Marama bean (*Tylosema esculentum*), a non-nodulating high protein legume indigenous to the Kalahari Sands: Studies of its N nutrition

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Abstract

Marama bean is a non-nodulating perennial legume native to the nutrient-poor soils of the Kalahari Sands. In Botswana and Namibia, it is the staple food of the Khoisan people. Marama bean, however, still have not been cultivated or established as an agricultural crop in these countries. This study investigated soil factors affecting the distribution and growth of marama bean in the field and how it would respond to additional N and P supply both under field and glasshouse conditions. The study then attempts to explore and understand the mechanisms employed by marama bean to acquire high nutrient concentration in its organs. Results from soil analysis showed that soils in which wild marama bean plants grow are poor in nutrients especially nitrogen. Total nitrogen was less than 0.06 % and soil organic matter was below 0.45 %. Macronutrients such as calcium and magnesium appears to play a significant role in the distribution of marama bean as soils obtained from marama bean-growing sites were richer in these elements compared to soils obtained from non-marama bean growing sites. Soil pH also appears to influence marama bean growth and distribution. When marama bean plants grown in the glasshouse were supplied with NO_3^-, growth was generally better at high than at low NO_3^- concentrations. Chlorophyll concentrations, tuber fresh weights, plant dry matter and % N in tubers significantly ($P \leq 0.05$) increased with increased nitrate supply. Nitrate application also increased concentrations of P, Ca, Mg, and K, but resulted in decreased concentrations of soluble sugars. Application of NO_3^- in the field also increased tuber fresh and dry weights of marama bean. Unlike N, P supply did not have a significant effect on marama bean growth . When marama bean preference for the form of N was

tested by supplying plants with 2 mM NO_3^-, 2 mM NH_4^+ or 1 mM NH_4NO_3 in the glasshouse, the results showed that it was able to utilize all forms of N efficiently.

When the source of N used by marama bean in the field and reference plants within its surroundings was analysed using the ^{15}N natural abundance method, the results showed similar $\delta\ ^{15}N$ values for marama bean and non-legume species growing within its vicinity. In a separate study using ^{15}N labeling technique, it was shown that marama bean took up more ^{15}N when the $^{15}NO_3^-$ concentration in the rooting medium was low compared to when it was high, with a larger proportion of assimilated ^{15}N stored in tubers than in leaves and stems.

There was no trend found between soil N levels and marama bean distribution and it can be concluded from this study that growth and distribution of marama bean plants in the field is not closely associated to soil N or that soil N is not the determinant factor of where marama bean grows. However, more sampling with many control sites in both countries need to be carried out to be able to draw definite conclusions. In addition, the influence of other factors, such as land use and habitat, competition, herbivory, dispersal ability and genetic factors on marama bean growth and distribution need to be investigated. The study on the response of marama bean to external supply of N and P indicated that it indeed benefited from additional N, but not from P, suggesting that N fertilization will be required for improved growth yields, if marama bean is to be cultivated as a crop plant. The fact that P supply did not have a significant effect on marama bean growth could mean that marama bean has a low P requirement.

Although it was found in this study that marama bean have similar $\delta^{15}N$ values as plant growing in its surrounding, it should be noted that $\delta^{15}N$ values are a tricky indicator of N

source and that high $^{\delta}$N signal could be due to other factors such as mycorrhizal association. Studies of N allocation to organs suggest that marama bean efficiently concentrates nutrients in its organs and utilizes the tuber as a buffer for N required during environmental fluctuations and plant re-growth

Chapter 1: General Introduction

1.1 Distribution and description of marama bean

Marama bean [*Tylosema esculentum* (Burchel) Schreiber] is a legume (subfamily Caesalpinoedeae) native to the semi-arid regions of Southern Africa. It tolerates the scorching heat and long drought periods of the Kalahari Desert of Botswana and Namibia where it is an important component of the diet of the Khoisan and nomadic Bushmen. It is also found in South Africa (Northern Cape Province, North-west Province and Limpopo Province), mostly in undulating grass-veld or savannas (NAS, 1979). Marama bean is commonly found creeping over the soil surface with vines carrying double lobed leaves that are soft and red-brown when young, but later turn leathery and grey-green with age (NAS, 1979). Golden-yellow flowers develop in mid-summer and the fruits ripen in late autumn (NAS, 1979).

1.2 Marama Bean as a Major Food Crop and Medicinal Plant of the Kalahari Bushmen

Marama bean is one of many plants native to Africa with great agricultural potential, but which still needs to be developed through plant breeding to improve growth and yield. With the increasing human population, improvements of drought tolerant crops such as marama bean could be of great benefit to indigenous populations. Marama bean produces edible seeds and tubers (NAS, 1979). The seed has a large protein content (30-39%) and oil content (36-43%), comparable to groundnut and soybean respectively (NAS, 1979; Bower *et al.*, 1988). The seeds are eaten boiled or roasted and has a delicious nutty flavour that has been compared to roasted cashew nuts (NAS, 1979). Marama bean could therefore easily serve as a cheap source of dietary protein for indigenous populations

living in the semi-arid regions where few conventional crops can survive. Like most grain legumes, the marama protein is rich in the amino acid lysine (5 %), but deficient in methionine (0.7 %) as indicated by Bower *et al.* (1988). The oil is present largely as mono-saturated or unsaturated fatty acids (Bower *et al.*, 1988) and hence can be included in the group of healthy foods. However, the legume faces the problem of over-exploitation from local people through over-harvesting of seeds as well as defoliation by game and livestock (NAS, 1979). The tuber (2 years or younger) is eaten raw, boiled or baked (NAS, 1979). It contains about 9 % protein which is about twice the value in conventional crops such as potatoes and sweet potatoes (Dakora *et al.*, 1999). People of the Kalahari also crush the plant leaves and make a thick paste that is used to treat wounds and, arthritis.

1.3 Marama bean as forage or fodder crop for game and livestock

The marama plant is a perennial prostrate vine with numerous herbaceous yellow-brown or red-brown stems and branches that creep along the soil surface in several directions. These viny stems can reach up to 6 m long and bears massive number of leaves. It has been reported that livestock and game in the Kalahari region feed on fresh foliage of marama bean (NAS, 1979). With high nitrogen content in their leaves, ranging from 1.3-2.9 % (Dakora *et al.*, 1999), and hence high protein content, marama bean vines can be used to supplement low-quality grass herbage in smallholder dairy farms to increase milk yields as well as meat protein content of livestock.

1.4 Marama bean as a source of water in the Kalahari Desert.

The marama tuber contains about 90% water by weight (Dakora *et al*,. 1999) and therefore is a great source of water to the indigenous populations, especially hunters in the Kalahari region which has unpredictable rainfall and water is scarce at most times of the year. According to local people, water is collected by crashing older tubers into a container and separating the liquid from the fibrous tissues using a sieve. Herdsmen looking after cattle in the Kalahari dig out young tubers and chew them as their immediate source of water. Drought, which easily affects legume plant growth, is likely to worsen with the projected rapid expansion of water-stressed areas (Postel, 2000). It is thus important to identify useful legume species with drought-tolerance ability and high water-use efficiency. The high water content of the marama bean tuber has been suggested to serve as a buffer for the plant to survive long periods of drought that are common in its natural habitat of the Kalahari desert.

1.5 Nitrogen nutrition in marama bean

The soils in which marama bean occurs naturally are sandy often with low organic matter content (Jobágy and Jackson, 2001; Ainsworth *et al.*) and poor nutrient levels, especially N. However, high nitrogen concentration (1.3 - 2.9%) was reported in the leaves, seeds and tubers of wild marama bean plants (Dakora *et al.*, 1999). It is not clear how and where marama bean obtained its nitrogen to explain these high values in its tissues. Dakora *et al.* (1999), combined glasshouse and field studies to evaluate nodulation and N_2 fixation in marama bean, using the ^{15}N natural abundance method. Their results showed lack of nodulation in plants examined both in the field-grown and glasshouse-

grown marama bean plants. Inoculating glasshouse-grown marama bean seedlings with rhizosphere soils collected from around wild marama bean plants did not result in nodule formation on roots of these plants.

Nitrogen isotope ratios ($^{15}N/^{14}N$) and discrimination ($\delta^{15}N$) have been successfully employed to establish the source of N used by plants (Shearer and Kohl, 1986). The $\delta^{15}N$ values of plant-available N forms fall within the range of < -5‰ for atmospheric ammonium to > +10 ‰ for N soil pools (Handley and Raven, 1992). An observed difference in $\delta^{15}N$ values between species in the field is thus hypothesized to reflect different N acquisition strategies. Plant species relying on N_2 fixation by bacterial symbionts have been shown to exhibit $\delta^{15}N$ values similar to atmospheric N_2 as opposed to plants relying entirely on soil N sources. Low $\delta^{15}N$ values around 0‰ or less usually suggests reliance on atmospheric N_2, whereas soil mineral N and the plants relying solely on soil mineral N have $\delta\ ^{15}N$ values well above 5 ‰ (Shearer and Kohl, 1986). The $\delta\ ^{15}N$ values of marama bean organs sampled from Namibia and South Africa were found to range from 7.9-8.96 ‰ (Dakora *et al.*, 1999), values comparable to those of plants dependent on soil N (Bergersen and Turner, 1983; Kohl and Shearer, 1980; Shearer and Kohl, 1986). Those results indicated that marama bean utilizes soil mineral N as the primary source of N nutrition. The high concentration of N in its organs could suggest that marama bean has a mechanism for concentrating N in its tissues.

1.6 Challenges in domesticating marama bean

Research needs to be done on many aspects of marama bean biology to allow its domestication. For example, there is a need to investigate the effects of altitude, soil

types, temperature, moisture, fertilization and altitude on marama bean and identify the optimal conditions for plant growth. This knowledge will enable the cultivation of marama bean in areas different from which it is found growing naturally. Also, cultural practices such as spacing, planting density, weeding, and pest and disease control all need to be studied if marama bean is to be cultivated as an agricultural crop. There is also a need for genetic manipulation and improvement using modern genomic tools. Furthermore it will be interesting to know if marama bean can be cultivated without irrigation within its habitat. However, encouraging the Khoisan inhabitants of the Kalahari to cultivate marama could be a particularly difficult task as they may not understand the importance of cultivating a crop that "has always been there, cultivated by God" as one Khoisan man is quoted to have said (NAS, 1979).

1.7 Aim of study

Since marama bean occurs in patchily localized stands, it was hypothesized that marama bean distribution is influenced by soil factors. It was also hypothesized that marama bean, which grows in nutrient-poor soils could benefit from additional nitrogen and phosphorus in soils and that marama bean might have a mechanism that it use to efficiently utilize nutrients from these soils. The objectives of this study were to i) investigate soil factors affecting marama bean growth and distribution in the field, ii) determine the form of nitrogen utilized by marama bean iii) determine the effect of N and P supply on marama bean growth in the glasshouse and in the field, iv) determine the source of N nutrition using the ^{15}N natural abundance method and v) explore the mechanisms by which marama bean accumulates high concentrations of N as a protein in its organs.

Chapter 2: General Materials and Methods

2.1 Studies of Marama Bean under Semi-Controlled conditions

2.1.1 Plant culture and nutrient application

Marama bean seeds used in all experiments weighed between 2.50 and 3.00 g. The seeds were sown in long polyvinyl chloride (PVC) tubes or 'pots' containing 10 g mixture of wet sand and vermiculite. Two seeds were sown per pot (1 cm deep) and pots were exposed to out door (natural) conditions. Before germination plants were watered with tap water and after germination, they were irrigated with de-ionized water until two weeks.

Nitrogen and phosphorus-free modified ½ strength Hoagland nutrient solutions were used to culture plants. The N-free nutrient solution consisted of 246.5 g $MgSO_4$, 111.0 g $CaCl_2$, 87.1 g K_2SO_4, 68.0 g KH_2PO_4, 87.1 g K_2HPO_4, 18.7 g sequestrene (138 Fe), 724.0 mg $MnCl_2.4H_2O$, 110.0 mg $ZnCl_2$, 70.0 mg $CuCl_2$, 25.0 mg $NaMoO_4$. $2H_2O$, 60.0 mg $CoCl_2.6H_2O$ and 5.7 g H_3BO_3 per 1000 mL as described by Hewitt (1966). In the P-free nutrient solution, KH_2PO_4 and K_2HPO_4 were omitted and 1.0 M KNO_3 was added to provide NO_3^- and K. The N-free stock solution contained 1 M of P, while the P-free stock solution contained 1 M of N. The stock solution was then diluted with de-ionised water to provide specific nutrient concentrations (1 mM, 2 mM, 5 mM and 10 mM of NO_3^- for N treatment and 1 mM, 2 mM, 5 mM and 10 mM of PO_4^- for P treatment). In all P treatments N concentration was always 2 mM while in N treatments, the P concentration was always 2 mM. All nutrient solutions were adjusted to a final pH of 6.8 with hydrochloric acid before given to plants.

2.1.2 Response of marama bean to different concentrations of N and P

The effects of nitrogen and phosphorus on marama bean growth were tested using sand cultures. Seeds were planted on 3 January 2001 (Year I experiments). Two weeks after germination, seedlings were thinned out to one seedling per pot and plants were given 300 mL N and P nutrient solutions three times a week. The treatments used were 0, 1, 2, 5 or 10 mM NO_3^- and 0, 1, 2, 5 or 10 mM PO_4^{3-} prepared from N and P-free modified ½ strength Hoagland nutrient solutions respectively. To avoid nutrient build up to toxic levels in the rooting medium, the sand was flushed once every two weeks with water. During late May when the temperatures drop and winter rainfall starts, watering of plants with nutrients solutions was stopped. At this time, shoot materials were harvested, oven dried and kept for total biomass determination. Watering with nutrient solution was resumed on 15 October 2001 when warm weather returned. The plants re-sprouted four weeks after watering was resumed. On 9 November 2001 confirmatory experiments (Year II experiments) with the same nitrate and phosphate treatments were set up, however, the number of replicates was increased from four to six per treatment. Germination occurred four weeks after planting and the application of nutrient solutions commenced two weeks after germination. During the onset of winter in May 2002, watering with nutrient solutions was also stopped. Commencement of watering resumed on 5 September 2002 as weather conditions became warm. Re-sprouting occurred four weeks after watering with nutrient solution was resumed.

2.1.3 Response of marama bean to different sources of N

Marama seeds were planted on 2 December 2001 (Year I experiments). Pots were placed outside as described above and watered with de-ionized water. Germination occurred two weeks after planting, and two weeks after germination plants were thinned out to one per pot leaving those of comparable sizes. They were given four different treatments, each containing six replicates randomly arranged, and were irrigated three times a week with 300 mL of ½-strength modified Hoagland nutrient solutions adjusted to contain 0 mM, 2 mM NO_3^-, 1 mM NH_4NO_3 or 2 mM NH_4Cl. Another experiment was set up for confirmation on 15 October 2002 (Year II experiments). However in the Year II experiments, germination occurred four weeks later. Two weeks after germination, plants were also supplied with the Hoagland nutrient solution containing N from different sources as described above.

2.1.4 Plant harvesting and growth analysis

Sixteen-months-old nitrate and phosphate-treated plants were harvested on 20 May 2002 and 24 March 2003 for Year I experiment and Year II experiments, respectively. Five-months-old plants treated with N from different sources were also harvested on the same dates. At harvest, all plants were divided into leaves, stems and tubers. The tubers were washed with de-ionized water and blotted dry with tissue paper and their fresh weights recorded. Means of 4-6 tubers of plants from the same treatment were calculated and reported as tuber yield. After this, plant organs from all experiments were oven-dried at 80 °C to constant dry weights and ground to a very fine powder. Mean (n = 4-6) total dry matter, tuber dry matter and shoot dry matter were then calculated for each treatment. Stems and leaves of plants from the N-source experiments after dry matter determination were combined and ground together because of their small amounts.

2.2 Field Studies of Marama Bean

2.2.1 Description of experimental study sites in Namibia and Botswana

Soil samples from marama bean-growing sites were collected from Namibia and Botswana on 13-15 May 2001 and 20 May 2002 respectively. In Namibia, soils were collected from two locations; Sandveld and Buitepos (see Fig. 2.1). At Sandveld, marama bean soils were collected from three sites; Sandveld Site 1, Sandveld Site 2 and Sandveld Site 3. These sites were about 5 km apart from each other and were all fenced off and not subject to grazing at the time of sampling. However, Buitepos was an open area near the road and subjected to grazing. Non-marama bean soils were collected from Sandveld Site 4 for use as control. This site was about 5 km south of Sandveld Site 1, and by eye, appears to look like Sandveld Site 3 in terms of topography. In Botswana, marama bean soils were sampled from seven sites namely: Charleshill, Chobokwane, Ghanzi, Groote Laagte Site 1, Makgobokgobo Site 1, Xhoga and Xanagas. These areas (except Makgobokgobo) were within the vicinity of Ghanzi (see Fig. 2.1) and were about 15 km apart. Non-marama bean soils were collected from Groote Laagte Site 2 and Makgobokgobo Site 2 for use as controls. Groote Laagte Site 2 was about 5 km away from Groote Laagte Site 1 and Makgobokgobo Site 2 was about 3 km from Makgobokgobo Site 1. The only observed difference between marama and non-marama sites (control sites) in the field is the absence of marama plants in non-marama sites.

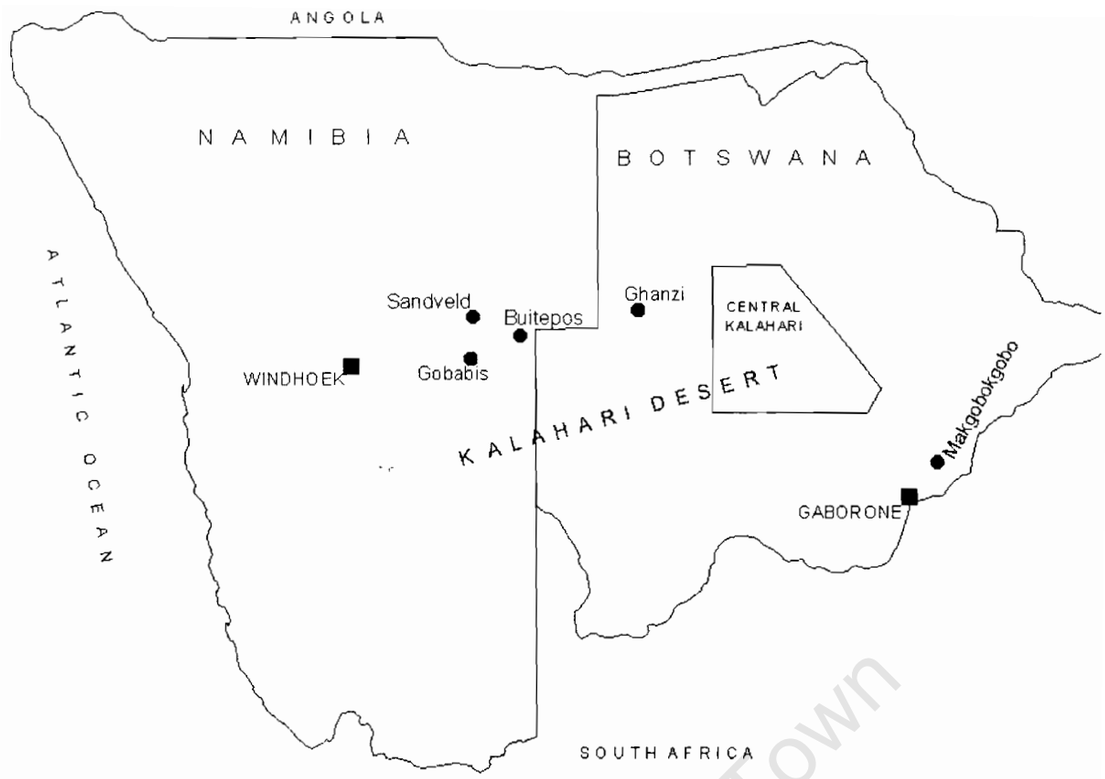

Fig. 2.1. Locations (●) where marama bean soils were sampled in Namibia and Botswana

2.2.2 Soil sampling

Soil samples were collected at different depths (0 – 40 cm, 40 – 80 cm, and 80 – 120 cm), using a soil corer. Three to four samples (for each soil depth) were collected from each site, and placed in plastic bags. They were then air-dried, sieved (2 mm) and their physical characteristics were described before they were analyzed for pH, organic matter, N and other mineral nutrients.

2.2.3. Sampling of field plants

To assess whether marama bean and its associated plant species feed from the same soil-N pools, wild marama bean plants were collected from Namibia (Sandveld and Buitepos)

and Botswana (Makgobokgobo) on 13 - 15 May 2001 and 20 May 2002 respectively. Whole marama bean plants were harvested at Sandveld and Buitepos, while at Makgobokgobo only shoots were sampled. Leaves of other plant species growing in the same area with marama bean were also sampled from both countries for comparisons. The plant species sampled included: *Acacia hebeclada, Bauhinia galpini, Geigeria ornativa, Grewia retinervis* and *Helichrysum melanacme*. The plant samples were processed for tissue elemental analysis by oven-drying at 60 ˚C to a constant weight and grinding to a very fine powder.

