and 54% at 3, 6, and 15 months after transplanting (MAT), respectively. These are higher than the commonly reported values of %Ndfa for* F. albida, *and indicate the potential of screening as a tool for obtaining higher $N_2$ fixation in NFTs. Total N fixed, unlike %Ndfa, which remained fairly constant during the three harvest periods, increased from 0.54 at 3 MAT to 1.41 g N $plant^{-1}$ at 15 MAT.*

**Keywords** *Bradyrhizobium* symbiosis, *Faidherbia albida* provenances, isotope dilution, $^{15}N$, $N_2$ fixation, *Parkia biglobosa*, reference tree, semiarid lands

Many soils in the tropics are either inherently infertile, or lose their fertility rapidly with crop removal. The growth of trees, in particular trees able to host rhizobia in their roots for atmospheric nitrogen fixation and called "nitrogen fixing trees (NFTs)," has been shown to enhance the restoration of soil fertility (Danso et al., 1992). For Senegal and much of the semiarid soils for which loss of soil fertility is a very disturbing factor, *Faidherbia albida* (Del) A. Chev. (syn. *Acacia albida*), a commonly occurring NFT in this region, has been examined for its role in restoring soil fertility (Charreau & Vidal, 1965). However, results reported by Ndoye et al. (1995) indicated suboptimal levels of $N_2$ fixation in *F. albida.* Similar results of low $N_2$ fixation in this NFT have been provided by Sanginga et al. (1990a and b). Research to improve the $N_2$ fixation capacity of *F. albida* is therefore necessary. For example, the demonstration of large genotypic variability existing in many NFTs has made it possible to select genotypes with enhanced $N_2$-fixing ability (Gauthier et al., 1985; Sougoufara et al., 1987; Liyanage et al., 1994). For NFTs, Sanginga et al. (1990b) and Ndoye et al. (1995) have examined and reported high genetic variability in the $N_2$ fixation abilities of different species, thereby offering hope for greater benefits in different farming systems. Sanginga et al. (1990b) further suggested that high growth potential is essential for enhanced $N_2$ fixation in *F. albida,* in support of similar observation for *Gliricidia sepium* (Jacq.) Steud (Liyanage et al., 1994). However, up to now, no experiment has been designed to estimate specifically the active nitrogen fixed in *F. albida.* Our study aims at assessing for the first time the N fixed in field conditions in a selected *F. albida* provenance with higher-than-average growth and $N_2$ fixation ability, using the $^{15}N$ isotope dilution technique.

## Materials and Methods

### *Experiment 1*

A greenhouse experiment was carried out at Dakar (Bel-Air experimental station, latitude 14° 44′ N; longitude 17° 30′ W) on an unsterilized sandy soil classified as an Arenosol in the USDA soil taxonomy (Soil Survey Staff, 1987). The pH was 7.0. This soil contained approximately $10^2$ native *Bradyrhizobium* $g^{-1}$ counted by infection test method using *F. albida* seedlings (Brockwell, 1982), 19 g C $kg^{-1}$, 0.25g N $kg^{-1}$, and 93% sand. The soil was sieved (<1 mm) and homogenized, and 20-kg portions were weighed into 30-cm diameter pots. To each pot, 1 g dry $K_2HPO_4$ was then added. Seeds of *F. albida* and *Parkia biglobosa* (Jacq) Benth. were scarified by immersion in concentrated sulfuric acid for 30 and 60 min, respectively, after which they were pregerminated for 2 days in petri dishes containing 0.8% soft agar. Seedlings were then transplanted, one per pot, followed by inoculation with a liquid inoculum of *Bradyrhizobium* strain ORS 136 from ORSTOM soil microbiology laboratory (10 mL $pot^{-1}$) containing $10^9$ cells $mL^{-1}$. There were a total of eight treatments, consisting of non-fixing *P. biglobosa* reference tree plus the following seven provenances of *F. albida:* Merina, Dangalma, Ndiongolor, Pire, and Kabrousse from Senegal, and Gomblora and Dem from Burkina Faso, each replicated five times. A solution of $(NH_4)_2SO_4$ containing 10.01 atom% $^{15}N$ excess was applied to supply 0.2 g N $pot^{-1}$. The pots were arranged randomly and watered daily to approximately field capacity (about 14% vol/wt). The plants were harvested 6 months after planting (MAP).

