Assessment of Symbiotic Nitrogen Nutrition in Marama Bean (*Tylosema esculentum* L.), a Tuber-Producing Underutilized African Grain Legume

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Abstract

Marama bean (*Tylosema esculentum* L.) is a legume indigenous to Southern Africa and produces protein-rich seed and tubers with potential for use as human food and animal fodder. The source of N used by the plant has been assessed. Field surveys combined with inoculation studies using rhizosphere soils and compost under different conditions show that marama bean does not have nodules or nodulate. Values from analysis of N isotope ratios ($^{15}N/^{14}N$ and $\delta^{15}N$ values), which indicate whether N is obtained from inorganic sources in the soil or from symbiotic fixation of atmospheric N_2 by *Rhizobium* bacteria, ranged from 6.8 to 8.8‰ (7.9±0.6‰) for shoots, 5.9 to 9.8‰ (7.9±1.6‰) for tubers and 8.05 to 10.12‰ (8.96±0.87‰) for seeds of plants sampled from the field at Roodeplaat near Pretoria, South Africa. Plants from Sandveld Research Station near Goubabis, Namibia, had mean values of 5.9±0.3‰ for shoots, 7.0±0.06‰ for tubers and 5.3‰ for seeds. These values are larger than those of three nodulating N_2-fixing *Acacia* species, which ranged from –4.0±0.16‰ for roots to –1.4±0.45‰ for leaves, and also higher than those of seeds of bambara groundnut (–0.17 to 4.5‰, mean = 1.9±0.5‰) with active symbiotic N_2

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fixation. The marama bean $\delta^{15}N$ values are comparable to those of soil N (5 to 15‰) and to two non-N_2-fixing cereal plants, sorghum (8.3 to 10.3‰, mean = 9.5±1.0‰) and pearl millet (7.9‰). The $\delta^{15}N$ values for marama bean plants were similar to those of the related species *Tylosema fassoglense* and *Bauhinia petersiana*. Absence of root nodules on field-grown plants and similarity of $\delta^{15}N$ to soil values and non-N_2-fixing cereals and dissimilarity to symbiotic N_2-fixing legumes together show that marama bean uses N from the soil, and not from symbiotic fixation of atmospheric N_2.

Keywords: Marama bean, rhizosphere soil, non-nodulation, $\delta^{15}N$ values, ^{15}N natural abundance, N nutrition

1. Introduction

Of the huge plant diversity in Africa, legumes have great potential for improving quality of life in the continent through their use as sources of protein-rich foods, timber, animal feed or as soil-improving biofertilizers. However, many of these legumes have remained undomesticated and virtually underutilized. Marama bean (*Tylosema esculentum* L.), which is found mainly in Southern Africa, is an example of a potentially important wild legume that has attracted little scientific research. Marama bean seed has a very large protein content (30–39%) and oil content (about 36%), comparable to soybean and groundnut, respectively. Additionally, this legume also produces edible tubers, which contain about 9% protein, almost twice the value in conventional root crops like potato and sweet potato. Another species, *Tylosema fassoglense*, also produces both edible grain and tuber, but only the seeds of the congeneric species *Bauhinia petersiana* are edible.

Because the grain and tubers of marama bean are rich in protein, its potential for human nutrition is large. To establish marama bean as a crop would require an understanding of the sources of N used by the plant, particularly its status with respect to N_2 fixation by symbiosis with rhizobia or other microorganisms. A number of approaches have been employed to assess the sources of N used by plants. Surveying for nodulation in field-grown plants (Allen and Allen, 1982), inoculation with soil from the rhizosphere of existing plants or with other sources to induce nodulation and/or the use of molecular markers to establish traits related to nodulation (Harrier et al., 1997) have been used. In our study, we assessed the presence of nodules in plants grown in the field, the ability of young plants to nodulate with different soils and conditions, and the use of N isotope ratios ($^{15}N/^{14}N$) and discrimination ($\delta^{15}N$) from field plants, to establish the source of N for marama bean. The isotope

ratio method is a technique which integrates over the plant's life time and the values correlate strongly with the source of N (Shearer and Kohl, 1986). Small $\delta^{15}N$ values indicate symbiotic fixation of N_2, whereas large $\delta^{15}N$ values indicate use of inorganic N from the soil.