2.2.4 Analysis of Growth and reproduction in wild marama bean plants.

Growth parameters of marama bean plants, including number of vines per plant, number of stems and leaves per vine, and vine length. The colour of the stem was also described. Reproductive units such as pod number per plant and seed number per pods or plant were determined in field plants. In order to determine whether marama bean produces a large proportion of single-seeded or double-seeded pods, seed pods were collected from a wild population irrespective of the plant and the number of each pod type counted. Tuber characteristics, which included tuber length, tuber circumference, tuber fresh weight, tuber dry weight and tuber water content, were also determined for field plants of unknown ages.

2.2.5 Response of marama bean to N and P in the field

Two field plots each measuring 25 m x 25 m (randomized complete block design) were set up at Nietvoorbij farm (33˚ 54´S, 18˚ 14´E) in Stellenbosch, Western Cape Region on 31 December 2002. The plots were divided into rows in which plants were planted with a spacing distance of 1 m. The plants were irrigated twice a week. Nitrogen and

phosphorus fertilizers were applied on 4 March 2003. A ring was formed around the plant and fertilizers were applied to plants in granular forms. Phosphorus was added as triple super-phosphate (TSP) at rates of 20 and 40 kg P ha^{-1}. Calcium ammonium nitrate, (in this study shortened to Ca-NH_4NO_3, and which is 27% N) and KNO_3 provided N also at the rates of 20 and 40 kg N ha^{-1}. A combination of TSP and KNO_3 (20 kg Pha^{-1} + 20 kg N ha^{-1}) was also applied to some plants, first the TSP was applied then KNO_3. Plants that serve as control were not supplied with N or P fertilizers. The rings were covered and all plants were subjected to irrigation. Plants were harvested after six months (13 June 2003) and subjected to treatments and analyses described in section 2.1.4.

2.2.6 Analysis of nutrient concentration in plant tissues

Finely ground plant materials were sent to a local laboratory for analysis of nutrient concentrations in tissues. Total nitrogen in tissues was determined using a Kjeldahl method (Bremner, 1965). Analyses of nutrients other than nitrogen were performed on dry ashed plant material (Giron, 1973). A weighed amount of plant material was placed in a crucible and ashed by heating in a muffle furnace at 550 ºC. The ash residue was then dissolved in a hydrochloric acid solution, filtered, diluted to a specific volume and nutrient element concentrations determined on ICP (Inductively Coupled Plasma) spectrophotometer

2.3 Statistical analysis

Differences in soil nutrient concentration between sites, and data collected from the effects of nitrogen and phosphorus supply on marama bean growth were analyzed statistically by a one-way ANOVA, two-way ANOVA or factorial ANOVA as appropriate, and mean separation was made with Duncan's Multiple Range Test using a

STATISTICA package. Where data are reported in percentage, square root transformation was performed before analysis, and for others actual data were used.

Chapter 3: A Biogeographic Analysis of Nutrient Concentrations in Marama Bean Soils

3.1 Introduction

Marama bean (*Tylosema esculentum* Burch. Schreiber) is a high-protein legume adapted to growing in the dry, very nutrient-poor soils of the Kalahari desert, generally characterized as low in organic matter, NO_3^--N, NH_4^+-N and total N (Jobágy and Jackson, 2001). Despite its potential as a crop for marginal soils, this tuber-forming grain legume has remained unresearched and undomesticated. The natural home of the marama bean is the Kalahari Desert, which covers the three Southern African countries of Botswana, Namibia and South Africa. Although the soils of marama bean endemicity are reported to be poor in nutrients (NAS, 1979), no hard data exist as evidence.

The grain and tuber of wild marama bean have been major food for the Khoisan people in Namibia, Botswana and South Africa, hence the need for its domestication. However, to establish any plant species as an agricultural crop requires a clear understanding of its environmental setting, including soil nutrient ecology. Assessing the nutrient concentrations in soils of undomesticated plant species does not only provide an indication of nutrient availability (Foth and Ellis, 1997), but also the amounts to supplement for optimal plant growth and yield under agricultural conditions. Because the supply of mineral nutrients to plants depends on their concentrations in soil (Finck, 1982), knowing the nutrient status of soils is thus a pre-requisite for plant introductions and domestication. This is usually achieved through biological tests or rapid chemical analyses of both plants and soils (Tisdale, 1993).

Data from plant and soil analysis often indicate whether plant growth is likely to be limited by low nutrient supply or not. Such site-specific use of plant and soil analysis is a common feature of modern agricultural practice and plantation forestry (Smethurst, 2000). The fact that marama bean occurs in patchily localized stands probably suggest specific requirements from soil such as nutrients, pH or biotic factors. Desert soils are reportedly low in total N (Jobágy and Jackson, 2001), the sources of which are mainly nitrate, and ammonium in precipitation, Aeolian deposition of nitrate salts, and biological assimilation of atmospheric N_2 by N_2-fixing organisms (Evans and Ehleringer, 1993; Jobágy and Jackson, 2001). Soil- water N generally follows a nutrient- type profile, with concentrations that decrease sharply with depth because of nutrient uptake and cycling (Jobágy and Jackson, 2001). However, recent studies (Walvoord *et al.*, 2003) shows that soil-water concentration profiles of nitrate in five arid to semi arid sites of the Western United States follow the conservative solute-accumulation profiles rather than the expected progressive nutrient depletion profiles. Maximum NO_3^--N concentrations in the subsoil below these nutrient-limited vegetation communities (West and Skuijins, 1978) can exceed 2000 mg per liter, ten times higher than N concentrations applied in hydroponic studies. The aim of this study was to characterize nutrient profiles of soils supporting growth of marama bean plants as a basis for understanding the species adaptation to a nutrient poor environment.

3.2 Materials and Methods

3.2.1 Determination of soil pH

A sample of soil (2 mm sieved) weighing 12.5 g was shaken with 25 mL of 0.01 M $CaCl_2$ for 15 minutes. The pH of the suspension was then measured with a portable ATC pH meter (Hannah Instruments, Portugal)

3.2.2 Determination of soil organic matter

Soil organic matter was determined by a weight loss-on-ignition method, which is based upon measuring the weight loss of the dry soil sample due to high temperature ignition as described by Walkley and Black (1934). A 10-g air-dried and 2 mm-sieved soil samples were placed in porcelain crucible, the mass of the crucibles together with the samples were recorded. The crucibles were placed in an oven at 80 °C for 24 h to drive out soil moisture. After 24 h, the masses of the crucibles were recorded again and the crucibles placed in a muffle furnace at 450 °C for 16 h. After 16 hours, once the furnace had cooled to below 100 °C, samples were removed from a furnace and allowed to further cooling in a dessicator. Samples were re-weighed and the amount of organic matter was determined Walkley and Black (1934). Organic matter for soil from Botswana could only be determined for Makgobokgobo Sites 1 and 2 due to little amounts of soil samples.

3.2.3 Determination of mineral nutrients in soils

Air-dried (2 mm sieved) soil samples were sent to a local soil laboratory for analysis of nitrate-N, ammonium-N, total N and other macro- and micronutrients.

Extraction of macronutrients

Ammonium-N and nitrate-N concentrations in soils were determined by colorimetry, using a Leco FP N-determinator as described by Bremner (1965) and total N was determined by a micro- Kjedahl method (Bremner, 1986; Jones *et al.,* 1991). Phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were extracted from soil by means of 1 % citric acid method of Dyer (1894). A soil sample of 20 g was shaken with 200 mL 1 % citric acid (heated to 80 ºC) in an Erlenmeyer flask and placed in an oven at 80 ºC. This was then shaken every 10 minutes and after 1 h the suspension was filtered through a What Mann paper (No. 40). A 50 mL cold filtrate was heated to dryness on a water bath and the residue heated for two hours to remove organic material. Five mL HCl and 5 mL HNO_3 were added to the residue, shaken and evaporated to dryness on a water bath. The residue was dissolved with 20 mL de-ionized water and 5 mL HNO_3 upon heating and filtered into a 100 mL volumetric flask. When the solution has cooled down to room temperature, it was diluted to 100 mL with de-ionized water. Determination of P, K, Ca, and Mg concentrations was done by direct aspiration on a calibrated simultaneous Inductively Coupled Plasma (ICP) spectrophotometer.

Extraction of micronutrients

Micronutrients in soil samples were extracted, using the di-ammonium EDTA method of Trielweiler and Lindsay (1969) as modified by Beyers and Coetzer (1971). A 5 g sample of finely crushed ($\leq$1 mm) air dry soil was placed in a centrifuge tube and shaken with 15 mL (50mL in case of manganese) of 0.02 M $(NH_4)_2$ EDTA solution for 11 hour at a constant temperature of 20 ± 2 ºC. A sample was then centrifuged at 2000 rounds per

minute for 5 minutes. The solution was filtered and the concentrations of micronutrients were determined by direct aspiration on a calibrated simultaneous ICP spectrophotometer. Boron was extracted with a solution of calcium chloride in hot water as described by FSSA (1974).

3.3 Results

3.3.1 Characteristics of soils from marama bean- and non-marama bean-growing areas

The soils collected from different sites in both Namibia and Botswana were fine aeolian sands characterized by the presence of dolomite or limestone concretions in the soil profile (Table 3.1). Marama bean soils from Sandveld Site 1 were brown and sandy with limestone/dolomite concretions on the surface and subsurface layer, while soils from Sandveld Site 2 and Buitepos had no limestone or dolomite concretions either on the surface or within the soil profile. The soil from Sandveld Site 3 was virtually white sand with carbonate concretions in the profile but not on the surface (Table3.1). The soils from the marama bean-growing sites in Botswana were characterized by brown sand with no dolomite or limestone concretions on the surface or in the soil profile. Of all the sites in Botswana, only Ghanzi was characterized by white sand with dolomite or limestone concretions in the soil profile (Table 3.1). Non-marama soils from Namibia (Sandveld Site 4) were very fine white sands that appeared wet, water-logged and clayish while those from Botswana soils (Groote Laagte Site 2 and Makgobokgobo Site 2) were generally brown sands with no limestone or dolomite concretions in the profile.

Table 3.1 Description of marama and non-marama bean soils collected from Namibia and Botswana

Site	Soil description
Namibia	
Marama bean soils	
Sandveld Site 1	Brown fine aeolian sand with limestone/dolomite concretions on top and subsurface layers
Sandveld Site 2	Brown fine aeolian sand with no limestone/dolomite concretions
Sandveld Site 3	White fine aeolian sand with limestone/dolomite concretions in soil profile but not on surface
Buitepos	Brown fine aeolian sand with no limestone/dolomite concretions
Non-marama bean soil	
Sandveld Site 4	White water-logged very fine sand with limestone/dolomite concretions in profile but not on surface layer. Appeared a bit clayish
Botswana	
Marama bean soil	
Charleshill	Brown fine aeolian sand with no limestone/dolomite concretions
Chobokwane	Brown fine aeolian sand with no limestone/dolomite concretions
Ghanzi	White fine aeolian sand with limestone/dolomite concretions in soil profile
Groote Laagte Site 1	Brown fine aeolian sand with no limestone/dolomite concretions
Makgobokgobo Site 1	Brown fine aeolian sand with no limestone/dolomite concretions
Xanagas	Brown fine aeolian sand with no limestone/dolomite concretions
Xhoga	Brown fine aeolian sand with no limestone/dolomite concretions
Non-marama bean soil	
Makgobokgobo Site 2	Brown fine aeolian sand with no limestone/dolomite concretions
Groote Laagte Site 2	Brown fine aeolian sand with no limestone/dolomite concretions

3.3.2 Soil pH

The pH of marama bean soils (0 –120 cm) collected from Namibia ranged from 5.08 ± 0.08 to 6.90 ± 0.26, while that of non-marama bean soils was 7.12 ± 0.20 (Table 3.2). A significant difference was found between marama bean soils from Sandveld Sites 1 and 2,

and Buitepos, and non-marama bean soils from Sandveld Site 4. The soils from Botswana showed mean pH values of 5.04 ± 0.21 to 7.40 ± 0.25 for marama bean-growing areas and 4.04 ± 0.03 to 4.89 ± 0.16 for non-marama bean soils (Table 3.2). The pH values of marama bean soils from Ghazi, Groote Laagte Site 1, Xanagas and Xhoga were significantly higher than those obtained for control soils from Groote Laagte Site 2 and Makgobokgobo Site 2. Marama bean soils collected from Ghanzi showed the highest pH value of 7.40 ± 0.25 (Table 3.2). The pH of marama bean soils from Groote Laagte Site 1 was significantly different from that of Groote Laagte Site 2, the control from the same location. No significant differences were found between marama and non-marama bean soils collected from Makgobokgobo. For both Namibia and Botswana, acidity values below pH 5 were recorded for only non-marama bean soils. Soil pH values did not change significantly with depth within sites (see Appendix 2A).

3.3.3 Soil Organic matter

The organic matter content of soils collected from Namibia was generally low. The mean organic matter content of marama bean soils (0 – 120 cm) differed significantly for the four Namibian sites. Buitepos showed the lowest organic matter content of 0.30 ± 0.02%, followed by Sandveld Sites 1 and 2 (0.38 ± 0.01%) and the highest (0.44 ± 0.02%) at Sandveld Site 3 (Table 3.2). However these values were significantly lower than those obtained for non-marama bean soils from Sandveld Site 4 (0.59 ± 0.03%). The soil organic matter content obtained for marama bean soils from Makgobokgobo Site 1 in Botswana was significantly higher than that of non-marama bean soils from near the same site (Table 3.2). As with pH, the organic matter content did also not change with soil depth within each site (Appendix 2A, B).

3.3.4 Soil Nitrogen

Total N

Total N in marama bean soils from Namibia was low, ranging from 0.03 to 0.04 % (Table 3.2). Total N of non-marama bean soils were similar to those of marama bean soils, although Sandveld Site 4 had slightly higher total N content compared to the other sites (Table 3.2). Total N in marama bean soils from Botswana ranged from 0.02 to 0.05%, and this did not differ significantly for different sites (Table 3.2). However total nitrogen did not change with soil depth (Appendix 2A, B).

Table 3.2 Soil pH, soil organic matter (SOM) and total N in the 0 – 120 cm profile of soil sampled from different sites in Namibia and Botswana. Values (Mean ± SE, n = 3-4) with the same letter within a column are not significantly different ($P < 0.05$), nd = not determined.

Site	pH	SOM (%)	Total soil N (%)
Namibia			
Marama bean soil			
Sandveld Site 1	6.21 ± 0.31b	0.38 ± 0.01c	0.04 ± 0.000001 a
Sandveld Site 2	5.08 ± 0.08a	0.38 ± 0.01c	0.03 ± 0.000003 a
Sandveld Site 3	6.90 ± 0.26bc	0.44 ± 0.02d	0.041 ± 0.000001a
Buitepos	5.76 ± 0.30b	0.30 ± 0.02b	0.03 ± 0.0000002a
Non-marama bean soil			
Sandveld Site 4	7.12 ± 0.20c	0.59 ± 0.03e	0.05 ± 0.0000003a
Botswana			
Marama bean soil			
Charleshill	5.60 ± 0.06b	nd	0.02 ± 0.000003a
Chobokwane	5.04 ± 0.21a	nd	0.02 ± 0.0000002a
Ghanzi	7.40 ± 0.25c	nd	0.02 ± 0.000005a
Groote Laagte Site 1	6.10 ± 0.11b	nd	0.04 ± 0.000004a
Makgobokgobo Site 1	5.15 ± 0.09ab	0.30 ± 0.01b	0.05 ± 0.000001a
Xanagas	6.03 ± 0.19b	nd	0.04 ± 0.000003a
Xhoga	5.78 ± 0.16b	nd	0.05 ± 0.000001a
Non-marama bean soil			
Groote Laagte Site 2	4.89 ± 0.16a	nd	0.03 ± 0.000007a
Makgobokgobo Site 2	4.04 ±0.03a	0.05 ± 0.001a	0.03 ± 0.0000004a

Levels of inorganic nitrogen across the soil profile

The concentration of the two inorganic N forms (nitrate-N and ammonium-N) in both marama and non-marama bean soils are shown in Table 3.3. Nitrate-N concentration differed with depth for marama bean soils collected from Sandveld Sites 1 and 3 (Table 3.3) and were generally higher in the 0-40 cm depth compared to the 40-80 cm depth, except for Sandveld Site 3. Nitrate-N levels in non-marama bean soils from Sandveld Site 4 followed the same pattern. In general, however, nitrate-N and ammonium-N levels in marama bean soils from Sandveld Site 2 decreased with soil depth. Marama bean soil from Buitepos had relatively high nitrate-N levels in its profile compared to the other marama bean soils from Sandveld.

Although a statistical analysis was not carried out to compare Botswana soils to Namibian soils, nitrate-N and ammonium-N concentrations in soils from Botswana were generally high than levels obtained for Namibian soils. But unlike levels obtained for Namibian soils that were generally similar in magnitude, the ammonium-N concentrations in Botswana soils were relatively higher than nitrate-N concentrations. In Botswana soils, levels of the two inorganic N forms varied across profiles within sites, but the variation was not consistent with depth. Soils from Charleshill and Chobokwane had low nitrate-N concentrations throughout the profile compared to the other sites (Table 3.3). Ammonium-N levels were also found to be lowest in soils from Charleshill. Both marama and non-marama bean soils from Makgobokgobo showed the highest concentrations of ammonium-N in the profile.

Table 3.3 Concentrations of nitrate-N and ammonium- N in the profiles of soil collected from different locations in Namibia and Botswana. Values (Mean ± SE, n = 3-4) with the same letter within a row under each N-type are not significantly different ($P < 0.05$).

Site	Levels of nitrate-N and ammonium-N (mg-N/kg)					
	NO_3^--N			NH_4^+-N		
	0 – 40 cm	40 – 80 cm	80 – 120 cm	0 – 40 cm	40 – 80 cm	80 – 120 cm
Namibia						
Marama bean soil						
Sandveld Site1	0.49 ± 0.09a	0.28 ± 0.06a	0.62 ± 0.07c	0.50 ± 0.10a	0.41 ± 0.08a	0.77 ± 0.08c
Sandveld Site 2	0.60 ± 0.10b	0.26 ± 0.07a	0.24 ± 0.01a	0.45 ± 0.10b	0.34 ± 0.05ab	0.29 ± 0.02a
Sandveld Site 3	0.42 ± 0.06a	0.41 ± 0.02ab	0.63 ± 0.00b	0.62 ± 0.12b	0.27 ± 0.07a	0.54 ± 0.04b
Buitepos	0.96 ± 0.10b	0.62 ± 0.14a	0.50 ± 0.12a	0.67 ± 0.11b	0.45 ± 0.01a	0.44 ± 0.04a
Non-marama bean soil						
Sandveld Site 4	0.69 ± 0.10a	0.53 ± 0.07a	0.68 ± 0.14a	0.47 ± 0.07b	0.33 ± 0.05a	0.33 ± 0.01a
Botswana						
Marama bean soil						
Charleshill	0.72 ± 0.07a	0.69 ± 0.06a	0.60 ± 0.06a	2.88 ± 0.44a	2.76 ± 0.40a	3.78 ± 1.55a
Chobokwane	0.90 ± 0.17b	0.57 ± 0.04a	0.58 ± 0.04a	5.33 ± 0.48b	3.68 ± 0.75a	4.64 ± 0.94ab
Ghanzi	4.10 ± 0.47a	4.36 ± 1.37a	-	5.68 ± 1.56b	3.78 ± 0.85a	-
Groote Laagte Site 1	1.67 ± 0.22a	1.14 ± 0.12a	1.17 ± 0.08a	4.86 ± 1.62a	3.31 ± 0.55a	4.60 ± 1.17a
Makgobokgobo Site 1	5.90 ± 0.00b	3.10 ± 0.21a	3.63 ± 0.81a	9.74 ± 0.00a	14.0 ± 2.80b	12.6 ± 1.50b
Xanagas	2.84 ± 0.49b	1.11 ± 0.33a	0.80 ± 0.13a	5.49 ± 1.14ab	6.35 ± 1.10b	4.97 ± 1.41a
Xhoga	1.51 ± 0.48ab	1.03 ± 0.30a	2.03 ± 1.16b	6.64 ± 0.86a	6.11 ± 0.64a	7.09 ± 1.45a
Non-marama bean soil						
Groote Laagte Site 2	1.37 ± 0.47a	1.22 ± 0.35a	1.22 ± 1.22a	2.98 ± 0.28a	5.48 ± 1.14b	4.09 ± 0.85b
Makgobokgobo Site 2	2.35 ± 0.90ab	2.70 ± 0.75b	2.00 ± 2.00a	13.0 ± 2.91b	13.1 ± 1.05b	9.56 ± 0.40a

3.3.5 Concentrations of macronutrients in marama bean soils

The concentrations of P, Ca and Mg in marama bean soils from Sandveld Sites 1 and 2, and from Buitepos were significantly lower compared to concentrations in non-marama bean soils from Sandveld Site 4 (Table 3.4). With the Botswana soils, however, P concentration in marama bean soils from Ghanzi, Xanagas and Xhoga, Ca from Ghanzi and Xhoga and K from Ghanzi, Xanagas, Xhoga, Charleshill, Chobokwane, Groote Laagte Site 1 and Makgobokgobo Site 1were significantly high than concentrations in non-marama bean soils from Groote Laagte Site 2 and Makgobokgobo Site 2 (Table 3.4). Changes in macronutrient concentrations in soils from Namibia with depth were not consistent although that of calcium and magnesium tended to be higher in the 80-120 cm layer (Appendix 2C). However Botswana soils from Charleshill, Chobokwane, Xanagas and Xhoga showed high P concentrations in the top 0 – 40 cm layer (Appendix 2D).