### *Experiment 2*

A field experiment was carried out at Mbao forestry station, 25 km east of Dakar. The soil was a sandy soil type and classified as an Entisol in the USDA soil taxonomy (Soil Survey Staff, 1987) with a pH of 7.6, 1 g C $kg^{-1}$ and 0.1 g N $kg^{-1}$. Scarified seeds of *P. biglobosa* and *F. albida* from Kabrousse were pregerminated as indicated in Experiment 1. After 2 days they were transplanted into plastic pouches (one seedling per pouch), and each pot was immediately inoculated with a 10-mL suspension of *Bradyrhizobium* strain MAO 232 containing $10^9$ cells $mL^{-1}$. This strain from West Africa MIRCEN *Rhizobium* collection was selected for its demonstrated $N_2$-fixing effectiveness on *F. albida* in preliminary comparison with other strains including ORS 136. After 2 months of growth in the nursery, all plants were transplanted into the field in a randomized completed block design with four replicates. The size of each plot was 3 m × 2 m, with 1 m spacing between planting holes. Within each plot, a 2 m × 1 m subplot was demarcated for the application of $^{15}N$-labeled fertilizer solution, $(NH_4)_2SO_4$ containing 9.60 atom% $^{15}N$ excess at the rate of 20 kg N $ha^{-1}$. Unlabeled $(NH_4)_2SO_4$ was applied at the same rate to the remaining trees outside the subplot. A basal fertilizer was then applied to each planting hole at the rate of 100 kg P $ha^{-1}$ as triple superphosphate and 42 kg K $ha^{-1}$ as KCl.

At 3, 6, and 15 months after transplantation, the two plants within each subplot were harvested.

### *Plant Analyses*

The harvested plant materials in both experiments were separated into different plant parts. Nitrogen (%N) and atom% $^{15}N$ excess were determined at the International Atomic Energy Agency (IAEA) laboratory in Seibersdorf, Austria, using an automated nitrogen analyzer coupled to a mass spectometer (Crasswell & Eskew, 1991). Nitrogen fixation was calculated using the isotope dilution equation (Fried & Middelboe, 1977). Data were statistically analyzed using the Newman and Keuls test.

## Results

### *Genetic Variability in $N_2$ Fixation Between* F. albida *Provenances in the Greenhouse*

There were significant differences between *F. albida* provenances in dry matter, nitrogen yield, atom% $^{15}N$ excess, and also in nitrogen fixation (Table 1). Although the provenance from Kabrousse had the highest total dry matter yield, it was not significantly different from the other provenances. This is in contrast to the leaves and stems, for which significant differences were found among provenances; for both parameters, the Kabrousse

**Table 1**

Dry weight, total nitrogen, %N15 atom excess, proportion (%Ndfa), and amount (Ndfa) of fixed nitrogen in different plant parts of seven *Faidherbia albida* provenances cultivated in pots containing 20 kg of non-sterile soil using *Parkia biglobosa* as the reference tree