2. Materials and Methods

Nodulation studies with field plants in Namibia and South Africa

In February 1995 and April 1997 field plants were surveyed for nodulation at Sandveld Agricultural Research Station, Namibia, by digging up individual plants and observing them for the presence of nodules. In 1995, similar surveys were carried out on field plants from west Pretoria (South Africa) and the Institute for Vegetable and Ornamental Research, Roodeplaat, in Pretoria (South Africa). In April 1997, 18-month-old marama bean plants, which were cultivated at the Roodeplaat Research Institute, were similarly surveyed for nodulation. Tuber yields, measured as fresh weight per plant, were recorded for the 1997 material harvested from Roodeplaat and Sandveld Station. Tuber water content was estimated after oven-drying known fresh weights.

Nodulation studies with rhizosphere soil from Namibia and Pretoria

In glasshouse studies done in South Africa, soils collected from around the rhizosphere of marama bean plants growing wild at Sandveld Research Station and Nina Junction, Namibia, were used to inoculate germinating seedlings of marama bean cultured in sand within plastic containers (5 l). Five grams soil was applied to each plant to test whether organisms in the soil would elicite nodule formation on the seedling roots. In all, there were 5 replicate pots per soil type. The seedlings were irrigated three times a week with a N-free modified Hoagland nutrient solution. Sixty days after sowing, the plants were harvested and observed for nodulation.

Nodulation studies with compost inoculant at Rothamsted

Seeds of marama bean were sown in a rooting mixture of coarse sand and perlite to a 20 cm depth in 50-l plastic containers. Following germination, the seedlings were inoculated with potting compost and supplied with N-free modified Hoagland nutrient solution three times a week. Three containers were used in this experiment. The plants were harvested at 6 months after sowing and observed for the presence of nodules on their roots.

Analysis of $^{15}N/^{14}N$ ratios and $\delta^{15}N$ in field plants of marama bean

Legumes obtaining their N from symbiosis with rhizobia and thus dependent on atmospheric N_2 for their N nutrition, have lower $\delta^{15}N$ values (close to 0‰ or less) than those utilizing soil N, which have higher $\delta^{15}N$ values around 5 to 15‰ (Shearer and Kohl, 1986). This distinct difference between the $\delta^{15}N$ signature of symbiotic N and soil N can be used to determine whether a leguminous plant is deriving its nitrogen from the atmosphere or from soil. The technique was applied in this study to ascertain if marama bean is a N_2 fixer.

Shoots and tubers of marama bean were collected from field plants at Roodeplaat in Pretoria and Sandveld Station in Namibia, stored in paper bags, and brought to Cape Town. These samples were then oven-dried at 80°C, weighed and finely ground for analysis of $^{15}N/^{14}N$ ratios using mass spectrometer. Leaf samples of a second species, *Tylosema fassoglense*, were obtained from the Namibian Botanical Institute and similarly analysed for ^{15}N content. Because *Tylosema esculentum* and *Tylosema fassoglense* were once classified as *Bauhinia* species, analysis of the $\delta^{15}N$ values of leaf, seed and pod samples from *Bauhinia petersiana*, collected from Newlands, Cape Town, South Africa, was done to provide a comparison with its two related species.

Analysis of $^{15}N/^{14}N$ ratios and $\delta^{15}N$ in cereals and nodulated legumes for comparison with marama bean

Three N_2-fixing tree legumes (*Acacia saligna, A. longifolia, A. cyclops*) growing in the Cape Town area were dug up from the field and separated into leaves, stems and nodulated roots. Four replicate seedlings were harvested for each species. The $\delta^{15}N$ values of samples were analyzed as described. Bambara groundnut (*Vigna subterranea* L. Verdc.) was collected from different locations in Tanzania. Fifty six different seed accessions were oven-dried and the $\delta^{15}N$ also analyzed as described. Grain from three sorghum (*Sorghum bicolor* L.) cultivars and pearl millet (*Pennisetum typhoides* subsp. *cereale* L.) obtained from Tanzania, were analyzed for $\delta^{15}N$ values. These cereals occur as companion plants in intercrop with N_2-fixing Bambara groundnut. The plant samples were oven-dried at 80°C, weighed, and ground into very fine powder for determination of $^{15}N/^{14}N$ ratios.