3.3.6 Concentrations of micronutrients in marama bean soils

The concentrations of Cu and Mn in marama bean soils from Namibia were low compared to those in non-marama bean soils (Table 3.5). In contrast, Zn and Mn concentrations in marama bean soils from Botswana were high compared to their levels in non-marama bean soils (Table 3.5). Overall, micronutrient concentrations were higher in Namibian soils than in Botswana soils.

The concentration of Cu and Zn in marama bean soils from Sandveld Site 3 in Namibia increased with soil depth, while Mn and Al decreased with depth (Appendix 2E). The concentrations of Cu, Mn and Al concentrations in soils from Botswana did not differ significantly with depth. Although significant changes were observed for Zn concentrations, these were not consistent (Appendix 2F).

Table 3.4 Concentrations of macronutrients in the profiles of soil collected from different locations in Namibia and Botswana. Values (Mean ± SE, n = 3-4) with the same letter within a column for each country are not significantly different at $P < 0.05$.

Site	Macronutrient concentrations (mg/kg) in 0 – 120 cm soil depth			
	P	Ca	Mg	K
Namibia				
Marama bean soil				
Sandveld Site1	3.56 ± 0.29b	898 ± 43.1 b	99.0 ± 2.1b	77.4 ± 6.67b
Sandveld Site 2	3.75 ± 1.59bc	122 ± 0.40a	37.5 ± 1.5a	57.8 ± 3.16a
Sandveld Site 3	3.56 ± 0.53b	1502 ± 4.0c	196.5± 5.85c	97.0 ± 3.22bc
Buitepos	2.67 ± 0.26a	198 ± 4.60a	45 ± 6.00a	133 ± 15c
Non-marama bean soil				
Sandveld Site 4	4.25 ± 0.20c	1750 ± 57.4c	226.5 ± 7.50c	66.1 ± 4.97ab
Botswana				
Marama bean soil				
Charleshill	1.33 ± 0.17a	276 ± 1.80b	69 ± 0.60a	80.7 ± 1.99c
Chobokwane	1.44 ± 0.18a	166 ± 4.00b	85.5 ± 0.60a	65.0 ± 3.24b
Ghanzi	7.00 ± 1.37c	1136 ± 6.00c	82.5 ± 1.50a	64.5 ± 3.69b
Groote Laagte Site 1	1.67 ± 0.17ab	296 ± 2.00b	268.5 ± 2.70c	72.0 ± 1.99bc
Makgobokgobo Site 1	1.00 ± 0.00a	298 ± 2.00b	102 ± 1.2b	73.5 ± 4.80bc
Xanagas	2.67 ± 0.53b	314 ± 6.00b	82.5 ± 1.80a	73.6 ± 2.19bc
Xhoga	2.11 ± 0.26b	810 ± 10.0c	123.0 ± 1.50b	85.9 ± 8.98c
Non-marama bean soil				
Groote Laagte Site 2	1.11 ± 0.11a	152 ± 20.0b	84.0 ± 0.90a	47.9 ± 3.34a
Makgobokgobo Site 2	1.25 ± 0.16a	32 ± 1.80a	48.0 ± 0.90a	38.3 ± 1.81a

Table 3.5 Micronutrients concentrations in marama and non-marama bean soils sampled from different locations in Namibia and Botswana. Values (Mean ± SE, n = 3-4) followed by similar letters within a column for each country are not significantly different at P < 0.05.

Micronutrient concentrations (mg/kg) in 0 – 120 cm soil depth				
Site	Cu	Zn	Mn	Al
Namibia				
Marama bean soil				
Sandveld Site1	0.51 ± 0.10b	1.41 ± 0.26b	47.4 ± 5.10c	18.5 ± 1.11b
Sandveld Site 2	0.20 ± 0.04a	1.00 ± 0.28ab	28.7 ± 2.21b	13.0 ± 0.96a
Sandveld Site 3	0.67 ± 0.10b	1.51 ± 0.49b	43.1± 7.31c	23.0 ± 2.60b
Buitepos	0.17 ± 0.02a	1.13 ± 0.20ab	18.7 ± 1.43a	12.2 ± 0.75a
Non-marama bean soil				
Sandveld Site 4	1.53 ± 0.23c	1.73 ± 0.48b	93.2 ± 27.2d	25.5 ± 3.74b
Botswana				
Marama bean soil				
Charleshill	0.31 ± 0.01b	0.54 ± 0.08a	24.3 ± 0.62bc	0.32 ± 0.01a
Chobokwane	0.19 ± 0.01a	0.84 ± 0.19b	7.70 ± 1.07a	0.33 ± 0.03a
Ghanzi	0.86 ± 0.09d	0.86 ± 0.17b	20.0 ± 2.74b	0.37 ± 0.01a
Groote Laagte Site 1	0.26 ± 0.01ab	1.06 ± 0.20a	18.7 ± 0.65b	0.30 ± 0.00a
Makgobokgobo Site 1	0.42 ± 0.02c	1.20 ± 0.26b	31.5 ± 1.07c	0.55 ± 0.03b
Xanagas	0.38 ± 0.02bc	0.51 ± 0.04a	19.1 ± 0.96b	0.27 ± 0.01a
Xhoga	0.79 ± 0.02d	1.05 ± 0.24b	64.6 ± 8.35d	0.39 ± 0.04a
Non-marama bean soil				
Groote Laagte Site 2	0.14 ± 0.01a	0.66 ± 0.08a	6.88 ± 1.00a	0.31 ± 0.04a
Makgobokgobo Site 2	0.37 ± 0.03bc	0.51 ± 0.05a	9.53 ± 0.90a	1.99 ± 0.14c

3.4 Discussion

As shown in Table 3.1, soils from patchy areas without marama bean plants ("non-marama bean soils") were by description not markedly different from soils that support large populations of marama bean plants ("marama bean soils") within the same site. Whether in Namibia or Botswana, marama bean soils were characterized by brown or white aeolian sand i) without limestone/dolomite concretions on the top and subsurface layers, ii) with limestone/dolomite concretions in the soil profile but not on the surface, and iii) with limestone/dolomite concretions on top and in subsurface profile.

The non-marama bean soils from Namibia differed in being water-logged and slightly clayish but also contained limestone/dolomite concretions (Table 3.1). However, the non-marama bean soils from Botswana were similar in physical characteristics to 6 out of 7 marama bean soils in not having any limestone/dolomite concretions. These characteristics of marama bean soil profile are consistent with those described by (De Frey, 1990).

In terms of soil properties, the pH and soil organic matter differed significantly ($P \leq 0.05$) between marama bean and non-marama bean soils (Table 3.2). For example, the pH of non-marama bean soil from Sandveld Site 4 in Namibia was markedly higher than those of the marama bean soils, and this was accompanied by significantly greater organic matter content as a consequence of the water-logging, which can inhibit mineralization (Brandy 1990).

Although in Botswana the pH and soil organic matter also differed between marama bean and non-marama bean soils, here both parameters were significantly lower in non-marama bean soils compared to marama bean-growing soils (Table 3.2).

Taken together, anoxia from water-logging at Sandveld Site 4 can affect root and tuber respiration through cytoplasmic acidosis and loss of membrane integrity (Greenway and Gibbs, 2003) leading to plant death and its absence in the site. Additionally, the low pH of non-marama bean soil from Makgobokgobo Site 2 can repress nitrification and thus reduce nutrient supply to plants or directly inhibit root growth. It is however unlikely that only these two factors caused the absence of marama bean plant populations in the non-marama sites.

Although the data on soil nutrient analysis have revealed broad differences between marama bean and non-marama bean soils (Tables 3.3, 3.4 and 3.5), site-specific comparisons could provide deeper insights into the factors affecting the biogeographic distribution of marama bean in the Kalahari regions of Southern Africa.

Plants in arid and semi arid systems have been shown to respond morpho;ogically and physiologically to areas of high nutrient availability within their rooting zones (Jackson *et. al.*, 1990). The concentrations of NO_3^-- N and NH_4^+- N (Table 3.3) did not follow the expected progressive nutrient depletion profiles and they also did not follow the recently discovered progressive nutrient accumulation profiles beneath desert soils (Walvoord *et al.*, 2003), although in this study soil resources profiles were only reported to a depth of 1.20 m.

A comparison of non-marama bean soils and marama bean soils did not reveal a consistent trend, for example, when P, Ca and Mg concentrations of the non-marama bean soils from Sandveld Site 4 were compared with those of marama bean soils from Sandveld Sites 1, 2 and 3, the latter showed significantly lower levels except for Sandveld Site 3, where the values are similar (Table 3.4). Even with the distant Buitepos

site, the levels of these macronutrients were markedly lower. Clearly, then, the absence of marama bean populations in Sandveld Site 4 could be closely linked to the differences in soil macronutrient concentrations. Interestingly, the description of Sandveld Sites 3 and 4 (Table 3.1) are near-identical, except that Site 4 was water-logged and slightly clayish. It is therefore not surprising that the macronutrient data for Sandveld Sites 3 and 4 are not different from each other. That water-logging could be a major factor responsible for the absence of marama bean in Sandveld Site 4 is probably re-enforced by the magnitude and commonality of macronutrient concentrations at the two sites.

A similar comparisons between non-marama bean soil from Makgobokgobo Site 2 with marama bean soil from Makgobokgobo Site 1 in Botswana shows that the latter is higher in the concentrations of Ca, Mg and K compared to the former. The concentrations of Mg and K showed a similar pattern for the two types of soil from Groote Laagte Sites 1 and 2.

The concentrations of micronutrients have also provided useful information that probably supports the role of nutrients in marama bean distribution. As shown in Table 3.5, the concentrations of Cu and Mn in non-marama bean soil from Sandveld Site 4 in Namibia were distinctly higher than those of marama bean soil from Sandveld Sites 1, 2 and 3, as well as the distant Buitepos site. There was however no clear pattern for Zn and Mn, although in this case, as with the macronutrients, the concentrations in non-marama soils were lower than those in marama bean soils (Table 3.3). Taken together, these data suggest a relationship between marama bean distribution and mineral profiles in soils. However, much more refined experimentation is required to specifically define the role played by nutrients in the distribution of marama bean. The preliminary data obtained in

this study would seem to suggest that cultivation of marama bean outside its area of endemicity is possible, so long as excessive soil moisture is avoided. It should however be noted that there are other factors such as competition, dispersal ability, genetic factors, as well as land use and habitat that can affect marama bean distribution in the wild. It is also recommended that, a transplant experiment is required in order to demonstrate that the soils that marama bean were not growing in are not appropriate for the bean's growth.

Chapter 4: Assessing N and P nutrition in Marama Bean Plants under Semi-Controlled Conditions

4.1 Introduction

Of all the nutrients, nitrogen and phosphorus are the one required in large amounts for plant growth. Nitrogen, in one form or another, accounts for about 80% of the total mineral nutrients absorbed by plants (Marschner, 1995). More over, inadequate nitrogen is often the growth-limiting nutritional stress in soils. Consequently, addition of N usually improves plant growth yield. While nitrogen is taken up mainly as ammonium and nitrate ions, phosphorus is absorbed as the orthophosphate ions; $H_2PO_4^-$, HPO_4^{2-} depending on the soil pH (Marschner, 1995). Phosphorus and nitrogen however, are deficient in most soils. Applying chemical fertilizers as a way of increasing nutrient supply promotes plant growth and yield. The use of glasshouse studies and pot experiments can assist in our understanding of plant response to exogenous supply of nutrients, especially when dealing with undomesticated species. The data from such controlled studies forms the basis for field experimentation, especially in relation to the quantities of nutrients required and the extra yields obtained from fertilization (Cooke, 1975). In short, it is essential to establish the relationship between nutrient supply and yield response of new undomesticated crop plants as done for conventional crops such as sugar beet and spring wheat which increased yields linearly with increased nitrogen supply (Boyd, 1970).

In the case of N, it is important to establish whether the supply of sole ammonium or NO_3^- is better for plant growth and higher yields, as growth may be faster with one ion species than the other. Available evidence shows that plant species differ in their growth

response to the different N ions. Although N assimilation is associated with the reduction of NO_3^- to NH_4^+, many plants show growth inhibition when NH_4^+ is supplied as the exclusive N source (Gerendás *et al.*, 1997; Raab and Terry, 1994; Jungk, 1977; Kirkby and Hughes, 1970). Growth inhibition has been attributed to various factors, such as NH_4^+-induced disorders in pH regulation and toxic effects of free ammonia (Claussen and Lenz, 1995; Goyal *et al.*, 1982). The results of a study by Lasa *et al.* (2001) showed spinach to be highly sensitive to NH_4^+ nutrition, while sunflower was moderately sensitive and pea tolerant. Often, plants fed NH_4^+-N tend to accumulate less biomass (Cramer and Lewis, 1993; Matsumoto and Tamura, 1981; Schortemeyer *et al.*, 1996), exhibit high total nitrogen content (Cox and Reisenauer, 1973; Cramer and Lewis, 1993; Lasa *et al.*, 2001), and show low concentrations of Ca, Mg, and K concentration in their tissues (Cox and Reisenauer, 1973; Marschner, 1995).

In general, plant growth with combined NO_3^- -N and NH_4^+-N is faster than with either one of the two species (Bigg and Daniel, 1978; Ganmore- Neumann and Kafkafi, 1980; Haynes and Goh, 1978; Lewis *et al.*, 1982). Nitrogen nutrition is known to be an important factor in the growth of tuber crops. For example, excessive nitrogen fertilization can extend the vegetative growth period and delay tuber development (Phillips *et al.*, 2004; Clark and Burge, 1999). However, the effect of nitrogen form on the growth of tuber crops is not well documented. In this study, the effect of nitrate and phosphate nutrition or N source (NH_4^+, NO_3^- or NH_4NO_3) on growth and tuber yield of marama bean plants was assessed. The study included measurements of chlorophyll, concentration, nitrate reductase activity (NRA), tissue carbohydrate concentrations, and mineral composition of marama bean organs.

4.2 Materials and Methods

4.2.1 Measurement of chlorophyll concentration in marama bean leaves

The procedure for chlorophyll determination was as described by Hiscox and Israelstam (1979), where di-methyl sulphoxide (DMSO) is used to extract the chlorophyll from fresh leaves. Fresh leaves (third leaf from the tip) were plucked from plants treated with nitrate of different concentrations and from plants treated with N from different sources to test whether nitrogen concentration and its sources have any effect on the chlorophyll concentration of marama bean leaves.

Leaf tissues weighing about 100 mg (the middle portion of the leaf) were cut into small fractions and placed in 15 mL plastic tubes containing 7 mL DMSO and incubated at 4 °C for 72 h. After incubation, the extract was diluted to 10 mL with DMSO, 3 mL of the extract was transferred to the cuvette and its absorbance was read on a spectrophotometer at 645 and 663 nm against the DMSO blank. The concentration of total chlorophyll was calculated by setting up simultaneous equations using the specific absorption coefficients for chlorophyll a and b as described by Arnon (1949).

4.2.2 Determination of nitrate reductase activity in organs of marama bean

Nitrate reductase activity (NRA) was assayed using the *in vivo* method of Jaworski (1971). The incubation mixture contained 0.1 M phosphate buffer (pH 7.7) and 0.1 M nitrate. The propanol concentration used was 1 % (v/v), this increase the permeability of the tissue to nitrate and nitrite (Blacquiére and Troelstra, 1986). The reaction was conducted anaerobically in the dark to prevent oxygen from competing with nitrite for endogenously generated reduced pyrimidine nucleotide and reduction of nitrite produced

to ammonia, by flushing with nitrogen gas. Duplicate samples of 0.4 g fresh marama leaves were cut into small pieces added to 10 mL of the incubation mixture and incubated in a water bath at 30 °C. From these, duplicate samples of 0.5 mL were taken after 20 min of incubation. To these, 1 mL sulphanilamide and 1 mL N-1-naphthyl-ethylene-diamine-dihydrochloride solution (NED) were added and mixed by vortex. The nitrite concentration (μmoles NO_2^-.g $Fwt^{-1}h^{-1}$) was calculated from the absorbance of the developing pink colour at 540 nm, using a nitrite standard curve.

4.2.3 Carbohydrate analysis

Soluble non-structural carbohydrates (sugar) were extracted from dried, finely ground samples of plant leaves, stems and tubers with 80 % v/v ethanol, and auto-extracted at 0 ºC for 72 h. The extracts were centrifuged and the supernatant adjusted to 25 mL. The ethanol insoluble non-structural carbohydrates (starch) were hydrolyzed from the air-dried pellets from the sugar extraction with boiling 3.2% HCl (3 mL) for 3 hours. Carbohydrate concentrations were calculated from the absorbance of the developing yellow colour of the phenol-sulphuric acid reaction at 490 nm, using a calibration curve for glucose as described by Buysse and Merckx (1993).

4.3 Results

4.3.1 Effects of nitrate application and N-source on the concentration of total chlorophyll in marama bean leaves

When marama bean plants were supplied with different concentration concentrations of nitrate, the concentration of chlorophyll in leaves increased with increasing nitrate supply (Fig. 4.1A), and was significantly different from that of the control for 2, 5 and 10 mM nitrate irrespective of the year of study. Chlorophyll concentrations in leaves of plants

from Year II experiments were generally higher than those obtained for plants from Year I experiments. The highest chlorophyll concentration was obtained when plants were supplied with 5 mM nitrate (25.31 and 34.56 mg.gFwt^{-1} for Years I and II respectively). When plants were fed with nitrogen from different N-sources, the chlorophyll concentration of leaves from Year I experiments was not affected by N-source (Fig 4.1 B). However with Year II experiments, chlorophyll concentration increased significantly with the supply of 2 mM nitrate or 1 mM ammonium nitrate compared to control (Fig. 4.1B), but was unaffected by the application of 2 mM ammonium.

4.3.2 Effects of nitrate application and N-source on nitrate reductase activity in leaves of marama bean plants

The NRA of leaves from both Years I and II experiments increased significantly with nitrate supply (Fig. 4.2). As was the case with chlorophyll concentration, leaf NRA data for Year II experiments were generally higher in magnitude than those obtained for Year I. Nitrate reductase activity of tubers determined for Year II experiments was significantly increased at only the 10 mM nitrate level (Fig. 4.2).

In order to confirm the results from the N-source experiments, the assay of nitrate reductase was performed several times for each set of experiments (4 times for Year I experiments and 3 times for Year II experiments) and the averages of these were computed as shown in Fig 4.3. During both years, nitrate reductase of marama bean leaves was significantly increased when ammonium was supplied as an exclusive source of N as well as by the application of 2 mM nitrate. The highest leaf NRA was obtained when plants were supplied with ammonium as an exclusive N source for both years.

Although there was a general increase in leaf NRA for both years when plants were supplied with 1 mM NH_4NO_3 compared to control, the increase was only significant for the first year.