| Plant parts | *F. albida* provenances[a] | Dry weight (g $plant^{-1}$) | Total N (g $plant^{-1}$) | $\%^{15}$Nae | %Ndfa | Ndfa (g $plant^{-1}$) |
|---|---|---|---|---|---|---|
| Leaves | Merina (S) | 7.4 b | 0.23 b | 0.78 a | 0.0 | 0.00 |
| | Dangalma (S) | 8.4 b | 0.26 ab | 0.75 a | 0.0 | 0.00 |
| | Ndiongolor (S) | 12.2 a | 0.33 ab | 0.51 bc | 26.3 ab | 0.09 b |
| | Pire (S) | 10.0 ab | 0.31 ab | 0.52 bc | 24.3 ab | 0.07 b |
| | Kabrousse (S) | 12.4 a | 0.38 a | 0.42 c | 39.9 a | 0.16 a |
| | Gomblora (BF) | 6.7 b | 0.23 b | 0.60 b | 13.8 b | 0.03 b |
| | Dem (BF) | 7.8 b | 0.28 ab | 0.53 bc | 23.3 ab | 0.06 b |
| | CV (%) | 24.2 | 25.3 | 15.3 | 41.1 | 54.6 |
| Stems | Merina (S) | 7.8 c | 0.09 b | 0.85 a | 0.0 | 0.00 |
| | Dangalma (S) | 9.1 bc | 0.11 b | 0.73 ab | 0.0 | 0.00 |
| | Ndiongolor (S) | 13.1 abc | 0.14 ab | 0.50 c | 23.2 a | 0.03 ab |
| | Pire (S) | 14.5 ab | 0.16 ab | 0.50 c | 23.6 a | 0.04 ab |
| | Kabrousse (S) | 18.4 a | 0.20 a | 0.39 c | 39.5 a | 0.08 a |
| | Gomblora (BF) | 7.5 c | 0.10 b | 0.58 bc | 10.5 a | 0.01 b |
| | Dem (BF) | 9.8 bc | 0.13 ab | 0.54 c | 16.2 a | 0.02 b |
| | CV (%) | 30.7 | 31.7 | 19.2 | 65.5 | 82.1 |
| Roots | Merina (S) | 20.5 a | 0.36 a | 0.73 a | 0.0 | 0.00 |
| | Dangalma (S) | 21.0 a | 0.37 a | 0.61 ab | 0.0 | 0.00 |
| | Ndiongolor (S) | 27.6 a | 0.52 a | 0.45 b | 28.4 a | 0.16 a |
| | Pire (S) | 24.7 a | 0.40 a | 0.45 b | 28.5 a | 0.12 a |
| | Kabrousse (S) | 27.9 a | 0.50 a | 0.40 b | 35.1 a | 0.20 a |
| | Gomblora (BF) | 20.4 a | 0.40 a | 0.53 c | 15.0 a | 0.06 a |
| | Dem (BF) | 19.5 a | 0.36 a | 0.51 c | 18.1 a | 0.08 a |
| | CV (%) | 36.4 | 36.0 | 20.8 | 67.5 | 83.8 |
| Total | Merina (S) | 35.6 a | 0.67 a | 0.78 a | 0.0 | 0.00 |
| | Dangalma (S) | 38.5 a | 0.73 a | 0.70 a | 0.0 | 0.00 |
| | Ndiongolor (S) | 52.9 a | 0.99 a | 0.49 b | 26.0 ab | 0.26 ab |
| | Pire (S) | 49.9 a | 0.87 a | 0.49 b | 25.5 ab | 0.24 ab |
| | Kabrousse (S) | 58.7 a | 1.08 a | 0.41 b | 38.2 a | 0.44 a |
| | Gomblora (BF) | 34.5 a | 0.74 a | 0.57 b | 13.1 b | 0.10 bc |
| | Dem (BF) | 37.1 a | 0.77 a | 0.53 b | 19.2 ab | 0.16 bc |
| | CV (%) | 30.2 | 29.9 | 15.9 | 50.4 | 70.3 |

[a] BF, Burkina Faso; S, Senegal.

For each plant part, values in the same column followed by the same letter do not differ significantly at $p = 0.05$.

provenance was highest, and that from Gomblora the lowest. As with total dry matter, no significant differences in total nitrogen of the whole tree were observed between the provenances. Total N was not equally distributed among plant organs; the greatest amount of nitrogen was located in the roots. Averaged for all seven provenances, the 0.41 g N accumulated in the roots represented almost 50%, while the leaves and stem contained 35% and 16%, respectively, of the total N in plants.

Atom% $^{15}N$ enrichment differed widely between provenances and among plant parts. For different plant parts or on a whole-plant basis, the provenances Merina and Dangalma contained the highest atom% $^{15}N$ excess values (0.78 and 0.70, respectively; Table 1), significantly higher than for other provenances, including the reference tree (0.65). The lowest atom% $^{15}N$ excess occurred in provenance Kabrousse, equivalent to almost half of that in Merina.