3. Results and Discussion

There was no evidence of nodulation in any of the field-grown plants examined at Roodeplaat near Pretoria, and Sandveld in Namibia either in 1995 or 1997 (Table 1). Also, rhizosphere soils collected from Namibia and
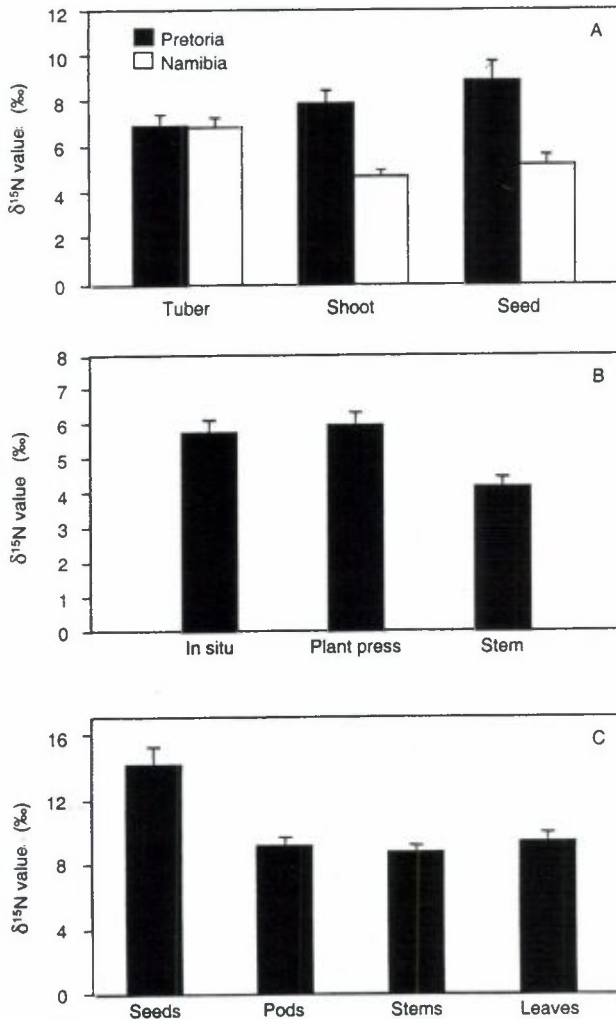
Figure 1. Values of $\delta^{15}N$ in organs of field plants: A) *Tylosema esculentum* L., B) *Tylosema fassoglense* L., and C) *Bauhinia petersiana* L. Mean±SD (n = 4–10).