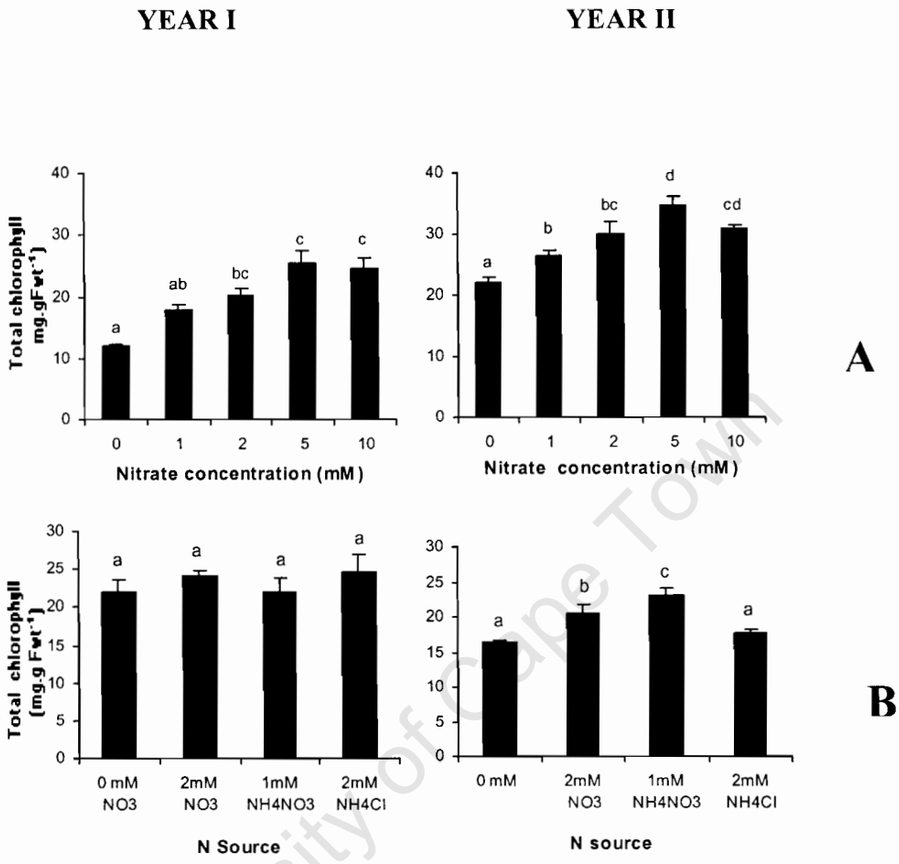

Figure 4.1. The effects of A) nitrate application and B) nitrogen source on chlorophyll concentration of marama bean leaves harvested from year I and year II pot experiments. Bars (means ± SE, n = 4-6) with different letters are significantly different ($P \leq 0.05$)

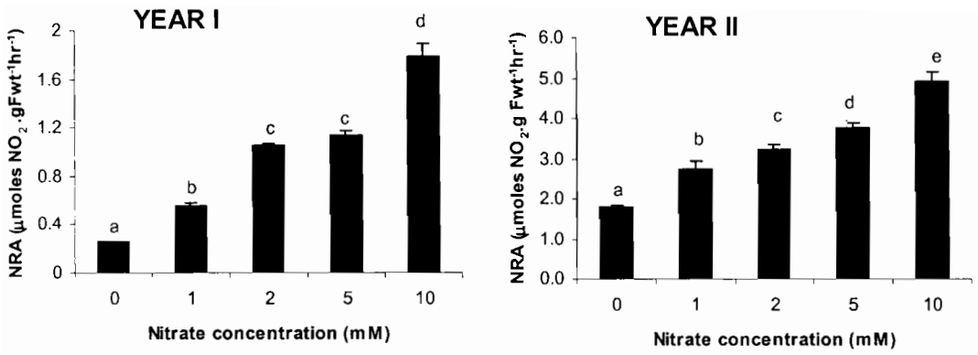

Figure 4.2. The effects of nitrate concentration on nitrate reductase activity of marama bean leaves harvested from year I and year II experiments respectively. Bars (means ± SE, n = 6) with different letters are significantly different ($P \leq 0.05$).

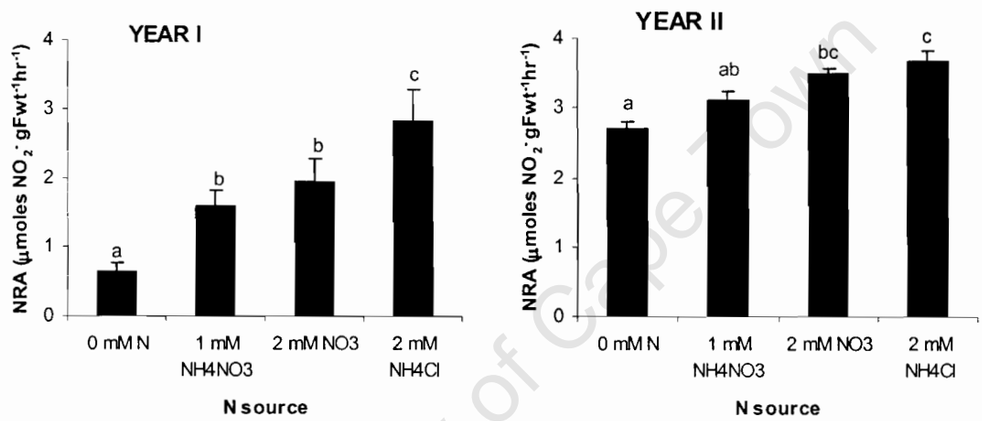

Figure 4.3. The effects of nitrogen source on nitrate reductase activity of marama bean leaves of pot-grown plants during two different years of growth. Bars (means ± SE, n = 16-24) with different letters are significantly different ($P \leq 0.05$).

4.3.3 Effects of nitrate application, N-source and phosphate application on marama tuber yield

When the effect of nitrate supply on marama bean was assessed, tuber yield of 15-months-old plants from Year I increased significantly with increasing NO_3^- concentration relative to the control plants, almost reaching a plateau at 2 mM nitrate with only minimal increments in leaf NRA at concentrations higher than this (Fig. 4.4A). In year I, the highest tuber yield (118.8gFwt.plant^{-1}) was obtained at 10 mM NO_3^-, while with Year II, the 2 mM NO_3^--fed plants produced the biggest tubers (122.1 g Fwt.plant^{-1}) as shown in Fig. 4.4

The effect of phosphate application on tuber yield was not consistent. The highest yields obtained for Year I (90.5 ± 23.7 gFwt.plant^{-1}) and Year II experiments (131.0 ± 13.0 gFwt.plant^{-1}) come from plants supplied with 5 mM phosphate; and this increase was significant for only Year II experiments when compared to control (Fig.4.4).

In the experiments involving different N-sources, tuber yield of marama bean plants increased significantly compared to control when supplied with either 1 mM ammonium nitrate or 2 mM nitrate in both Year I and II. However, the supply of NH_4^+-N did not have any significant effect on tuber yield of marama bean plants (Table 4.1). Infact, for year II, the application of NH_4^+ as an exclusive N source decreased the marama bean tuber yield compared to control.

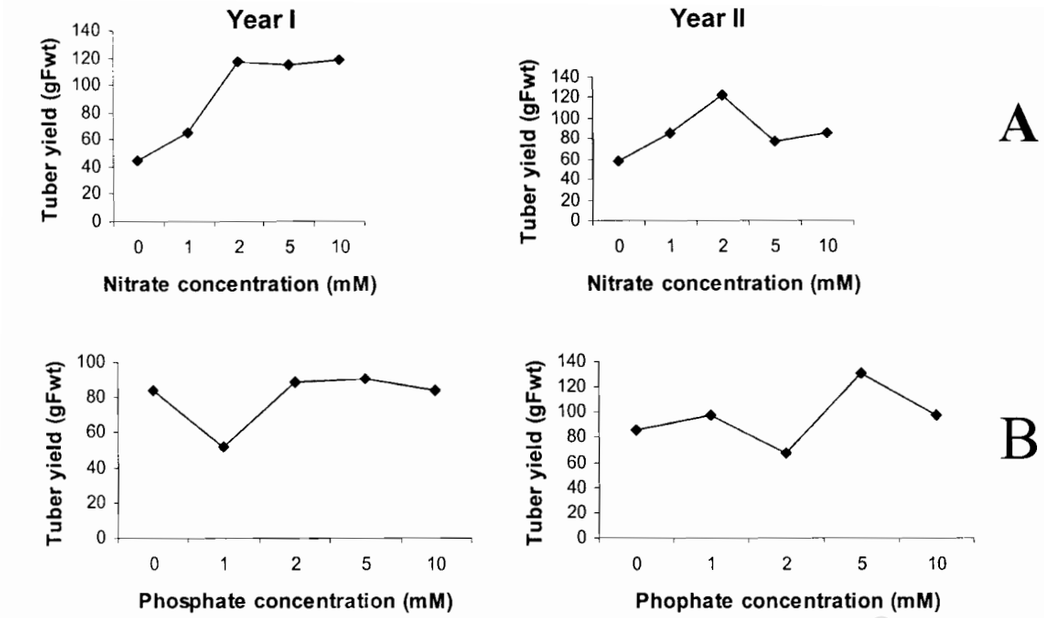

Figure 4.4 The effect of nitrate and phosphate application on tuber yield of pot-grown marama bean plants during two different growth years, n = 6.

Table 4.1 The effect nitrogen source on marama bean tuber yield**.** Values (Mean ± SE, n = 6) followed by the same letter within a column are not significantly different (P ≤0.05).

	Year I tuber yield (g Fwt)	Year II tuber yield (g Fwt)
N-source		
0 mM N	25.13 ± 3.85a	34.11 ± 2.44ab
1 mM NH_4NO_3	34.02 ± 3.00bc	39.15 ± 1.84b
2 mM NO_3^-	37.09 ± 5.74c	37.52 ± 3.70b
2 mM NH_4^+	30.36 ±3.54ab	29.28 ± 1.23a

4.3.4 Effects of nitrate application, N-source and phosphate application on marama bean growth and dry matter yield

Applying 2, 5 or 10 mM NO_3^- significantly increased shoot, tuber and total dry matter of plants from Year I experiments (Fig. 4.5). Shoot dry matter of plants from Year II

experiments were significantly increased when plants received 2 mM or 10 mM NO_3^-. However, tuber dry matter and total dry matter values were highest with 2 mM NO_3^- supply to Year II plants (Fig. 4.5).

When marama plants were supplied with nitrogen from different N-sources, dry matter of shoots from Year I experiments was significantly increased by the application of 2 mM NO_3^-. Tuber and total dry matter also increased when plants were supplied with 2 mM NO_3^-, but they were not significantly different from that of control plants (Fig .4.6). This effect was not reproduced in plants from Year II experiments. Dry matter accumulation in plants from Year II experiments was significantly increased with provision of 1 mM ammonium nitrate. However, when either 2 mM NO_3^- or ammonium alone was supplied, plant dry matter was not significantly affected compared to control (Fig. 4.6).

Phosphate application produced mixed results. Feeding 1 mM phosphate to marama bean significantly reduced dry matter in Year I experiments. But shoot, tuber and total dry matter were not significantly affected by supply of 2, 5 or 10 mM phosphate (Fig. 4.7). However, shoot dry matter in Year II experiments was significantly increased in plants receiving 1 mM phosphate, in contrast to the results obtained for Year II experiments. Although supplying 10 mM phosphate significantly increased shoot dry matter, tuber and total plant biomass was not significantly affected by phosphate application (Fig. 4.7).

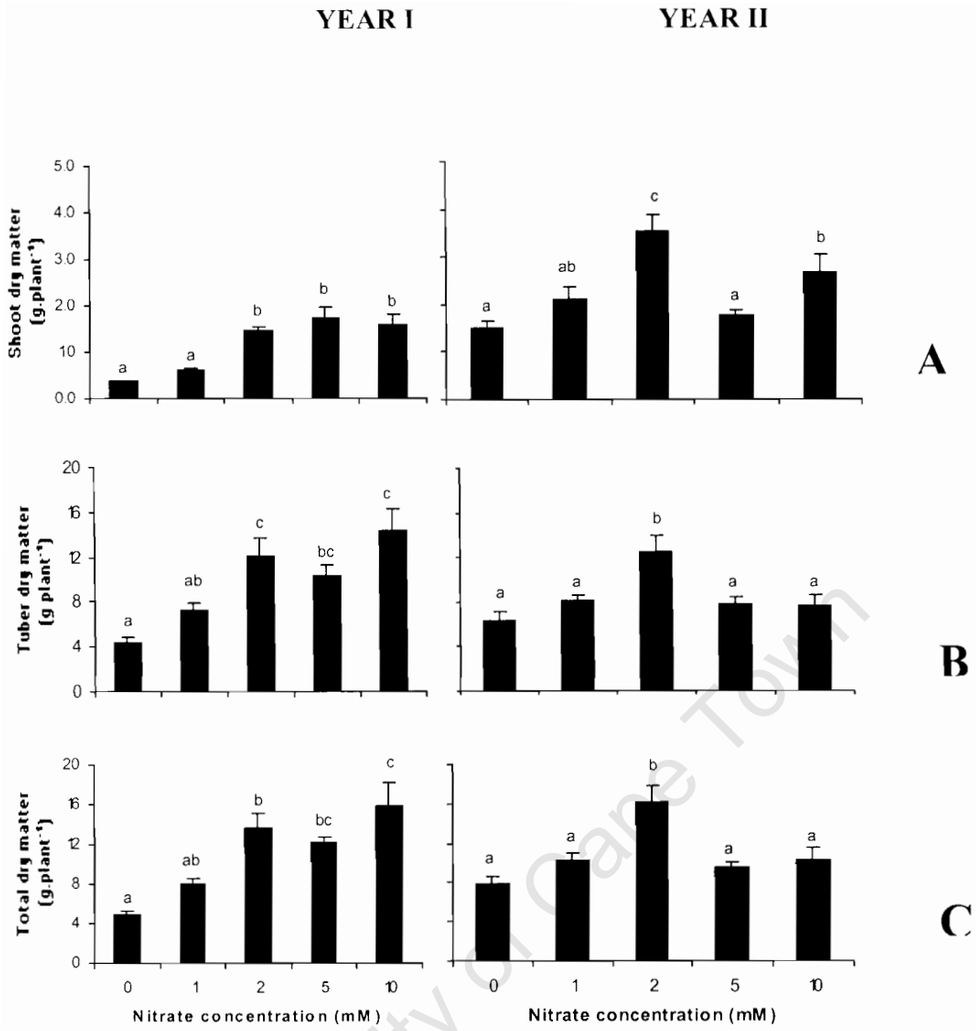

Figure 4.5. Dry matter of A) shoots, B) tubers and C) marama bean plants exogenously supplied with nitrate and harvested from year I and year II experiments. Bars (means ± SE, n = 4-6) with different letters are significantly different ($P \leq 0.05$).

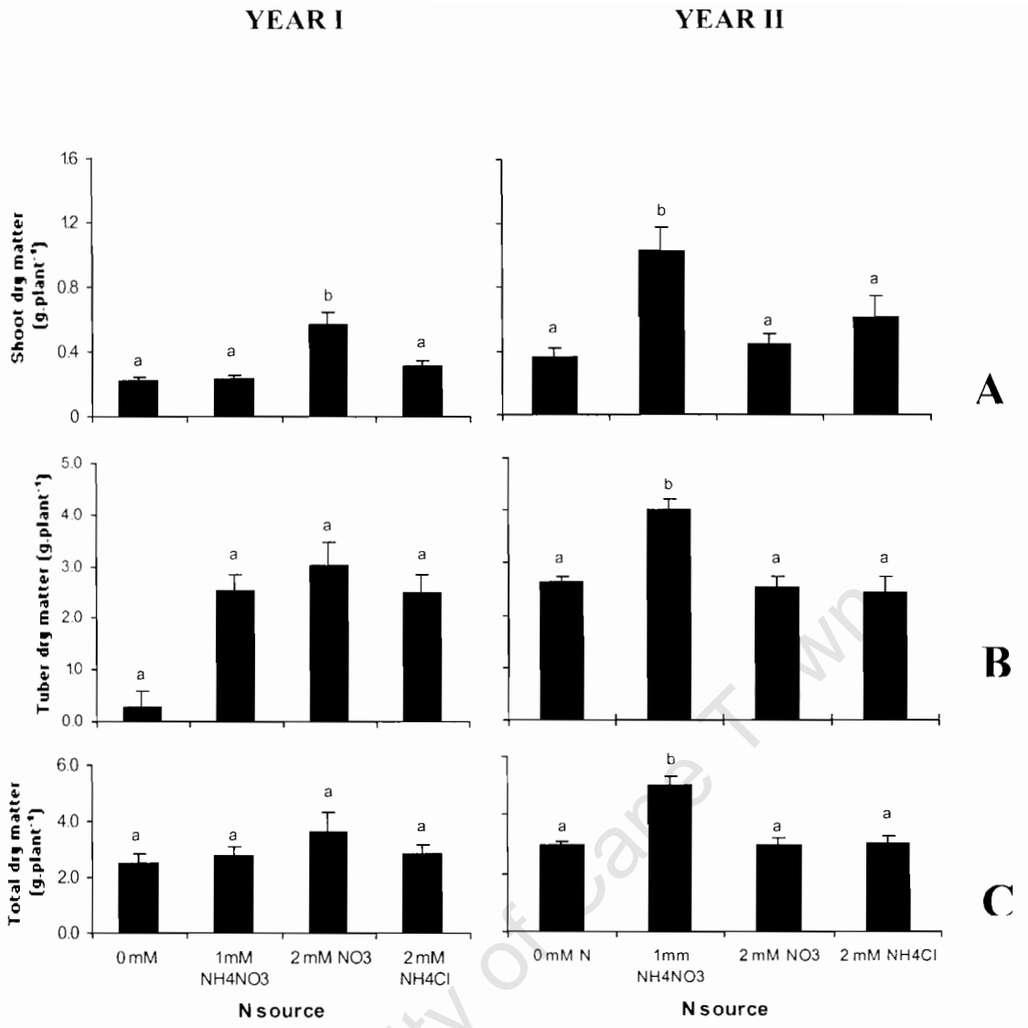

Figure 4.6. Dry matter of A) shoots, B) tubers and C) marama bean plants exogenously supplied with nitrogen from different sources and harvested from year I and year II experiments. Bars (means ± SE, n = 6) with different letters are significantly different ($P \leq 0.05$)

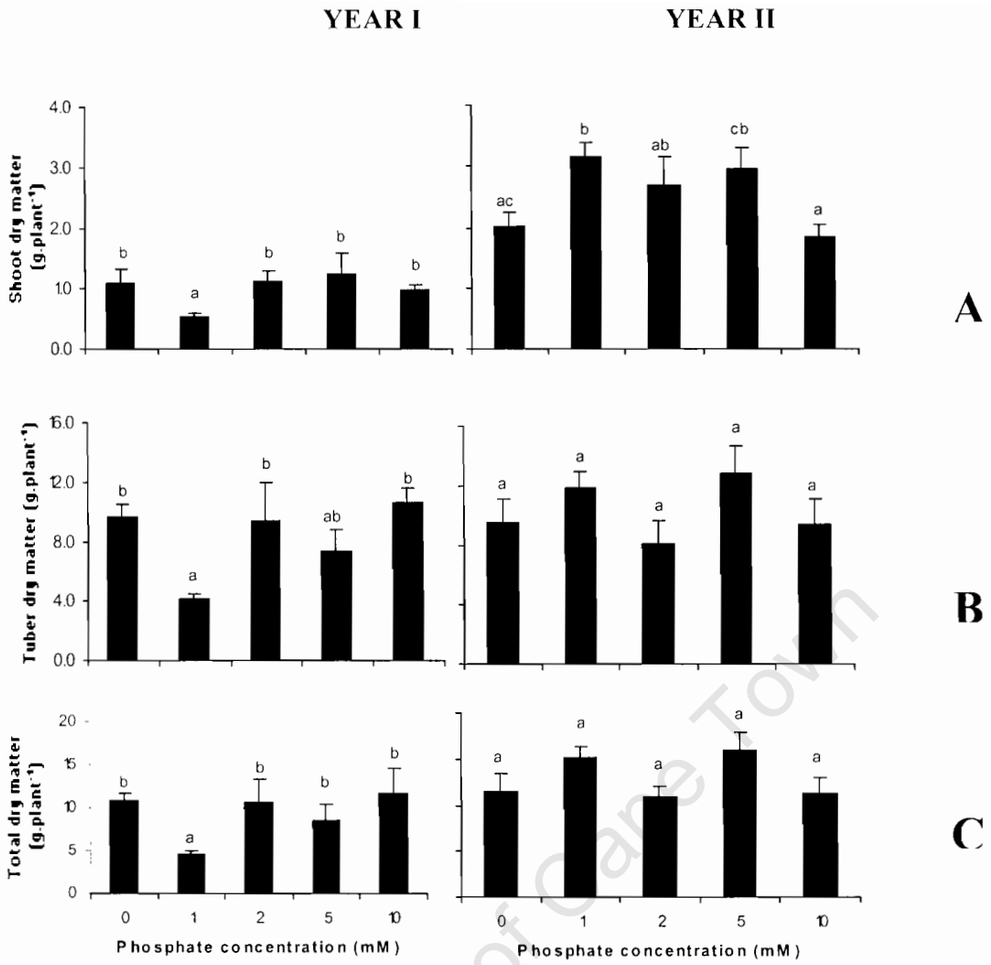

Figure 4.7. Dry matter of A) shoots, B) tubers and C) marama bean plants exogenously supplied with phosphate and harvested from year I and year II experiments. Bars (means ± SE, n = 4-6) with different letters are significantly different ($P \leq 0.05$).

4.3.5 Effects of nitrate application on carbohydrate concentration of marama bean plants

The plants from Year II experiments showed higher sugar concentrations than those from Year I experiments. As shown in Table 4.2, the tissue sugar concentrations decreased

significantly with increasing NO_3^- supply to marama bean plants. The concentration of non-soluble carbohydrates (starch) in organs of marama bean followed the same pattern as the sugars, in that they were not only higher in Year II than Year I, their levels were also higher in tubers followed by stems and leaves. Relative to control, leaf starch concentrations in Year I experiments were markedly reduced with 1, 5 or 10 mM NO_3^- supply (Table 4.2). However, starch concentrations of stems and tubers from Year I were significantly lower in plants supplied with 1, 2 or 5 mM NO_3^- relative to control plants. The concentration of starch in leaves and stems from Year II experiments were not affected by NO_3^- supply, although the levels in tubers were significantly reduced relative to control plants (Table 4.2).