Significant differences in the nitrogen-fixing potential (NFP)—which we define as the proportion of a plant's total nitrogen requirement that is derived from atmospheric $N_2$ fixation (%Ndfa)—occurred among provenances. Although provenances Merina and Dangalma had the highest nodule dry weights (460 and 420 mg plant$^{-1}$, respectively), they did not fix any nitrogen. The provenance Kabrousse, with 290 mg of dry nodules plant$^{-1}$, had the highest %Ndfa: 39.9, 39.5, 35.1, and 38.2 in the leaves, the stems, the roots, and the whole plant, respectively (Table 1); the corresponding respective amounts were 0.16, 0.08, 0.20, and 0.44 g N fixed plant$^{-1}$. However, within each provenance %Ndfa did not differ significantly among plant parts. Averaged for all provenances, %Ndfa in the whole plant was 17.4, quite similar to the 18.2, 16.1, and 17.9 in the leaves, stem, and roots. However, for all the provenances, an average of almost 52% of the total N fixed occurred in the roots, while total N fixed in leaves was more than two times that in the stem. Variability in total N fixed between provenances was highest for the leaves (>50-fold range), followed by in the stem (about 8-fold range) and least (about 4-fold range) in the roots.

### *Field Estimation of $N_2$ Fixation*

The best $N_2$-fixing provenance, Kabrousse, was selected for field testing and examined 3, 6, and 15 months after transplanting. The amounts of N fixed at these periods were 540, 790, and 1410 mg N plant$^{-1}$, respectively (Table 2). Based on the planting density of 100 plants ha$^{-1}$ in most *F. albida* forest park, these values would correspond to 54, 79, and 141 g N ha$^{-1}$. The total N fixed in roots increased with time and represented 31.5, 48, and 54.6% of the total N fixed in the whole tree at 3, 6, and 15 months, respectively, while the total N fixed in leaves was 46, 21.5, and 14.9% of the total N fixed in the whole tree.

## Discussion

Although the $^{15}N$ isotope dilution technique is potentially the best available for measuring $N_2$ fixation, this is contingent upon the suitability of the reference plant used (Fried et al., 1983; Witty, 1983). A previous study (Ndoye et al., 1995) indicated that *P. biglobosa* and *Tamarindus indica* L. were appropriate reference trees for measuring $N_2$ fixation in *Acacia* species including *F. albida.* Thus our decision to select *P. biglobosa* as the reference tree in this study. However, the atom% $^{15}N$ excess values indicated that two of the provenances, those from Merina and Dangalma, were significantly higher than that of the reference tree *P. biglobosa.* We were thus faced with the choice of using these non–$N_2$-fixing genotypes or *P. biglobosa* as reference trees. The former had the advantage

**Table 2**

Proportion (%Ndfa) and amount (Ndfa) of nitrogen derived from fixation in different plant parts of 3, 6, and 15 months of field-grown *Faidherbia albida* originated from Kabrousse (Senegal) using *Parkia biglobosa* as reference tree

| Plant parts | $\%^{15}$Nae | | | %Ndfa | | | Ndfa (g plant$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 MAT[a] | 6 MAT | 15 MAT | 3 MAT | 6 MAT | 15 MAT | 3 MAT | 6 MAT | 15 MAT |
| Leaves | 0.89 a | 1.22 a | 1.01 a | 60.6 a | 55.6 a | 55.6 a | 0.25 a | 0.17 b | 0.21 b |
| Stems | 0.87 a | 1.07 a | 1.08 a | 47.8 a | 59.0 a | 54.5 a | 0.10 a | 0.15 b | 0.34 b |
| Roots | 0.74 a | 1.21 a | 1.08 a | 54.1 a | 59.6 a | 51.4 a | 0.17 a | 0.38 b | 0.77 ab |
| Total | 0.83 a | 1.18 a | 0.83 a | 54.7 a | 57.8 a | 54.7 a | 0.54 a | 0.79 a | 1.41 a |
| CV (%) | 48.3 | 61.5 | 40.8 | 40.2 | 44.6 | 34.9 | 81.9 | 63.6 | 70.9 |

[a] MAT, months after transplantation.

For each harvesting time, values in the same column followed by the same letter do not differ significantly at $p = 0.05$.

that these are likely to be similar in growth and physiological attributes to the other provenances. However, our decision to stick to *P. biglobosa* is twofold, to provide relative comparison with our previous study (Ndoye et al., 1995), and a greater assurance that the estimates we are making are conservative estimates and less likely to be overestimated.