Roodeplaat near Pretoria did not result in nodule formation on roots of marama bean plants grown under glasshouse conditions in South Africa. Nodulation was not observed at Rothamsted, when marama bean seedlings were inoculated with potting compost under glasshouse conditions. Corby (1974) also did not find nodules on *T. fassoglense* plants growing in the field in Zimbabwe. Thus, marama bean is probably non-nodulating, and therefore cannot use atmospheric N_2 and must depend on soil N for its N nutrition.
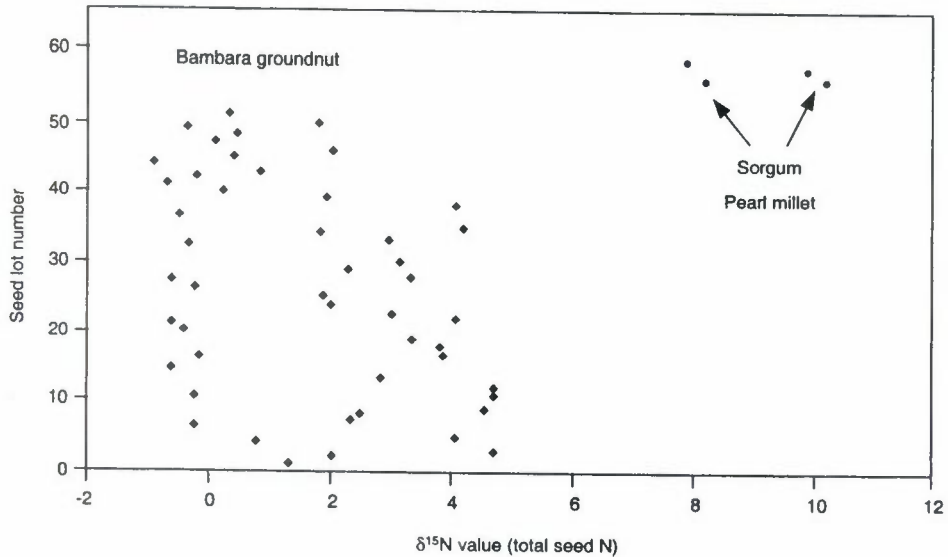
Figure 2. Range of $\delta^{15}N$ values for seed lots of the non-N_2-fixing cereals, Pearl millet and sorghum, and the N_2-fixing Bambara groundnut collected from 51 locations in Tanzania.

The ^{15}N natural abundance method indicates the source of N used by plants, and has been routinely used to assess the symbiotic status of field plants (Shearer and Kohl, 1986; Muofhe and Dakora, 1999). Its application here reveals that the $\delta^{15}N$ values of shoots and tubers of marama bean were large (Fig. 1) compared to those of nodulated N_2-fixing *Acacia* species (*A. saligna, A. longifolia* and *A. cyclops*), which ranged from −3.14±1.41‰ to −1.42±0.45‰ for leaves, −3.09±0.41‰ to -2.48±0.68‰ for stems, and −4.04±0.16‰ to −1.9±0.49‰ for nodulated roots (Table 3). The $\delta^{15}N$ values of seed from symbiotic bambara groundnut (Fig. 2) collected from Tanzania ranged from −0.17 to 4.5‰ (mean 1.9±0.5‰). The $\delta^{15}N$ signatures of the shoots of ten marama bean plants sampled from Roodeplaat ranged from 6.8 to 8.8‰ (mean 7.9±0.6‰), while their tuber values were 5.9 to 9.8‰(average 7.9±1.6‰; see Fig. 1A). The data for plant samples from Namibia were similar, with an average $\delta^{15}N$ value of 5.9±0.3‰ for shoots and 7.0±0.06‰ for tubers (Fig. 1A). The large $\delta^{15}N$ values of marama bean are more similar to those of soils (Shearer et al., 1978), and to the $\delta^{15}N$ values we measured for sorghum (8.3 to 10.35‰, average 9.5±1.0‰) and pearl millet (7.9±0.5‰; see Fig. 2), two non-N_2-fixing cereal plants that depend solely on soil for their N nutrition. These N isotope analyses therefore clearly show that marama bean does not obtain its N by symbiotic N_2 fixation, as expected from the absence of root nodules. As

T. esculentum, T. fassoglense and *B. petersiana* are considered congeneric by some botanists (Allen and Allen, 1981), it is perhaps not surprising that their $\delta^{15}N$ values are similar (Fig. 1). We suggest that the three species depend on soil N, and not on symbiotic fixation of atmospheric N_2, for their N nutrition.