4.3.6 Effects of phosphate application on carbohydrate concentration of marama bean

An assessment of P supply on marama bean plants showed that sugar concentrations were highest in tubers, followed by stems, and finally leaves. However the species response to P was not consistent. Leaf sugar concentrations of Year I plants were significantly reduced when fed 10 mM phosphate compared to control. In addition, while stem sugar concentrations increased significantly in plants receiving 1 mM phosphate, it decreased at higher levels, 5 and 10. The concentration of sugar in tubers also reduced when plants were supplied with 1, 2, and 10 mM phosphate, and increased significantly with the supply of 5 mM phosphate. However, phosphate application had no significant effect on the sugar concentration of plants from Year II experiments. Similarly, the starch concentration of marama bean plants was not affected by phosphate supply in both Year I and II experiments (Table 4.3).

4.3.7 Effects of nitrate supply and nitrogen source on total nitrogen concentration of marama bean tissues

Nitrogen concentration measured as % N, was generally higher in shoots than in tubers with NO_3^- supply. As shown in Table 4.4, tissue concentration of N increased significantly in shoots and tubers at higher levels of NO_3^- supply compared to control plants. Except for tubers, which showed an increase in N concentration with the provision of 2 mM NO_3^- and 2 mM NH_4^+, there was no response by other organs to the source of N supplied to plants (Table 4.4).

Table 4.2 Effects of nitrate application on sugar and starch concentration of marama bean plant organs. Values (Mean ± SE, n = 4-6) followed by the same letter within a column are not significantly different ($P \leq 0.05$).

	Carbohydrate content (mg/g dry matter)		
Year I			
	Leaves	Stem	Tuber
Nitrate supply (mM)		*Sugar*	
0	132 ± 1.9d	189 ± 4.7d	355 ± 32.3b
1	114 ± 6.0cd	137 ± 11.7c	272 ± 19.6a
2	99.1 ± 4.3cb	109 ± 3.6bc	252 ± 4.3a
5	87.6 ± 4.8ab	85.6 ± 4.9ab	236 ± 3.5a
10	72.8 ± 1.0a	74.4 ± 2.1a	221 ± 7.6a
Nitrate supply (mM)		*Starch*	
0	38.9 ± 0.20c	48.2 ± 0.73c	82.2 ± 3.77c
1	15.7 ± 0.42a	19.8 ± 0.55a	71.1 ± 3.53bc
2	39.1± 0.25c	31.7 ± 1.30b	48.1 ± 0.67a
5	30.6 ± 0.40b	46.6 ± 8.79c	58.1 ± 0.46ab
10	30.0 ± 0.38b	49.7 ± 0.11c	70.7 ± 4.08bc
Year II			
Nitrate supply (mM)		*Sugar*	
0	188 ± 7.0d	325 ± 21.8c	692 ± 109d
1	169 ± 4.8c	231± 11.2d	558 ± 35.7c
2	129 ± 5.2b	184 ± 2.65c	415 ± 48.7b
5	117 ± 5.5b	143 ± 13.7b	315 ± 14.4ab
10	71.8 ± 4.8a	101 ± 6.67a	254 ± 41.2a
Nitrate supply (mM)		*Starch*	
0	39.1 ± 3.42a	97.1 ± 7.1a	137 ± 9.2d
1	34.3 ± 1.00a	86.0 ± 4.3a	110 ± 8.0c
2	41.1 ± 3.03a	82.8 ± 2.9a	84.1± 1.9b
5	35.0 ± 0.89a	64.3 ± 5.3a	72.9 ± 6.2b
10	35.9 ± 0.78a	86.3 ± 4.0a	53.7 ± 1.7a

Table 4.3 Effects of phosphate application on sugar and starch concentration of marama bean plant organs. Values (Mean ± SE, n = 4-6) followed by the same letter within a column are not significantly different ($P \leq 0.05$)

	Carbohydrate content (mg/g dry matter)		
Year I			
	Leaves	**Stem**	**Tuber**
Phosphate supply (mM)			
	Sugars		
0	16.0 ± 0.90bc	21.3 ± 1.97c	376 ± 4.00d
1	21.7 ± 2.62c	27.7 ± 0.66d	175 ± 2.50b
2	11.9 ± 0.42ab	10.8 ± 0.48ab	100 ± 1.18a
5	18.9 ± 0.53c	7.87 ± 0.28a	400 ± 0.65e
10	8.35 ± 0.41a	11.3 ± 0.17b	324 ± 4.66c
Phosphate supply (mM)			
	Starch		
0	16.3 ± 0.60ab	20.2 ± 0.58a	50.0 ± 3.10ab
1	15.2 ± 0.49ab	16.1 ± 0.31a	52.3 ± 2.73b
2	17.0 ± 0.29b	17.6 ± 0.55a	43.8 ± 0.51ab
5	13.3 ± 0.42a	19.3 ± 1.16a	43.8 ± 2.72ab
10 mM	15.1 ± 1.24ab	17.2 ± 1.41a	42.1 ± 0.66a
YEAR II			
Phosphate supply (mM)			
	Sugar		
0	133 ± 9.9a	48.4 ± 8.2ab	378 ± 66a
1	118 ± 2.7a	72.1 ± 9.3b	518 ± 85a
2	133 ± 8.3a	37.0 ± 4.2a	434 ± 95a
5	118 ± 1.7a	64.9 ± 12ab	382 ± 46a
10	121 ± 3.1a	79.9 ± 16b	401 ± 29a
Phosphate supply (mM)			
	Starch		
0	39.1 ± 3.42a	32.0 ± 3.44a	78.5 ± 13.4a
1	34.3 ± 1.00a	42.7 ± 6.52a	111 ± 17.4a
2	41.1 ± 3.03a	36.0 ± 5.62a	93.2 ± 19.4a
5	35.0 ± 0.89a	41.8 ± 5.63a	75.6 ± 5.52a
10	35.9 ± 0.78a	45.9 ± 8.40a	88.9 ± 3.66a

Table 4.4 Effects of nitrate supply and nitrogen source on total nitrogen content in shoots and tubers of marama bean plants. Values (Means ± SE, n = 6) followed by the same letter within a column under each treatment are not significantly different ($P < 0.05$)

	Total N content in Shoots (%)	Total N content in Tuber (%)
Nitrate supply (mM)		
0	1.56 ± 0.19a	0.54 ± 0.05a
1	1.80 ± 0.21ab	1.48 ± 0.22b
2	1.57 ± 0.21a	0.61 ± 0.09a
5	2.09 ± 0.26b	1.35 ± 0.15b
10	2.02 ± 0.22b	1.62 ± 0.10b
N source		
0 mM N	1.96 ± 0.16a	1.42 ± 0.13a
1 mM NH_4NO_3	2.22 ± 0.10a	1.56 ± 0.13a
2 mM NO_3^-	2.11 ± 0.08a	1.62 ± 0.10ab
2 mM NH_4^+	2.17 ± 0.19a	1.91 ± 0.33b

4.3.8. Effects of nitrate supply on plant mineral composition

Plants fed different concentrations of NO_3^- were analyzed for both macro- and micro-nutrients. As shown in Table 4.5, the concentrations of P, Ca and Mg increased in 2 and 10 mM-fed plants relative to control. The concentrations of Zn, Mn and to some extent Cu, also increased at higher NO_3^- levels compared to control. Supplying 1 to 10 mM NO_3^- increased K concentration in tubers of both Year I and II experiments, but did not affect the levels of other macronutrients (Table 4.5). The concentrations of Zn, Mn, Fe, and to some extent Cu decreased significantly relative to control in the tubers of plants from Year I and II experiments (Table 4.5).

4.3.9 Effects of phosphate on plant mineral composition

The effect of P supply on tissue concentration of minerals was less precise compared to N application. Although there was a clearly significant increase in P and K concentrations in shoots of Year II plants with increasing P supply, shoot K levels were unaffected in Year II plants (Table 4.6). The shoot concentration of Zn was also increased by 1 and 2 mM phosphate, but decreased at higher 5 and 10 mM levels in Year I plants (Table 4.6).

As with shoots, the P and K concentrations of tubers increased significantly with increasing P supply to plants in both Year I and II experiments. In Year II but not Year I, the tuber level of Fe increased in response to 2 and 5 mM phosphate (Table 4.6). Zinc similarly showed increased accumulation in tubers when plants were supplied with 1 to 5 mM phosphate but not the 10 mM level.

The decreased concentrations of soluble sugars in organs, especially photosynthetic tissues, could imply that N taken up by marama bean plant is used up by the plant for growth or that it is rapidly incorporated with sugars into organic-N for storage in tubers. This may then lead to a decrease in soluble sugars as well as represent a mechanisms by which this legume accumulates N in tubers as reserves for re-growth and seed protein formation.

Table 4.5 Effects of nitrate application on nutrient concentration in marama bean plant tissues. Values (Means ± SE, n = 4-6) followed by the same letter within a row are not significantly different ($P < 0.05$).

	Nitrate concentration (mM)				
Concentration of nutrient in tissue	0	1	2	5	10
Year I					
Shoots					
P %	0.05 ± 0.00 a	0.05 ± 0.00 a	0.08 ± 0.02b	0.05 ± 0.00a	0.11 ± 0.02b
K %	0.40 ± 0.00a	0.40 ± 0.00a	0.54 ± 0.08a	0.40 ± 0.00a	0.50 ± 0.05a
Ca %	0.18 ± 0.03a	0.34 ± 0.03a	1.69 ± 0.16b	0.24 ± 0.11a	1.42 ± 0.12b
Mg %	0.05 ± 0.01a	0.10 ± 0.01b	0.37 ± 0.08c	0.06 ± 0.02a	0.27 ± 0.02bc
Cu mg/kg	1.69 ± 0.42a	1.53 ± 0.11a	3.19 ± 0.73ab	2.27 ± 1.21ab	5.65 ± 1.01b
Zn mg/kg	18.4 ± 3.49a	16.1 ± 2.40a	22.8 ± 3.60a	35.6 ± 7.70b	67.2 ± 1.45c
Mn mg/kg	8.93 ± 1.43a	7.50 ± 0.00a	17.0 ± 1.00b	7.50 ± 0.00a	25.9 ± 0.66c
Fe mg/kg	138 ± 28.0b	75.3 ± 9.1a	127 ± 9.0b	68.0 ± 23.7a	165 ± 37.7b
Tuber					
P %	0.50 ± 0.11b	0.28 ± 0.05a	0.29 ± 0.03a	0.23 ± 0.04a	0.22 ± 0.03a
K %	1.90 ± 0.16a	1.31 ± 0.21a	1.59 ± 0.16a	1.73 ± 0.21a	1.81 ± 0.15a
Ca %	0.44 ± 0.01b	0.30 ± 0.05a	0.42 ± 0.04b	0.36 ± 0.01ab	0.45 ± 0.02b
Mg %	0.20 ± 0.02a	0.16 ± 0.03a	0.23 ± 0.00a	0.21 ± 0.01a	0.23 ± 0.01a
Cu mg/kg	4.10 ± 0.33c	2.54 ± 0.23b	2.95 ± 0.42b	1.88 ± 0.17a	2.09 ±0.31ab
Zn mg/kg	40.3 ± 3.91b	26.5 ± 5.42a	28.0 ± 4.36a	23.1 ± 4.37a	21.4 ± 3.78a
Mn mg/kg	6.10 ± 0.31b	3.39 ± 0.16a	4.13 ± 0.31a	3.51 ± 0.61a	3.21 ± 0.22a
Fe mg/kg	209 ± 48.0b	105 ± 9.75a	141 ± 11.2a	112 ± 19.2a	101 ± 9.75a
Year II					
Tuber					
P %	0.22 ± 0.01a	0.21 ± 0.01a	0.22 ± 0.01a	0.25 ±0.01a	0.22 ± 0.01a
K %	0.70 ± 0.23a	1.55 ± 0.05b	1.50 ± 0.05b	1.36 ± 0.07b	1.47 ± 0.08b
Ca %	0.37 ± 0.02a	0.39 ± 0.03a	0.41 ± 0.03a	0.34 ± 0.02a	0.34 ± 0.02a
Mg %	0.20 ± 0.01a	1.57 ± 0.03b	0.24 ± 0.02a	0.23 ± 0.01a	0.27 ±0.01a
Cu mg/kg	0.35 ± 0.01a	3.13 ± 0.22b	2.86 ± 0.21b	3.21 ± 0.16b	2.10 ± 0.20b
Zn mg/kg	44.9 ± 0.45c	45.7 ± 6.79c	33.7 ± 2.38bc	16.1 ±1.73a	26.6 ± 4.64b
Mn mg/kg	4.53 ± 0.33c	4.73 ± 0.21c	3.67 ± 0.26b	3.21 ± 0.17b	2.07 ± 0.14a
Fe mg/kg	140 ± 18.7c	124 ± 4.27bc	106 ± 2.00ab	96.9 ± 9.0ab	85.7 ± 6.11a

Table 4.6 The effect of phosphate application on nutrient concentration in marama tissues. Values (Means ± SE, n = 4-6) followed by the same letter within a row a re not significantly different (P < 0.05).

	Phosphate concentration (mM)				
Concentration of nutrient in tissue	0	1	2	5	10
Year I					
Shoots					
P %	0.05 ± 0.00a	0.07 ± 0.01a	0.45 ± 0.04b	0.44 ± 0.05b	0.45 ± 0.07b
K %	0.42 ± 0.02a	0.40 ± 0.00a	0.54 ± 0.09a	0.52 ± 0.06a	0.50 ± 0.06a
Ca %	0.53 ± 0.01b	0.31 ± 0.07a	0.83 ± 0.09b	0.46 ± 0.03ab	0.37 ± 0.06a
Mg %	0.17 ± 0.03b	0.05 ± 0.01a	0.17 ± 0.02b	0.16 ± 0.03b	0.12 ±0.02b
Cu mg/kg	2.09 ± 0.20a	1.47 ± 0.21a	3.61 ± 0.44b	2.28 ± 0.47a	2.42 ± 0.28a
Zn mg/kg	45.5 ± 2.20b	60.6 ± 2.65c	69.8 ± 6.16c	32.9 ± 3.98ab	18.8 ± 3.28a
Mn mg/kg	7.99 ± 0.66ab	9.19 ± 0.91b	13.6 ± 2.24b	6.05 ± 0.60a	5.88 ± 0.94a
Fe mg/kg	84.8 ± 13.1a	88.3 ± 11.9ab	119 ± 5.89b	77.5 ± 3.04a	74.2 ± 10.8a
Tuber					
P %	0.10 ± 0.01a	0.46 ± 0.02b	0.63 ± 0.07b	0.63 ± 0.06b	0.60 ± 0.09b
K %	1.36 ± 0.10a	2.04 ± 0.12b	2.07 ± 0.31b	2.13 ± 0.12b	2.55 ± 0.35b
Ca %	0.32 ± 0.01a	0.45 ± 0.02b	0.39 ± 0.04ab	0.42 ± 0.07ab	0.35 ± 0.02ab
Mg %	0.21 ± 0.01a	0.27 ± 0.02a	0.23 ± 0.05a	0.21 ± 0.02a	0.19 ± 0.01a
Cu mg/kg	2.53 ± 0.39a	2.74 ± 0.29a	2.77 ± 0.09a	2.80 ± 0.35a	3.57 ± 0.62a
Zn mg/kg	33.7 ± 0.55a	60.4 ± 6.50b	46.2 ± 4.60ab	50.5 ± 7.22b	26.9 ± 3.52a
Mn mg/kg	2.58 ± 0.66a	3.91 ± 0.11b	2.84 ± 0.53ab	2.17 ± 0.28a	2.02 ± 0.20a
Fe mg/kg	113 ± 5.50a	167 ± 25.7b	104 ± 15.6a	94.9 ± 15.2a	106 ± 10.5a
Year II					
Shoots					
P %	0.14 ± 0.01a	0.56 ± 0.009b	0.75 ± 0.12bc	1.01 ± 0.17c	1.17 ± 0.23c
K %	0.60 ± 0.14a	1.52 ± 0.06bc	1.35 ± 0.0b	2.18 ± 0.29c	2.90 ± 0.39c
Ca %	1.18 ± 0.11b	1.07 ± 0.07b	1.12 ± 0.09b	0.46 ± 0.08a	0.93 ± 0.08ab
Mg %	0.47 ± 0.05a	0.47 ± 0.05a	0.48 ± 0.04a	0.40 ± 0.03a	0.40 ± 0.02a
Cu mg/kg	11.0 ± 0.60b	7.43 ± 0.46ab	7.16 ± 0.52ab	5.58 ± 0.72a	8.06 ± 0.83ab
Zn mg/kg	48.1 ± 7.65b	49.7 ± 9.65b	19.7 ± 11.8a	33.8 ± 4.84b	46.6 ± 4.51b
Mn mg/kg	18.9 ± 3.00a	15.15 ± 2.02a	13.2± 1.87a	13.08 ± 1.62a	18.3 ± 2.00a
Fe mg/kg	306.3± 35.1c	185.1 ± 15.2a	263.7 ± 307ab	144.6 ± 13.7a	278.4 ± 39.9b
Tuber					
P %	0.10 ± 0.01a	0.37 ± 0.01b	0.52 ± 0.04bc	0.75 ± 0.07c	0.67 ± 0.04c
K %	1.35 ± 0.06a	1.63 ± 0.06a	2.15 ± 0.11ab	2.52 ± 0.09b	2.84 ± 0.22b
Ca %	0.40 ± 0.03a	0.41 ± 0.01a	0.45 ± 0.04a	0.34 ± 0.02a	0.38 ± 0.01a
Mg %	0.25 ± 0.01a	0.19 ± 0.02a	0.25 ± 0.02a	0.20 ± 0.01a	0.16 ± 0.01a
Cu mg/kg	6.27 ± 1.03b	7.31 ± 0.72b	5.17 ± 0.46ab	3.33 ± 0.17a	3.31 ± 0.28a
Zn mg kg	28.3 ± 5.33b	22.7 ± 4.28ab	29.3 ±4.44b	19.5 ± 2.11a	14.9 ± 1.21a
Mn mg kg	2.75 ± 0.34a	1.89 ± 0.16a	2.41 ± 0.23a	4.22 ± 1.50b	3.12 ± 0.15ab
Fe mg kg	65.2 ± 5.57a	65.2 ± 3.26a	104.5 ± 13.6b	92.3 ± 16. 0b	57.2 ± 2.65a

4.4 Discussion

Analysis of mineral nutrients in marama bean soils collected from Namibia and Botswana revealed very low concentrations of plant-available N (see Chapter 3). The levels of NO_3^--N and NH_4^+-N were extremely low ranging from 0.24 to 14.0 mg-N/kg. Studies of the species' preference for the two forms of N (i.e. NO_3^--N and NH_4^+-N) showed that marama bean can efficiently utilize both N forms for growth. A series of experiments revealed increased chlorophyll concentrations and NRA with increasing nitrate supply (Fig.4.1 A) and with different sources of N supply compared to control (Fig.4.1 B). The increased NRA with higher concentrations of NO_3^- or NH_4NO_3 is consistent with the findings of several studies (Andrews *et al.*, 1984; Atkins *et al.*, 1980; Geiger *et al.*, 1999; Pate *et al.*, 1980; Wallace, 1986). Data from N source experiments for Year I and Year II showed that, with this legume, 2 mM NH_4^+ could stimulate NRA to levels significantly higher than that of 2 mM NO_3^- (Fig. 4.3). This finding is inconsistent with standard information in the literature (Andrews *et al.*, 1984; Atkins *et al.*, 1980; Wallace, 1986). Since nitrate reductase enzyme is an inducible enzyme, its favoured response to ammonium application was not fully understood. It is however, very important to note that the in situ nitrate reduction rates are controlled by other factors such as the limitation by reducing powers, changing environmental conditions and by the efficiency of nitrate translocation from roots to the site of reduction. It can, however, be inferred that this activation of NRA by ammonium nutrition could be due to nitrification of the ammonium ion which has been reported to occur at warm temperatures (Ilies and Mavinic, 2001; Kim et al., 1997). This trait may speculatively be useful in N acquisition by marama bean

in its N-poor environment. Future studies should include measurements of NRA at different temperatures to explain the activation of NRA in this legume species.

Growth response of marama bean plants to different concentrations of NO_3^- (Figs. 4.4 A) were predictable and conformed predictably with the findings of previous studies (Andrews *et al.*, 1984; Atkins, 1982; Atkins *et al.*, 1980; Pate *et al.*, 1980; Sprent, 1980; Wallace, 1986), in that, growth of marama bean plants increased with N supply and concentration (Figs 4.4 and 4.5). The provision of 2 mM NO_3^- seemed to be the optimal nitrate concentration for marama bean growth (Figs 4.4A). However, unlike NO_3^-, the application of phosphate at different concentrations produced an inconsistent pattern of growth response (Fig. 4.4B). Although the data for P supply were less precise in pattern, supplying mineral nutrients to marama bean in cropping systems is likely to promote growth and increase both grain and tuber yields. Such improved N nutrition is also likely to increase the protein levels in seeds and tubers, and thus promote its market value as a new crop for food and livestock development. The provision of exogenous NO_3^- in the field markedly decreased the concentrations of nutritionally important nutrients such as P, Cu, Zn, Mn, and Fe (Jovaní *et al.*, 2001) in edible tubers, but not in shoots (Table 4.5). Unlike NO_3^-, supplying different concentrations of phosphate significantly increased nutritionally-important nutrients such as P, Ca, K and Zn in tubers, indicating that with field research on N and P management, the decreasing nutrient quality promoted by NO_3^- nutrition can be overcome by phosphate supplementation.