Nitrogen fixation improvement programs for such NFTs could be based on two approaches, enhancement in (a) %Ndfa and (b) total N yield, with N yield being predominantly under the genetic control for dry matter yield. Using %Ndfa to compare the $N_2$ fixation potentials of the *F. albida* provenances grown under controlled conditions in the greenhouse, our results demonstrated great differences among the seven *F. albida* provenances, ranging from zero to 32% on the whole plant basis. Similarly large variations have been reported in other tree species such as *Casuarina* spp. (Fleming et al., 1987; Sanginga et al., 1990a; Sougoufara et al., 1987), *G. sepium* (Awonaike et al., 1992; Liyanage et al., 1994), *Leucaena leucocephala* (Lam) de Wit, and *A. albida* (Sanginga et al., 1990b). Consequently, Sanginga et al. (1990b) recommended that, to derive greater N benefits from *F. albida* in the field, it will be essential to screen and use provenances with high $N_2$ fixation capacity and therefore low in soil N uptake.

The importance of selecting genotypes with higher yields in $N_2$ fixation programs has been shown convincingly in the study of Liyanage et al. (1994), who found that total N yield contributed more to the variability in the agronomically important parameter of total N fixed among *G. sepium* provenances than did %Ndfa. The best approach will therefore be to select $N_2$-fixing genotypes that combine high yield with high %Ndfa. On the basis of both %Ndfa and dry matter (total N) yield, the provenance from Kabrousse was most outstanding (Table 1). As a result of its high fixation, it had a lower need for soil N uptake, as indicated by the %Ndfs: 57.6% vs. an average of 80% for the other provenances.

Because of possible differences between fixing and reference plants in the relative translocation of the N accumulated at different times to different plant organs, it is recommended to calculate $N_2$ fixation in whole plants rather than in individual plant parts. However, where atom% $^{15}$N in the different plant parts of a reference plant are observed to be similar (Zapata et al., 1987a and b), it should be justified to calculate $N_2$ fixed in different plant parts. This is particularly important in trees for two reasons. One, when it is an advantage to examine whether the $N_2$ fixed in an easily accessible plant part could be used to represent $N_2$ fixed in the whole plant (Liyanage et al., 1994; Danso et al., 1995). The second reason is that it allows an assessment of the effect of tree removal on the N balance in soil to be made.

As with *Robinia pseudoacacia* L. (Danso et al., 1995), our data indicate that for both the greenhouse and field studies, %Ndfa did not differ significantly between different plant parts and the whole tree, for which reason %Ndfa in an easily accessible plant part like the leaves can be used for measuring the %Ndfa by the whole tree of *F. albida.* This was in contrast to total N fixed; on average, total N fixed in the greenhouse was underestimated by 48% to 85%, depending on whether the roots or stems, respectively, were the organs used. The underestimates observed in the field were similarly high, but depended on the age of the tree. This was despite the fact that full recovery of roots is not easy under field conditions, as indicated in the study of Liyanage et al. (1994). From our data, ignoring the roots resulted in underestimating the amount of N fixed in field-grown *F. albida* by about 50%. Thus, roots cannot be ignored in field assessments of N fixed in *Acacia* species including *F. albida,* as already reported by Ndoye et al. (1995), and they constitute an essential source of soil N fertility restoration and organic matter build-up in soil.

Despite the selection made, the best *Faidherbia* provenance, Kabrousse, for up to 15 months fixed only 141 g N $ha^{-1}$, significantly lower than what has been measured for many NFTs, such as *Acacia holosericea* A. Cunn. (Cornet et al., 1985), *L. leucocephala* (Sanginga et al., 1989), and *G. sepium* (Liyanage et al., 1994). This is despite the fact that $N_2$ fixation accounted for more than 50% of the total N accumulated in *F. albida.* The deduction from this study is that *F. albida*'s genetic potential for growth is low and a major factor in how much N is fixed. However, we are not aware of $N_2$ fixation measurement in *F. albida* beyond 15 months. For such a perennial NFT, this is important because some trees may show slow early growth that could accelerate later. For example, unpublished results (Hafedh Nasr, personal communication) on *Acacia cyanophylla* Lindl. showed that growth and $N_2$ fixation were low in the first 2 years, with an almost fivefold increase in yield between the second and third years. We plan to initiate such long-term studies for *F. albida.* Besides, with continual depletion of soil N with age, there is a high probability that %Ndfa may increase further.

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