Soils which support growth of marama bean are generally low in N. However, Tredoux et al. (1998) have reported that there are large NO_3 concentrations in deep groundwaters of arid and semi-arid regions and that such NO_3 can accumulate naturally in subsurface soils, leading to increased availability to plants. We suggest that growth of marama bean, with deep roots and large storage capacity, would allow accumulation of large amounts and concentrations of N as protein in the plant. The very large N concentrations of the seed (Table 1) and large size would allow extensive and rapid root growth, even into N-poor surface soils (provided water were available) as well as formation of photosynthetically active cotyledons and leaves of high N concentrations. In deed, root growth appears rapid: at Rothamsted roots reached 50 cm (bottom of the container) within three weeks of germination. Tubers start to form very early after a few leaves are made, and grow rapidly reaching weights of 3 to 10 kg after 18 months for plants grown at Roodeplaat (Table 2). Tubers then become massive, reaching up to 160 kg as estimated for plants of indeterminate age at Sandveld, Namibia (Table 2). Given the large tuber N concentration and size (Tables 1 and 2), their capacity as a reserve for N to support regrowth after drought, defoliation (grazers) and for flowering and seed production is considerable. This buffers the plant against environmental fluctuations in N supply. Whether marama bean uses NO_3 from surface or subsurface soil which is suggested to accumulate in arid and semi-arid regions (Tredeux et al., 1998), is yet to be determined. Whatever the case, the high $\delta^{15}N$ values obtained in this study are consistent with a non-nodulation trait in these species. Detailed analysis of the N-sources used by marama bean and the changes in the N-containing components during the growing cycle will provide valuable insights into the way that this plant exploits soil N resources, and is essential for any domestication of this potentially valuable legume.

Although the N nutrition of marama bean is intriguing, its ability to tolerate drought also arouses interest as it has been reported to thrive in desert regions that experience zero rainfall in some years (NAS 1979). As shown in Table 2, the rapid formation of large tubers (up to 10 kg tuber fresh weight per 18-month-old plant) would be a mechanism by which the legume stores water for re-growth during drought. The water content is 87–92% of tuber fresh mass (Table 2): this represents a considerable reservoir and large buffering capacity during dry periods. Additionally, marama bean exhibits leaf behaviour features that are likely to conserve water. The leaves are markedly heliotropic. Leaflets fold tightly together when conditions are very bright and hot, and the leaves orientate so as to present the least surface to the sun. In

Table 1.   Nodulation response of marama bean plants to inoculation with rhizosphere soil, and concentration of nitrogen in organs of field plants growing in Roodeplaat (South Africa) and Sandveld Station (Namibia).

Soil inoculum	Nodulation		N concentration in organ (%)*		
	1995	1997	Leaves	Tubers	Seeds
Roodeplaat	–	–	2.9±0.4	3.1±0.03	3.1±0.1
Sandveld	–	–	1.3±0.2	1.9±0.4	2.8±0.3
Rothamsted	–	–	ND	ND	ND

*Samples for the N analysis were obtained from field plants. The Rothamsted experiment used compost as inoculum. Values are Mean±SD (n = 4–10). – = absence of nodules on roots. ND = not determined.

Table 2.   Yield and water economy of tubers of marama bean plants harvested from Roodeplaat (South Africa) and Sandveld Station (Namibia) in 1997.

Plant no.	Roodeplaat*			Sandveld Station#		
	Tuber fwt. (kg/plant)	H_2O/fwt. tuber (kg)	(%)	Tuber fwt. (kg/plant)	H_2O/fwt. tuber (kg)	(%)
1	9.8	8.7	89	64.4	56.0	87
2	6.3	5.6	89	13.7	12.6	92
3	5.5	4.7	86	34.3	30.1	88
4	3.2	2.9	91	160.0	139.5	87

*18-month-old plants; #plants of unknown age.

Table 3. ^{15}N content of roots, shoots, and leaves of N_2-fixing *Acacia* species collected from fields around Cape Town

Acacia species	δ^{15}N value (‰)		
	Leaves	Stems	Roots
Acacia saligna	−1.42±0.45	−2.72±0.02	−1.92±1.07
A. longifolia	−1.53±0.67	−2.48±0.68	−1.92±0.49
A. cyclops	−3.14±1.41	−3.09±0.41	−4.04±0.16

Values are Mean±SD (n = 3).

early morning and evening, the leaflets lie flat thereby presenting the maximum area to the sun. Such behaviour may minimize energy load and thus decrease the potential for photoinhibition of photosynthesis and transpiration in an arid environment where C3 plants might otherwise be at a disadvantage. Given the prospect of global warming and its attendant drought, the efficient water-harvesting and storage in the protein-rich tubers of marama bean, should make this legume an ideal crop of the future.

Acknowledgement

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