Besides the changes in tissue nutrient profile, NO_3^- supply also altered the concentrations of soluble sugars in organs of marama bean plants (Table 4.2). For example, increasing the NO_3^- supply to roots from 0 mM to 10 mM showed progressively decreased

concentrations of soluble sugars in all organs. Except for tubers, where the decrease was gradual, leaf and stem sugar levels were generally two-fold lower in 10 mM NO_3^--fed plants compared to the zero-NO_3^- controls (Table 4.2). These results are inconsistent with those obtained for tobacco plants (Geiger *et al.*, 1999) where soluble sugars in leaves rose with increasing concentrations of NO_3^- supplied to the roots. As with sugars, starch levels in tubers and, to some extent, in leaves also increased with increasing NO_3^- concentrations (Table 4.2), a finding consistent with that obtained for tobacco plants (Geiger *et al.*, 1999).

The decreased concentrations of soluble sugars in photosynthetic organs with increasing NO_3^- nutrition could imply that N taken up by marama bean plants is rapidly incorporated with sugars into organic-N for storage in tubers hence the leaf and stem decrease in soluble sugars. In a sense, this could represent a mechanism by which the legume accumulates N in tubers as reserves for re-growth and formation of high-protein seed. This argument is supported by the relatively greater tuber dry matter (Fig. 4.5 B) compared to shoot biomass (Fig. 4.5 A) when plants were grown in different concentrations of NO_3^-. However, for a C3 legume, which grows in the sunny hot Kalahari desert, a photosynthetically-driven N accumulation in tubers may not be a sustainable mechanism for enhanced N nutrition due to the expected high rates of photorespiration (Noctor *et al.*, 2002)

The growth response of marama bean to N and P supply under semi-controlled conditions is interesting in many ways. It has been argued by Bieleski and Lauchli (1983) that growth rates of plants from nutrient-poor soils, such as marama bean from the Kalahari desert, are genetically controlled to be at low level, in keeping with the low nutrient

supply from the soil. However the growth response obtained here for N and P application is inconsistent with that hypothesis. In fact, other studies also found *Aspalanthus linearis,* originally from the nutrient-poor soils of the Cedarberg, South Africa, to increase its growth and N_2 fixation with exogenous N and P supply under both field and glasshouse conditions (Muofhe and Dakora, 1999 b, c). Another study (Muofhe and Dakora, 1999 b, c) also did not support the notion that growth of plants from nutrient-poor environments is genetically pre-determined by the low nutrient condition. On the contrary, the findings obtained here and in other studies (Muofhe and Dakora, 1999 b, c) suggest that the cultivation of under-developed crops from nutrient poor marginal areas can be expanded through the use of mineral fertilizers or via planting in agriculturally-fertile soils. Therefore, there is a potential for developing marama bean into a new crop.

Chapter 5: Field Assessment of Plant Growth and N Nutrition in Wild and Cultivated Marama Bean Plants

5.1 Introduction

In Africa, a large number of native plant species with great production potential are being exploited for a variety of uses. If such species could be manipulated for yield enhancement and nutritional upgrading (e.g. increasing seed protein content) it is possible that they could become new crops with great market potential and yields that surpass that of conventional crops (Ignacimuthu and Babu, 1987). Marama bean (*Tylosema esculentum*), a legume native to the Kalahari desert in Southern Africa is one of such plant species. Marama bean is adapted to a wide range of climatic conditions and to the nutrient-poor soils of these semi-arid areas. It produces edible grain and tubers that are highly nutritious (Keegan and Van Staden, 1981) and are an important part of the diet of the native inhabitants. These attributes indicate that marama bean is probably worthy of cultivation as a new crop. However to domesticate marama bean as a crop requires a deeper understanding of its N nutrition, especially in relation to how and where it obtains its N under the near- desert conditions.

The use of the ^{15}N natural abundance technique to quantify contribution of biological nitrogen fixation to any plant is based on the observation that N derived from soil is generally slightly different from that of the air (Shearer and Kohl, 1986). Plants obtaining all N from the soil generally show a positive $\delta^{15}N$ signal. The technique is applied by estimating the ^{15}N abundance of the N_2-fixing species and analyzing the ^{15}N abundance of non-N_2-fixing species in its surrounding (Boddey, 2000). The natural abundance vary

among N pools in the ecosystem. For example, soil processes of mineralization, nitrification and denitrification (Koba *et al.*, 1998) are frequently accompanied by strong fractionation that may leave product pools depleted in ^{15}N and substrates pools ^{15}N enriched. Additions of atmospheric N_2 or other gaseous forms of N further modify $\delta^{15}N$ signatures of mineral and organic N pools in soil (Heaton *et al.*, 1997), resulting in varying $\delta^{15}N$ signatures of plant available N forms (Yoneyama, 1996). Observed differences in $\delta^{15}N$ in different plant species are hypothesized to reflect different N acquisition strategies (Hobbie *et al.*, 2000), including the role of mycorrhizal symbiose and the utilization of mineral or organic soil N forms by plants (Michelsen *et al.*, 1996). Differences in $\delta^{15}N$ within plant organs have also been reported (Shearer and Kohl, 1980). This distribution of ^{15}N among plant organs is of interest, because the variation of among plant parts may shed light on metabolic processes within the plant. The aim of this work was to i) estimate growth including reproductive potential in field plants, ii) determine the source of N nutrition using the ^{15}N natural abundance method and, iii) evaluate plant response to N and P fertilizer application under field conditions.

5.2. Materials and Methods

5.2.1 Determination of $\delta^{15}N$ Values and %N in Leaves of Marama Bean and Reference Plants.

Leaves of marama bean plants and that of its associated species were oven-dried at 60 ºC to constant dry weights. Leaves from one sample plant were pooled together as one sample, three to four plants were used per species as replicates. After weighing, the

samples were finely ground for analysis of $\delta^{15}N$ and %N using a Finnigan MAT 252 mass spectrometer (Bremen, Germany).

5.2.2 Distribution of $\delta^{15}N$ Values and %N in marama bean vines.

The vines of four marama bean plants were collected from Makgobokgobo Site 1 in Botswana and separated into individual branches. The number of leaves per branch and number of leaves per main stem were counted. Because there were different number of branches per vine, (ranging from 7 – 13), the first branch closest to the base of the stem was termed "lower branch" and the last branch closest to the apex of the plant called "upper branch", the same fashion was employed to obtain a "lower quarter branch" and an "upper quarter branch". Similarly, the first two leaves on the branch were referred to as "lower leaves" and the last two at the end of the branch, the "upper leaves". Leaves on the main stem were also named in the same way. These individually labeled branches and leaves were oven-dried at 60 ºC and their dry weights determined. The samples were then ground to a fine powder and analyzed for $\delta^{15}N$ and %N using a mass spectrometer.

5.3 Results

5.3. 1 Growth and reproduction in wild marama plants

The average number of vines per plant of the four plants sampled at Sandveld was 11.0 ± 2.45 and that of branches per vine was 30.8 ± 6.57 (Table 5.1). Vines had leaves with the mean value of 57.0 ± 3.89 per vine, and average vine length per plant was 4.13 ± 0.37 m (Table 5.1). The average number of pods per plant was 23 ± 10 with vines bearing 7.33 ± 3.48 pods on average (Table 5.2). A total of 587 pods were collected from a wild

population of marama bean. Of these, 59.3% were single-seeded, and 40.7 % double-seeded (Fig. 5.1). In this study, no pods were found to have more than two seeds.

Table 5.1 Number of vines and vine length per plant or branches and leaves per vine, podsper plant and pods per vine of wild marama bean plants measured at Sandveld, Namibia, on 24 March 2001.

Plant parmeter	Mean
Number of vines/plant	11.0 ± 2.45
Number of branches per vine	30.8 ±6.57
Number of leaves per vine	57.0 ± 3.89
Vine length per plant (m)	4.13 ± 0.37
Number of pods per plant	23 ± 10
Number of pods per vine	7.33 ± 3.48

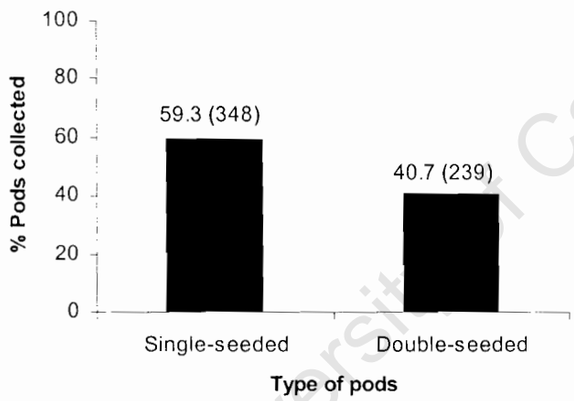

Figure 5.1 Single vs. double-seeded pods from Sandveld, Namibia

5.3.2 Characteristics of marama bean tubers

The average sizes of tubers of plants dug up from different sites in Namibia ranged from 41.0 ± 5.34 to 52.0 ± 1.53 cm long and were 51.3 ± 2.60 to 71.5 ± 9.31 cm thick. Mean tuber fresh weights ranged from 3.38 ± 0.69 to 7.33 ± 2.45 kg per tuber, and mean dry

weights 0.51 ± 0.07 to 0.87 ± 0.26 kg per tuber. The water content was above 80 % of tuber fresh weight (Table 5.2).

5.3.3 Leaf δ^{15}N and %N of marama bean and reference plant species

Table 5.3 shows the variation in δ^{15}N values of leaf tissues sampled from marama bean (*Tylosema esculentum*) and other plant species growing in the same site. The δ^{15}N of marama bean leaves from Sandveld were significantly different from those of *Grewia retinervis* and *Acacia hebeclada* sampled from the same site. δ^{15}N of marama bean leaves sampled from Makgobokgobo and Buitepos were not significantly different from those of associated species (Table 5.3). However, the δ^{15}N values of plant species collected from Buitepos were slightly higher than those of the same species obtained from Sandveld and Makgobokgobo. The %N of marama bean plants from Buitepos was 2.63 ± 0.21 % compared to 1.65 ± 0.02% for Sandveld in Namibia, and 2.46 ± 0.10 % for Makgobokgobo in Botswana (Table 5.3).

Table 5.2 Characteristics of marama bean tubers harvested from wild plants of unknown ages in Namibia, between 13 and 15 May 2001. Values are Mean ± SE, n = 3 - 4 .

Site	Mean tuber length (cm)	Mean tuber circumference (cm)	Mean tuber Fwt. (kg/plant)	Mean tuber Dwt. (kg/plant)	Mean water content (%)
Sandveld Site 1	50.3 ± 3.17	60.3 ± 4.81	4.90 ± 0.57	0.67 ± 0.08	86.0 ±0.00
Sandveld Site 2	41.0 ± 5.34	59.7 ± 7.69	3.38 ± 0.69	0.51 ± 0.07	80.3 ± 8.76
Sandveld Site 3	52.0 ± 1.53	51.3 ± 2.60	3.97 ± 0.69	0.59 ± 0.12	85.3 ± 1.33
Buitepos	50.5 ± 5.69	71.5 ± 9.31	7.33 ± 2.45	0.87 ± 0.26	88.0 ± 1.35

5.3.4 N concentration, N allocation and $\delta^{15}N$ distribution in wild marama bean vines.

The $\delta^{15}N$ values and N content or concentrations in leaves were always higher than those obtained for branches (Fig. 5.2). However, there was no significant difference between branch and leaf $\delta^{15}N$ values sampled from different positions on the main stem. The total N of leaves and branches did also not differ significantly with position on the stem, although $\delta^{15}N$ values and total N of leaves from the upper branch were generally higher than the rest (Fig. 5.2).

On a per branch basis, leaves on the lower quarter branch had the highest N content while the upper branch leaves generally showed the lowest N content. The $\delta^{15}N$ values and the leaf N of leaves sampled from the main stem, increased with their position away from the base of the stem, but the lower older leaves on the branch showed the lowest values, while the upper had the highest (Fig. 5.2).

Table 5.3 $\delta^{15}N$ values of leaves of reference plants associated with marama bean at Buitepos, Sandveld and Makgobokgobo. The values are Mean ± SE (n = 3 - 4). Values followed by different letters within a column are significantly different ($P \leq 0.05$), nd = not determined (i.e. plants were not found at that site).

	Leaf $\delta^{15}N$ value (‰)			Leaf nitrogen concentration (%)		
Species	Buitepos	Sandveld	Makgobokgobo	Buitepos	Sandveld	Makgobokgobo
Tylosem. esculentum	6.85 ± 0.31a	5.90 ± 0.30b	5.32 ± 0.10a	2.63 ± 0.21a	1.65 ± 0.02a	2.46 ± 0.10a
Bauhinia petersiana	6.62 ± 0.13a	nd	nd	2.73 ± 0.08a	nd	nd
Helicrysum melanacme	4.72 ± 0.33a	nd	nd	2.94 ± 0.19a	nd	nd
Grewia retinervis	nd	4.17 ± 0.15b	4.91 ± 0.10a	nd	2.26 ± 0.03a	2.16 ± 0.03a
Geigeria ornativa	5.74 ± 0.21a	2.41 ± 0.53a	nd	2.24 ± 0.13a	2.05 ± 0.10a	nd
Acacia hebeclada	nd	3.42 ± 0.14a	nd	nd	2.43 ± 0.07a	nd

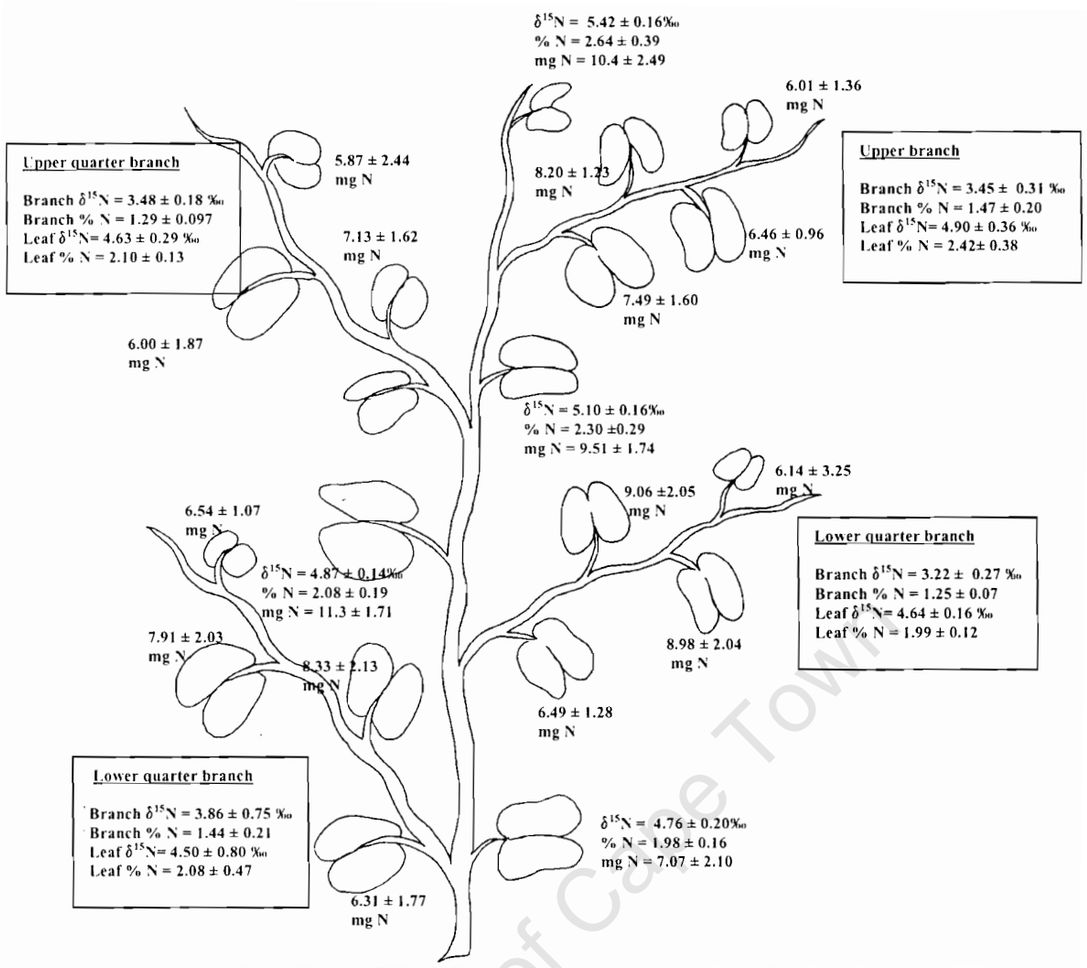

Figure 5.2 Distribution of % N, δ ^{15}N and the variation of nitrogen content (mg N per leaf) in marama bean vines (n = 4 for branches and n = 8 for vine leaves and branch leaves).

5.3.5 Effects of N and P application on fresh and dry weights of marama bean tubers.

Compared to control, the tuber fresh weights of marama bean plants supplied with 40 kg N ha^{-1} as KNO_3 or 20 and 40 kg N ha^{-1} as calcium ammonium nitrate (hereby abbreviated as Ca-NH_4NO_3) were significantly increased (Table 5.4). In fact, the highest tuber yield (99.04 ± 12.14 g $plant^{-1}$) was obtained from plants supplied with 40 kg N ha^{-1} as calcium ammonium nitrate. Except for plants receiving 20 kg N ha^{-1} (KNO_3) or 20 kg P ha^{-1} (TSP), all other treatments significantly out yielded the control in tuber production on a dry weight basis.

Table 5.4 The effect of source and level of fertilizers on NRA, tuber fresh weight and tuber dry weight of field-grown marama plants. Values (Mean ± SE, n = 4 -5) followed by the same letter within a column are not significantly different (P ≤0.05).

Fertilizer level	Fertilizer source	Tuber fresh weight g $plant^{-1}$	Tuber dry weight g $plant^{-1}$
0 kg ha^{-1}	-	20.11 ± 2.31a	2.16 ± 0.36a
20 kgN ha^{-1}	KNO_3	35.78 ± 5.11ab	3.86 ± 0.68ab
40 kgN ha^{-1}	KNO_3	59.43 ± 3.27b	7.41 ± 0.63c
20 kgN ha^{-1}	Ca-NH_4NO_3	56.93 ± 8.88b	6.99 ± 1.91bc
40 kgN ha^{-1}	Ca-NH_4NO_3	99.04 ± 12.14c	12.3 ± 1.47d
20 kg N + 20 kg P ha^{-1}	KNO_3 + TSP	48.84 ± 8.53ab	5.42 ± 1.04bc
20 kg P ha^{-1}	TSP	45.43 ± 11.8ab	4.46 ± 0.54ab
40 kg P ha^{-1}	TSP	41.10 ± 11.8ab	5.82 ± 1.43bc

5.3.6 Effects of N and P application on mineral composition of marama bean tubers

Applying different amounts of N and P to field plants of marama bean did not significantly affect the concentration of macronutrients in tubers (Table 5.5). However, with micronutrients, the concentration of Zn decreased significantly in tubers with the provision of 40 kg N ha^{-1} as KNO_3, 20 and 40 kg N ha^{-1} as Calcium ammonium nitrate ($Ca\text{-}NH_4NO_3$), as well as 40 kg P ha^{-1} as TSP when compared to the control (Table 5.5). The level of Mn was similarly decreased by the first three treatments and by 20 kg P as TSP. In contrast the concentration of Cu increased over the control with the supply of 40 kg N as KNO_3, 40 kg P as TSP and 20 kg N + 20 kg P ha^{-1} as KNO_3 and TSP (Table 5.5).

Table 5.5. Mineral concentrations in marama tubers of field grown plants supplied with varying levels of N and P. Values (Mean ± SE, n = 5) followed by different letters within a column are significantly different ($P \leq 0.05$).

Fertilizer source and level	Macronutrient concentration (%)					Micronutrient concentration (mg/kg)				
	N	P	K	Ca	Mg	Cu	Zn	Mn	Fe	B
0 kg ha^{-1}	1.74 ± 0.20a	0.29 ± 0.03a	1.29 ± 0.11a	0.36 ± 0.04a	0.17 ± 0.01a	6.60 ± 0.70ab	47.7 ± 5.09b	6.53 ± 0.62b	136 ± 13.8a	8.70 ± 0.61a
20kgN ha^{-1} (KNO_3)	1.60 ± 0.33a	0.20 ± 0.03a	1.32 ± 0.14a	0.35 ± 0.04a	0.19 ± 0.02a	5.17 ± 0.88a	52.6 ± 12.5b	4.70 ± 0.14ab	97.3 ± 9.76a	8.39± 0.37a
40kgN ha^{-1} (KNO_3)	1.70 ± 0.54a	0.24 ± 0.03a	1.40 ± 0.07a	0.45 ± 0.03a	0.19 ± 0.02a	10.2 ± 2.46b	31.8 ± 6.32a	4.43 ± 0.33a	125 ± 21.4a	9.77 ± 1.36a
20 kgN ha^{-1} (Ca-NH_4NO_3)	1.27 ± 0.24a	0.20 ± 0.02a	1.55 ± 0.14a	0.41 ± 0.04a	0.21 ± 0.02a	6.31 ± 1.00ab	31.8 ± 6.20a	4.44 ± 0.48a	124 ± 14.6a	10.3 ± 0.72a
40 kgN ha^{-1} (Ca-NH_4NO_3)	1.62 ± 0.27a	0.21 ± 0.01a	1.39 ± 0.02a	0.36 ± 0.03a	0.19 ± 0.01a	6.56 ± 0.42ab	27.4 ± 2.1a	4.06 ± 0.26a	140 ± 22.2ab	8.77 ± 0.64a
20 kg N +20 kg P ha^{-1} (KNO_3 + TSP)	1.81 ± 0.23a	0.29 ± 0.02a	1.87 ± 0.18a	0.41 ± 0.05a	0.24 ± 0.08a	9.12 ± 1.68b	77.0 ± 5.96c	6.36 ± 0.55b	188 ± 34.0b	10.5 ± 0.97a
20 kg P ha^{-1} (TSP	0.90 ± 0.14a	0.23 ± 0.05a	1.39 ± 0.05a	0.33 ± 0.01a	0.17 ± 0.01a	6.16 ± 0.43ab	40.6 ± 8.56b	4.43 ± 0.11a	145 ± 16.7ab	9.46 ± 0.99a
40 kg P ha^{-1} (TSP)	1.55 ± 0.39a	0.33 ± 0.04a	1.22 ± 0.09a	0.38 ± 0.03a	0.17 ± 0.01a	8.03 ± 1.52b	23.3 ± 1.47a	6.09 ± 1.30ab	134 ± 15.1a	9.05 ± 1.12a

5.4 Discussion

Growth analysis of wild marama bean plants

Data from growth analysis of wild plants depict the marama bean as a versatile crop. The ability of this legume to produce about 11.0 ± 4.2 vines per plant each measuring about 4.1 ± 0.7 m and containing 30.8 ± 11.4 branches clearly indicates the extent of the above-ground biomass production within a growing season. These features make the marama bean an ideal candidate for multiple uses in cropping systems. As a creeper, the highly foliaged vines of marama bean can serve as a cover crop, effectively controlling soil erosion and weeds, while maintaining soil moisture. On average, the vines alone can produce up to57.0 ± 3.89 leaves per vine. This potentially represents a huge photosynthetic source for servicing the tubers, which are a major sink. Excavation of wild marama bean plants of unknown ages showed tubers of varying sizes and weights. Tuber length ranged from 41.0 ± 10.7 to 52 ± 2.6 cm, tuber circumferences 51.3 ± 4.5 to 71.5 ± 18.6 cm, tuber fresh weights 3.4 ± 1.3 to 7.3 ± 4.8 kg per plant, tuber dry weights 0.51 ± 0.15 to 0.87 ± 0.53 kg per plant and tuber water content 80.3 ± 17.5 to 88.0 ± 2.7 % (Table 5.3).

A previous study (Dakora *et al.*, 1999) has shown that fresh weights of tubers from wild marama bean plants can be up to 160 kg per plant, clearly indicating their size as a sink for photosynthates and water. Such a huge tuber mass can only be associated with older plants and/or improved nutrient supply (Table 5.5). The 80-88 % water content is close to the 87- 92 % obtained by Dakora *et al.* (1999) and, represents a considerable large buffering capacity for re-growth during drought. The use of tuber as a water reservoir

would no doubt be a mechanism by which marama bean is able to thrive in years when there is zero rainfall in the Kalahari desert (NAS, 1979).

N nutrition in marama bean: the mystery of N source for the high protein concentration in leaves, grain and tubers

As indicated elsewhere (Dakora *et al.*, 1999) and in Chapter 3, marama bean is an indigenous legume that produces protein rich grain and tuber. Its origins are restricted to the N-poor soils of the Kalahari Desert where it has been shown to be a non-nodulating legume (Dakora *et al.*, 1999). As shown in Chapter 3, soil NO_3^--N, NH_4^+-N and total N are extremely low (less than 0.06 %). The legume's non-symbiotic status combined with the low endogenous soil-N level raises the question as to where marama bean obtains its N for high protein accumulation in grain and tubers.

One hypothesis proposed by Dakora *et al.* (1999) was that marama bean probably obtains its N from deep NO_3^- capture beyond the reach of other plants species, or from naturally accumulated deep groundwater NO_3^- in subsurface soils.

In this study, marama bean plants together with other species in the same site were assessed for their source of N using ^{15}N isotope analysis. According to the ^{15}N natural abundance methodology, legumes obtaining their N nutrition from rhizobial fixation of atmospheric N_2 have lower $\delta^{15}N$ (Shearer and Kohl, 1986; Muofhe and Dakora, 1999a) while those utilizing soil-N have higher $\delta^{15}N$ (Shearer and Kohl, 1986; Dakora *et al.*, 1999).

As shown in Table 5.3, the $\delta^{15}N$ values of marama bean leaves sampled from Buitepos in Namibia were not significantly ($P \leq 0.05$) different from those of *Helicrysum melanacme*,

Geigeria ornativa and *Bauhinia galpini*, a close relative from the same phylogenetic tribe (Table 5.3). Similar studies conducted at Sandveld in Namibia, and at Makgobokgobo in Botswana show that the $\delta^{15}N$ signatures of marama bean leaves were not different from those of *Grewia retinervis* sampled from the same site from the two countries. In fact, the $\delta^{15}N$ values were similar for each species in the two countries (Table 5.3), thus confirming the precision of the technique as well as re-enforcing the commonality of N-source used by marama bean and other associated plant species in the same locality within each country. But the fact that *Bauhinia galpini*, a close phylogenetic relative of marama bean was found to have similar $\delta^{15}N$ values as the test legume (6.62 ± 0.13 ‰ for *B. galpini* vs 6.85 ± 0.31 ‰ for marama bean) strongly supports our view that marama bean does not obtain its N from a different source compared to other associated non-fixing species in the same site. The leaf $\delta^{15}N$ value of *Acacia hebeclada* was however significantly lower than that of marama bean (Table 5.3) because the former is N_2-fixing legume, which generally shows ^{15}N depletion (Muofhe and Dakora, 1999a) as a consequence of ^{15}N discrimination by nitrogenase enzyme. *Geigeria ornativa* is the only non-legume from the same site whose $\delta^{15}N$ value differed significantly from that of marama bean. This could imply that *G.ornativa* feeds on a different soil-N pool compared to marama bean. However recent evidence (Wheeler *et al.*, 2000) shows that plant species that are mycorrhizal can exhibit reduced (negative) $\delta^{15}N$ values in organs. But whether *G. ornativa* is mycorrhizal or not, remains to be determined. It is however reported that reference plants are generally the significant sources of error for isotope methods (dilution method and natural abundance method), largely as a result of temporal and spatial variation in $\delta^{15}N$ signature of soil N pools (Danso *et al.*, 1993).

Figure 5.2 shows the data for N concentration, N allocation, N content and $\delta^{15}N$ values of vines, branches and leaves. In general, the $\delta^{15}N$ of vine leaves as well as N concentration increased from bottom to the tip, resulting in greater N content in leaves closer to the vine tip. In contrast, the youngest leaves on branches showed lower N content compared to the older branch leaves (Fig. 5.2). The variation of $\delta^{15}N$ between parts of a single plant may be due variously to ammonium or nitrate acquisition, (Evans *et al.*, 1996; Evans, 2001, preferential nitrate reduction in roots or shoots (Pate *et al.*, 1993; Unkovich *et al.*, 2000) and N_2 fixation (Shearer *et al.*, 1980). These data have implications for the choice of leaf selected for photosynthetic measurements as well as for rates of decomposition following leaf fall. Taken together, these data have provided no evidence that marama bean feeds from a different soil-N pool compared to associated non-legume species. The high N concentrations of marama bean plants must be due to mechanisms other than differential N-sourcing.

Potential of marama bean as a new crop

On average, wild marama bean plants can produce up to 23 pods per plant, of which about 41 % are double-seeded and 59% are single seeded (Table 5.2; Fig. 5.1). This contrast with earlier reports (NAS, 1979) that marama bean can produce up to 6 seeds per pod. These observations suggest that grain yield of marama bean has the potential to increase under agronomic conditions. However, because both grain and tuber are edible, a balance has to be struck between cultivating the plant for grain and for tubers. Data from a preliminary field study (Table 5.4) have shown that improved nutrient supply can increase tuber yields. As shown in Table 5.4, applying 20 and 40 kg N per hectare as KNO_3 or Ca-NH_4NO_3 significantly increased tuber yields, whether measured as fresh

weights or dry weights. Supplying 20 and 40 kg N P per hectare as triple super phosphate also increased tuber fresh weight, clearly indicating that tuber yields of marama bean can be increased from improved agronomic practices such as N and P supply.

Besides tuber yields that increased with N and P application, the concentrations of some micronutrients were also altered (Table 5.6). The tuber levels of Cu, Zn, Mn, and Fe were generally increased by the combined application of 20 kg N as KNO_3 and 20 kg P as triple superphosphate. This bio-fortification of micronutrients in tubers has implications for improved human health when tubers are eaten as food. In addition to its dietary value, marama bean can also be used as a forage crop. As shown in Fig. 5.2, marama bean leaves have high N concentrations and therefore greater tissue content of crude protein. Cultivation of this legume as a crop will not only conserves and stabilizes sandy soils against erosion, but also serves as forage for game and livestock development. This is in addition to the value of the tubers as feed for animals and source of water for hunters and wildlife. The use of raw tubers both as food and source of water adds value to this African legume as an important species for defence during military activities.

Chapter 6: Accumulation and Partitioning of $^{15}NO_3^-$ in Marama Bean Plants Grown under Glasshouse and Field Conditions: Probing for Mechanisms that Enhance N Nutrition in this Non-nodulating Legume

6.1 Introduction

Nitrogen isotopes have been used to successfully study nitrogen uptake, accumulation and allocation and distribution within plant parts (Friedrich and Schrader, 1979; Ingemarsson *et al.*, 1987a; Oscarson *et al.*, 1987; Schrader, 1978), and those findings show that rates of N uptake and accumulation can be highly variable depending on the stage of crop development. Understanding the processes that regulate N uptake, accumulation and distribution in crop plants is of major importance in increasing yields and improving grain quality, in addition to increasing N-use efficiency from improved N fertilization strategies. Under sub-optimal N supply, N uptake by crops depends on its availability and distribution in the growth medium, as well as on root distribution (Gastal and Lemaire, 2002). Under ample N supply, however, the uptake is controlled by the plant's growth rate via internal regulation (Gastal and Lemaire, 2002). N uptake, and partitioning in crop plants also depend on the carbon allocation between organs as well as the N composition of those organs, which in turn depends on soil N and the internal N status of the species (Gastal and Lemaire, 2002). It is thus the uptake of N and its allocation between plant organs that regulate N accumulation in this plant species.

Tolley-Henry and Raper (1986 b) tested the hypothesis that resupplying nitrogen after a period of nitrogen stress leads to restoration of the balance between root and shoot growth and normal functional activity in soybean plants. In their study, they showed that

reduced nitrogen was redistributed from the leaves into the stems and roots of continually nitrogen-stressed soybean plants. When nitrogen stress was relieved, the distribution of reduced nitrogen within the plant organs returned to levels similar to those of non-stressed plants.

Previous studies (see Chapters 4 and 5) have shown that marama bean can utilize both the NO_3^- and NH_4^+ forms of N. It was also shown that marama bean does not source N from soil pools different from those of closely associated plant species in the same locality (Chapter 5). The aim of this study was to further explore the mechanisms by which marama bean accumulates high concentrations of N as protein in leaves, grain and tubers.

6.2 Materials and methods

6.2.1 Application of $^{15}NO_3^-$ to marama bean plants grown under glasshouse conditions

To study N allocation to organs, after N starvation, seeds of marama bean were planted on 12 December 2001 in PVC tubes containing a mixture of sand and vermiculite under glasshouse conditions and irrigated with de-ionized water. Three weeks after germination, plants were supplied with 300 mL of modified ½ strength N- free Hoagland nutrient solution thrice a week. At four months of age, the plants were exposed to three N-treatments with 28 replicates. The treatments were 0, 2 and 5 mM $^{15}NO_3^-$ (supplied as KNO_3 containing 2% ^{15}N enrichment). The N treatments were started four months after germination in order to exhaust cotyledon-N and thus deprive external N from the plants.

Plants were then irrigated 3 times a week with 300 mL of modified ½ strength Hoagland nutrient solution containing the respective $^{15}NO_3^-$ concentrations (i.e. 0, 2 and 5 mM).

6.2.2 Application of $^{15}NO_3^-$ to marama bean plants under field conditions

Four 82 days-old marama bean plants were chosen at random from the field experiment set up at a farm in Stellenbosch , South Africa, on 31 December 2002 (see Chapter 5) and supplied with 1 L of 20 mM KNO_3 containing 2 % ^{15}N at 13H00 on 4 March 2003.

6.2.3 Plant harvesting and processing

Four subsequent destructive harvests were carried out on glasshouse-grown marama plants. During each harvest seven plants were harvested from each treatment (0 mM NO_3, 2 mM or 5 mM $^{15}NO_3$). The first harvest was carried out on 1 April 2002 at 14H00, six hours after the first irrigation with $^{15}NO_3^-$ (6 h of absorption). Subsequent harvests were carried out at 8:00 am on 3 April (48 h of absorption), 7 April (144 h of absorption) and 13 April (288 h of absorption). Plants from the first and second harvests were irrigated only once with respective $^{15}NO_3^-$ concentrations, those from the third harvest three times, and those from the fourth harvest six times.

Field-grown plants supplied with 1 L of 20 mM $^{15}NO_3^-$ in the field were harvested at 15H00 on 7 March 2003 (74 h after $^{15}NO_3^-$ application). Control plants from the field were also harvested for comparisons.

Plant materials from both experiments were separated into leaves, stems and tubers and oven-dried at 60 ºC for 72 h. After recording dry weights, plant parts were ground to fine

powder for measurements of % N, atom % excess (A % E) and ^{15}N concentrations in tissues using an Integra carbon–nitrogen analyzer coupled to a continuous flow isotope ratio mass spectrometer (PDZEuropa, Northwich, Cheshire , UK). The standard used was ammonium sulfate at 0.36679 atom % ^{15}N calibrated against IAEA N1. Accumulation rate of ^{15}N, ^{15}N content and ^{15}N concentration in marama bean organs were also calculated.

6.3 Results

6.3.1 ^{15}N atom % excess and % N in marama bean organs grown in the glasshouse

Atom % excess of ^{15}N was significantly increased in tissues of marama bean plants supplied with 2 and 5 mM $^{15}NO_3^-$ compared to control (Table 6.1). The ^{15}N enrichment was quite uniform in different organs of marama bean plants.

Plants that received one irrigation level of $^{15}NO_3^-$ harvested after 6 h and 48 h of absorption showed low atom % excess compared to those receiving 3 or 6 irrigation levels and harvested after 144 and 288 h of $^{15}NO_3^-$ absorption (Table 6.1). The interaction between ^{15}N concentration and absorption time was significant for all plant organs supplied with 2 and 5 mM $^{15}NO_3^-$ harvested after 144 h and 288 h of absorption (Table 6.1).

Application of 5 mM $^{15}NO_3^-$ significantly increased leaf and stem %N compared to control (Table 6.1). Tuber %N was unaffected by $^{15}NO_3^-$ supply. Tissues of plants exposed to $^{15}NO_3^-$ for 6 h showed high %N than plants exposed to $^{15}NO_3^-$ for a longer period (Table 6.1). There was a decrease in %N with $^{15}NO_3^-$ absorption time observed

until at the 144th h, after which the stem and tuber %N showed an increase again as observed in plants exposed to $^{15}NO_3^-$ for 288 h. The interaction between absorption time and ^{15}N concentration was significant in stems and leaves, but not in tubers (Table 6.1). The highest %N was obtained for leaves of plants exposed to 5 mM $^{15}NO_3^-$ for 48 h. Percent N tended to be high in leaves than in stems and tubers (Table 6.1).

Table 6.1 Percent N and atom % excess in tissues of marama bean organs supplied with 0, 2 or 5 mM NO_3^- and harvested at different times after $^{15}NO_3^-$ feeding. Values followed by different letters within a column under each treatment are significantly different ($P \leq 0.05$). *Numbers in parentheses represent irrigation levels.

^{15}Nconcentration/ Absorption time	Atom % excess			%N		
	Leaf	Stem	Tuber	Leaf	Stem	Tuber
Main effects						
^{15}N concentration						
0 mM	0.368 ± 0.0001a	0.368 ± 0.0001a	0.368 ± 0.001a	3.25 ± 0.06a	2.69 ± 0.28b	3.03 ± 0.19a
2 mM	0.405 ± 0.007b	0.416 ± 0.010b	0.409 ± 0.009b	3.42 ± 0.06a	2.09 ± 0.13a	2.58 ± 0.16a
5 mM	0.423 ± 0.001c	0.406 ± 0.010b	0.410 ± 0.010b	3.72 ± 0.06b	3.10 ± 0.17c	3.06 ± 0.19a
Harvest time (h)						
6 (1)*	0.374 ± 0.001a	0.371 ± 0.001a	0.369 ± 0.003a	3.62 ± 0.06b	3.27 ± 0.20b	3.51 ± 0.20b
48 (1)	0.381 ± 0.003a	0.379 ± 0.002a	0.377 ± 0.002a	3.59 ± 0.08b	2.43 ± 0.14a	2.63 ± 0.13a
144 (3)	0.409 ± 0.007b	0.415 ± 0.010b	0.411 ± 0.010b	3.34 ± 0.05a	2.21 ± 0.19a	2.20 ± 0.15a
288 (6)	0.430 ± 0.014c	0.420 ± 0.012b	0.426 ± 0.014b	3.34 ± 0.04a	2.60 ± 0.22a	3.21 ± 0.24b
Interactions						
0 mM x 6h	0.368 ± 0.0001a	0.368 ± 0.0001a	0.368 ± 0.0001a	3.46 ± 0.11b	3.79 ± 0.36c	3.93 ± 0.29a
0 mM x 48h	0.368 ± 0.0001a	0.368 ± 0.0001a	0.368 ± 0.0001a	3.33 ± 0.09ab	2.07 ± 0.06ab	2.49 ± 0.16a
0 mM x 144h	0.368 ± 0.0001a	0.368 ± 0.0001a	0.368 ± 0.0001a	3.27 ± 0.10ab	2.35 ± 0.23b	3.27 ± 0.25a
0 mM x 288h	0.368 ± 0.0008a	0.368 ± 0.0005a	0.369 ± 0.0001a	3.06 ± 0.13a	2.55 ± 0.33bc	3.42 ± 0.43a
2 mM x 6h	0.372 ± 0.001ab	0.370 ± 0.002a	0.369 ± 0.001a	3.71 ± 0.07c	2.85 ± 0.20bc	3.36 ± 0.39a
2 mM x 48h	0.379 ± 0.001ab	0.383 ± 0.006a	0.382 ± 0.004a	3.42 ± 0.11b	2.16 ± 0.15ab	2.51 ± 0.29a
2 mM x 144h	0.425 ± 0.005c	0.464 ± 0.050c	0.446 ± 0.011bc	3.36 ± 0.11a	1.40 ± 0.10a	1.95 ± 0.16a
2 mM x 288h	0.444 ± 0.018c	0.445 ± 0.060c	0.441 ± 0.024bc	3.18 ± 0.10ab	1.97 ± 0.20ab	2.50 ± 0.21a
5 mM x 6h	0.381 ± 0.002ab	0.376 ± 0.002a	0.370 ± 0.0004a	3.69 ± 0.11bc	3.17 ± 0.38bc	3.25 ± 0.37a
5 mM x 48h	0.397 ± 0.001b	0.387 ± 0.003ab	0.381 ± 0.002a	4.02 ± 0.07d	3.07 ± 0.23bc	2.90 ± 0.21a
5 mM x 144h	0.433 ± 0.004c	0.413 ± 0.020b	0.418 ± 0.021b	3.40 ± 0.07b	2.89 ± 0.31bc	2.39 ± 0.33a
5 mM x 288h	0.479 ± 0.060d	0.447 ± 0.050c	0.469 ± 0.024c	3.78 ± 0.14cd	3.27 ± 0.43c	3.70 ± 0.44a

6.3.2 ^{15}N Accumulation rate in glasshouse-grown marama bean plants

The accumulation rate (μg.g $DW^{-1}h^{-1}$) in marama plants supplied with 2 and 5 mM $^{15}NO_3^-$ was slightly increased at high ^{15}N concentration, but not significant. Leaves showed high accumulation rate at both ^{15}N concentrations unlike stems and tubers (Table 6.2). Plants exposed to $^{15}NO_3^-$ for 6 h showed high N accumulation rate in their tissues than those exposed for longer periods. Irrigating marama plants with more $^{15}NO_3^-$ did not increase tissue N accumulation rate as observed for plants that received 3 (exposed to $^{15}NO_3^-$ for 144 h) and 6 irrigations (exposed to$^{15}NO_3^-$ for 288 h).

At each ^{15}N concentration, N accumulation rate was highest in plants allowed 6 h of $^{15}NO_3^-$ absorption and lowest in plants allowed 288 h of absorption (Table 6.2).

6.3.3 ^{15}N content in glasshouse-grown marama bean plants

When marama bean plants supplied with 2 or 5 mM $^{15}NO_3^-$ were analyzed for ^{15}N content in organs, leaves and stems showed high N content at high concentration than at low concentration, but the difference was only significant in stems (Table 6.3). Tuber ^{15}N content on the other hand was high at low ^{15}N concentration than at high ^{15}N concentration (Table 6.3). Overall, ^{15}N content was high in plants supplied with 2 mM (0.240 ± 0.01 mg^{15}N.plant^{-1}) than those supplied with 5 mM $^{15}NO_3^-$ (0.208 ± 0.01 mg^{15}N. plant^{-1}).

Tuber ^{15}N content increased with the time of $^{15}NO_3^-$ absorption and irrigation levels, but inconsistent results were obtained for leaves and stems (Table 6.3). On a whole plant basis, ^{15}N content increased with time of absorption and/or irrigation (Table 6.3).

The interaction between $^{15}NO_3^-$ concentration and absorption time was significant in leaves irrigated with 5 mM $^{15}NO_3^-$ harvested after 144 h of absorption, whereas in tubers, it was significant after 288 h. On whole-plant basis, plants exposed for longer hours to showed high N content at both concentrations of $^{15}NO_3^-$ (Table 6.3).

Table 6.2 Accumulation rates of ^{15}N in organs of marama bean organs supplied with 2 and 5 mM $^{15}NO_3^-$ and harvested at different times after $^{15}NO_3$ feeding. Values followed by different letters within a column under each treatment are significantly different ($P \leq 0.05$). Numbers in parentheses represent irrigation levels.

^{15}N Concentration/	Accumulation rate $\mu g^{15}N.g\ DW^{-1}.h^{-1}$		
Absorption time (h)	Leaf	Stem	Tuber
Main effects			
$^{15}NO_3^-$ Concentration			
2 mM	6.78a	5.00a	5.90a
5 mM	7.11a	5.92a	5.91a
Harvest time (h)			
6 (1)*	23.2c	18.72c	20.4c
48 (1)	3.01b	2.08b	2.14b
144 (3)	1.01a	0.64a	0.64a
288 (6)	0.56a	0.40a	0.48a
Interactions			
2 mM x 6 h	22.9c	17.6c	20.6c
2 mM x 48 h	2.70b	1.69b	1.99b
2 mM x 144 h	0.99a	0.45a	0.60a
2 mM x 288 h	0.49a	0.30a	0.37a
5 mM x 6 h	23.5c	19.9c	20.1c
5 mM x 48 h	3.32b	2.47b	2.30b
5mM x 144 h	1.02a	0.83a	0.68a
5 mM x 288 h	0.63a	0.50a	0.59a

Table 6.3 ^{15}N content in organs of marama bean plants supplied with 2 or 5 mM $^{15}NO_3^-$, and harvested at different times after $^{15}NO_3^-$ feeding. Values followed by different letters within a column under each treatment are significantly different ($P \leq 0.05$). *Numbers in parentheses represent irrigation levels.

$^{15}NO_3^-$ Concentration/ Absorption time (h)	^{15}N content (mg/organ)			
	Leaf	Stem	Tuber	Total
Main effects				
$^{15}NO_3^-$ Concentration				
2 mM	0.015 ± 0.001a	0.011 ± 0.001a	0.214 ± 0.01b	0.240 ± 0.01b
5 mM	0.018 ± 0.004a	0.015 ± 0.001b	0.175 ±0.01a	0.208 ± 0.01a
Harvest time (h)				
6 (1)*	0.016 ± 0.001ab	0.012 ± 0.001	0.172 ± 0.010a	0.181 ± 0.01a
48 (1)	0.012 ± 0.001a	0.012 ± 0.001	0.157 ± 0.01a	0.182 ± 0.01a
144 (3)	0.023 ± 0.007b	0.014 ± 0.001	0.219 ± 0.02b	0.256 ± 0.02b
288 (6)	0.015 ± 0.001ab	0.013 ± 0.002	0.229 ± 0.02b	0.258 ± 0.02b
Interactions				
2 mM x 6 h	0.016 ± 0.001ab	0.012 ± 0.002	0.176 ± 0.017a	0.204 ± 0.018ab
2 mM x 48 h	0.013 ± 0.001a	0.012 ± 0.001	0.173 ± 0.019a	0.198 ± 0.019a
2 mM x 144 h	0.017 ± 0.002ab	0.011 ± 0.001	0.275 ± 0.032a	0.302 ± 0.031b
2 mM x 288 h	0.014 ± 0.002a	0.011 ± 0.002	0.232 ± 0.023a	0.257 ± 0.022b
5 mM x 6 h	0.015 ± 0.001a	0.014 ± 0.002	0.168 ± 0.013ab	0.197 ± 0.014a
5 mM x 48 h	0.012 ± 0.002a	0.012 ± 0.002	0.141 ± 0.017a	0.165 ± 0.019a
5mM x 144 h	0.029 ± 0.014b	0.017 ± 0.002	0.163 ± 0.016ab	0.209 ± 0.014ab
5 mM x 288 h	0.016 ± 0.001ab	0.016 ± 0.003	0.227 ± 0.035b	0.258 ± 0.036b

6.3.4 ^{15}N concentration in glasshouse-grown marama bean plants

The concentration of ^{15}N ($mg^{15}N.g\ DW^{-1}$) was high in stems and tubers supplied with 5 mM than with 2 mM $^{15}NO_3^-$ concentration, however, the difference was not significant in leaves (Table 6.4). At each $^{15}NO_3^-$ concentration leaves showed high ^{15}N concentration than stem and tubers. Concentration of ^{15}N in marama plant tissues was highest in plants harvested after 288 h of absorption and received the highest level of irrigation (Table 6.4). The interaction between $^{15}NO_3^-$ concentration and absorption time showed high stem

and tuber ^{15}N concentrations after 6 h of absorption and was also increased in leaves and tubers supplied with 5 mM $^{15}NO_3^-$ harvested after 288 h of absorption (Table 6.4).

Table 6.4 Concentration of ^{15}N in tissues of marama bean plants supplied with 2 or 5 mM $^{15}NO_3^-$ and harvested at different times after $^{15}NO_3^-$ feeding. Values followed by different letters within a column under each treatment are significantly different ($P \leq 0.05$). *Numbers in parentheses represent irrigation levels.

NO_3^- Concentration/	mg ^{15}N. g DW^{-1}		
Absorption time (h)	Leaf	Stem	Tuber
Main effects			
$^{15}NO_3^-$ Concentration			
2 mM	0.137 ± 0.003a	0.084 ± 0.004a	0.103 ± 0.005a
5 mM	0.157 ± 0.004a	0.125 ± 0.006b	0.124 ± 0.008b
Harvest time (h)			
6 (1)*	0.139 ± 0.003a	0.112 ± 0.008ab	0.122 ± 0.010bc
48 (1)	0.145 ± 0.005a	0.100 ± 0.006ab	0.103 ± 0.006ab
144 (3)	0.145 ± 0.003a	0.092 ± 0.006a	0.092 ± 0.006a
288 (6)	0.161 ± 0.008b	0.114 ± 0.005b	0.138 ± 0.011c
Interactions			
2 mM x 6 h	0.138 ± 0.003a	0.105 ± 0.007b	0.124 ± 0.014b
2 mM x 48 h	0.130 ± 0.004a	0.081 ± 0.006ab	0.095 ± 0.011ab
2 mM x 144 h	0.143 ± 0.005a	0.065 ± 0.006a	0.086 ± 0.006a
2 mM x 288 h	0.142 ± 0.008a	0.086 ± 0.005ab	0.108 ± 0.005ab
5 mM x 6 h	0.141 ± 0.005a	0.119 ±0.014a	0.120 ± 0.013a
5 mM x 48 h	0.160 ± 0.003b	0.119 ±0.009a	0.110 ± 0.007a
5mM x 144 h	0.147 ± 0.003a	0.118 ±0.011a	0.097 ± 0.011a
5 mM x 288 h	0.180 ± 0.009b	0.143 ± 0.014a	0.169 ± 0.013b

6.3.5 Atom % excess and %N in marama bean organs grown under field conditions

Marama bean plants grown under field conditions showed significantly increased ^{15}N atom % excess in their tissues when supplied with 20 mM of $^{15}NO_3^-$ (0.892 ± 0.09 and 0.709 ± 0.06 A %E for shoots and tubers respectively) compared to control (0.368 ± 0.0001 A% E) as shown in Table 6.5.

The %N in marama bean tissues varied between 4.14 ± 0.004 and 4.92 ± 0.11%. It was always high in tubers than in shoots and was not affected by the supply of 20 mM $^{15}NO_3^-$ (Table 6.5).

Table 6.5 Percent ^{15}N and atom % excess in marama bean plant organs supplied with 0 and 20 mM $^{15}NO_3^-$ under field conditions. Values followed by different letters within a column are significantly different ($P \leq 0.05$).

	Atom % excess		%N	
^{15}N Concentration	Shoots	Tuber	Shoots	Tuber
0 mM	0.368 ± 0.0001a	0.368 ± 0.0001a	4.17 ± 0.21a	4.92 ± 0.11a
20 mM	0.892 ± 0.09b	0.709 ± 0.06b	4.14 ± 0.04a	4.61 ± 0.41a

6.3.6 Accumulation rate, content and concentration of ^{15}N in marama bean plants under field conditions

When marama plants were supplied with 20 mM 2% $^{15}NO_3^-$ under field conditions, the accumulation rate was slightly high in shoots (4.98 μg.gDW^{-1}. h^{-1}) than in tubers (4.35 μg.gDW^{-1}. h^{-1}), but the difference was not significant (Table 6.6). On the other hand, ^{15}N content was high in tubers (0.478 ± 0.10mg.tuber^{-1}) than in shoots (0.286 ± 0.06 mg. shoot^{-1}) and the difference was significant (Table 6.6). The ^{15}N concentration (mg.gDW^{-1}) in marama bean tissues behaved in a similar fashion as the accumulation rate, being slightly high in shoots than in tubers (Table 6.6).

Table 6.6 Accumulation rate, content and concentration of ^{15}N in marama bean plants supplied with 20 mM 2% $^{15}NO_3^-$ under field conditions. Values followed by the same letters within a column are not significantly different ($P \leq 0.05$). (-) = Not determined.

Plant organ	^{15}N Accumulation ($\mu g.gDW^{-1}.h^{-1}$)	^{15}N content ($mg^{15}N.organ^{-1}$)	^{15}N concentration ($mg\ ^{15}N.gDW^{-1}$)
Shoot	4.98a	0.286 ± 0.06	0.368 ± 0.07a
Tuber	4.35a	0.478 ± 0.10	0.322 ± 0.02a
Total	-	0.764 ± 0.16	-

6.4 Discussion

Supplying marama bean with 2 or 5 mM $^{15}NO_3^-$ each containing the same level of ^{15}N in the glasshouse showed no differences in atom % excess of organs analysed, however they both differed significantly from zero (0 mM) $^{15}NO_3^-$ control (Table 6.1).

When glasshouse-grown marama bean plants dependent on only seed-N for four months were supplied with different concentrations of $^{15}NO_3^-$ either as single dose or 3-6 successive doses, different rates of ^{15}N accumulation were observed for leaves, stems, and tubers. Although rates of accumulation were unaffected by $^{15}NO_3^-$ concentration, there were marked differences between harvests (Table 6.2). The ^{15}N accumulation was highest within the first 6 h, followed by 48 h of exposure to single dose of $^{15}NO_3^-$ (Table 6.2). Rates of ^{15}N accumulation in organs markedly decreased with time even though the plants were supplied with 3-6 many times more $^{15}NO_3^-$ than the first and second harvest. Interestingly, the $^{15}NO_3^-$ concentration x harvest time interaction was significant ($P \leq 0.05$) and those data showed that ^{15}N accumulation in leaves, stems and tubers was

highest within the first 6 h of supplying 2 or 5 mM $^{15}NO_3^-$ and the rate of storage in organs was the same for the two $^{15}NO_3^-$ concentrations (Table 6.2). The decrease of ^{15}N accumulation with time could be due to saturation of organs with ^{15}N. These results also suggest that multiple applications of N do not necessarily improve uptake and utilization of N. Table 6.3 shows the data for ^{15}N accumulation and partitioning between organs of marama bean plants. The ^{15}N content of stems and tubers (but not leaves) differed betweens the two $^{15}NO_3^-$ concentrations. Although leaves and stems were generally unaffected, the ^{15}N content of tubers significantly ($P \leq 0.05$) increased with time (dose) of $^{15}NO_3^-$ application (Table 6.3), leading to differences in ^{15}N concentrations of organs (Table 6.4).

Interestingly, plant total ^{15}N content was greater at 2 mM than at 5 mM $^{15}NO_3^-$, suggesting the ability of N-deprived marama bean to scavenge N from low N-sources for storage and subsequent use. Nevertheless, what was more intriguing is the fact that most of the assimilated ^{15}N was stored in tubers than in leaves and stems. Late N application has been shown to result in greater N partitioning to perennial organs as compared to N taken up earlier in the season in forest trees (Millard, 1996; Sanchez *et al.*, 1991; Weinbaum *et al.*, 1984). About 95 % of the ^{15}N uptake at first harvest (i.e. after 6 h exposure) was stored in tubers, with only 5 % allocated to leaves and stems. As shown in Table 6.3, 86-95 % of the ^{15}N assimilated by marama bean plants was stored in tubers with the remaining 5-14 % deposited in leaves and stems. These results also showed that the reduction in nutrient solution N supply decreased the allocation of exogenous N to shoots. Consequently, the reduction of N supply resulted in preferential N allocation to tubers. This change in N partitioning in favour of roots under N stress agrees with

previous results observed in alfalfa (Meuriot *et al.*,2003; Fishbeck and Philips, 1981; MacDowall, 1983) and other legumes such as white clover (Davidson, 1969), or soybean (Rufty *et al.*, 1984) and in perennial grasses (Bélanger *et al.*,1992) .

As with the data from glasshouse, field studies also showed that ^{15}N partitioned to tubers represented 63 % of the total plant uptake, with only 37 % allocated to leaves and stems (Table 6.6). These ^{15}N data suggest that efficient uptake of N and its accumulation in tubers is one mechanism by which marama builds an N reserve to support re-growth after drought and for production of protein- rich seeds. These findings therefore support the suggestion by Dakora *et al.* (1999) that the huge tubers formed by marama bean probably function as a sink for N and water which are re-allocated to developing leaves and seeds during reproduction or periods of environmental fluctuations. Staswick (1994) argued that the effect of N availability during plant growth was related to altered source/sink relationships for N rather than having a direct regulatory role.

Taken together, these studies shows that marama bean does not obtain its N from symbiotic fixation with soil rhizobia, nor from a source different from that utilized by associated plant species in the same site. However, marama bean efficiently scavenges N from low concentrations and rapidly builds a reserve in large tubers to serve as a buffer for the formation of protein-rich organs, including seeds. These adaptive responses to low soil N environments also allow marama bean to go dormant and perenniate, while awaiting more favourable conditions for shoot growth.

Chapter 7: General Discussion and Conclusions

The data from this study showed that soils supporting growth of marama bean are very poor in nutrients, especially N, which is less than 0.06%, and organic matter below 0.45%. As a result, supplying N and P significantly increased plant growth and tuber yield under both field and glasshouse conditions. This indicates that, despite the species adaptation to low-nutrient conditions, the application of mineral fertilizers to marama bean in an agricultural setting is likely to promote and increase tuber yield. This therefore offers a great opportunity for the tuber-producing grain legume to be domesticated as a new crop for the Khoisan people of Southern Africa, and perhaps a new market crop for the world. Waalvoord *et al.* (2003) reported that a large nitrate pool located deep (>1 m) beneath desert soils. This trend was not reflected in our results from soil analysis, however it should be acknowledged that desert subsoil nitrate inventories are spatially highly variable, thereby requires substantial measurement efforts to reduce uncertainty in global exatrapolations.

In this study, it was shown that marama bean could utilize all forms of N at concentration studied for. Ammonium nutrition is known to depress plant growth as compared to nitrogen nutrition even at concentration lower than 2 mM (Chaillou *et al.*, 1986; Kirkby and Mengel, 1967; Kirkby, 1968). This phenomenon was not obtained with marama bean, showing its ability to efficiently use all forms of N at concentrations given. It is however, of utmost importance in future to test for the levels of ammonium at which it would become toxic to marama bean. It will also be necessary to test at what seedling stage marama bean would be most prone to ammonium toxicity and its effect on tuber sugar concentration. Schortemeyer *et al.* (1997) had reported that root concentration of

water-soluble carbohydrates of maize hybrids Melga and Melina in the early seedling stage did not differ under ammonium and nitrate nutrition. These comparisons of relative value of ammonium and nitrate salt as N sources for crop growth are essential in the establishment of fertilization practices. The accumulation of carbohydrates at low N and P supply in marama bean implies that nutrient deficiency limits the utilization of carbohydrates by the plant.

In addition to its use as a food crop, marama bean could potentially be produced as fodder for game and livestock because of its high tissue N concentration and thus elevated crude protein content of leaves, vines, grain and tubers (Dakora *et al.*, 1999). Although this high protein content of edible grain and tubers could suggest greater acquisition of N from sources such as biological N_2 fixation, recent evidence shows that marama bean is not a symbiotic legume (Dakora *et. al*, 1999). Thus the N acquired must come from another source. The use of ^{15}N natural abundance also revealed that this legume does not source N from a different N-pool compared to other plants growing in the same locality, suggesting that it must be obtaining its N from the soil just like other plants in its surrounding. However, $\delta^{15}N$ values must be handled cautiously as they can be influenced by other factors such as temporal and spatial variation in $\delta^{15}N$ signature of soil N pools (Danso *et al.*, 1993) and mycorrhizal association (Wheeler *et al.* 2000). Levels of isotopic enrichment depend dynamically on the stoichiometry of reactions as well as on specific abiotioc conditions. Thus, the $\delta^{15}N$ of a specific N pool is not a constant unchanging tracer. This fact together with analytical problems in measuring $\delta^{15}N$ and the complexicity of the N cycle itself limit the influence of making inferences based on observations of ^{15}N abundance. Because of this complexity, Högberg (1997)

suggested that data on $\delta^{15}N$ can only be used alone when certain requirements are met, e.g. when a clearly discrete N sources in terms of amount and isotopic signatures are studied for. Nevertheless, $\delta^{15}N$ studies offer the advantage of giving insights into the N cycle without disturbing the system by adding ^{15}N tracer.

Further experiments in this study involving $^{15}NO_3^-$ feeding showed that marama bean takes up more N at low NO_3^- concentrations than at high NO_3^- levels. Additionally, 65-95% of ^{15}N assimilated was stored in tubers, implying that the tuber acts as an N reserve for the formation of new vegetative materials and for pod-filling. In fact, the ability of marama bean to thrive in nutrient-poor Kalahari sands seems to lie in its efficient nutrient-concentration mechanism. Because of the high protein content of marama bean tubers and grain, further research on this legume has great prospect of developing it into a new market crop for Africa. More field studies with many replicate sites in Namibia and Botswana, however, are needed to strengthen findings obtained from this study. I recommend that it is essential to carry out transplant experiments and determine $\delta^{15}N$ values of soils at different depths in which marama bean grow in the field, in order to explain the source of N used by marama bean in the field.

References

Adu-Gyamfi (Ed.). 2002. Food security in nutrient-stressed environments: Exploiting plants'genetic capabilities. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 340

Ainsworth E.A, Davey P.A., Bernacchi C.J., Dermody O.C., Heaton E.A., Moore D.J., Morgan P.B., Naidu S.L., Ra H.S.Y. and Zhu X.G. (2002). A meta-analysis of elevated [CO_2] effects on soybean (*Glycine max)* physiology, growth, and yield. *Global Change Biology* **8**: 695-709.

Andrews M., Sutherland J.M., Thomas R.J. and Sprent J.I. (1984). Distribution of nitrate reductase activity in six legumes: the importance of the stem. *New Phytologist* **98**: 301-310

Atkins C.A. (1982). *Ureide metabolism and the significance of ureides in legumes*. In: Advances in Agricultural Microbiology (ed. NS Subba Rao). pp. 53-88. Oxford and IBH Publications, New Delhi.

Atkins C.A., Pate JS., Griffiths G.J. and White S.T. (1980). Economy of carbon and nitrogen in nodulated and non-nodulated (NO_3^--grown) cowpea (*Vigna Unguiculata* (L) Walp.). *Plant Physiology*, **66**: 978-983

Arnon D.I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, **24**: 1-15

Bealagere G., Gastal F. and Warembourg F.R. (1192). The effects of nitrogen fertilization and the growing season on carbon partitioning in a sward of tall fescue (Festuca arudinacea Schreb). *Annals of Botany*, **70**:239-244

Bergersen F.J. and Turner G.L. (1983). An evaluation of ^{15}N methods for estimating

Nitrogen-fixation in a subterranean clover-perennial ryegrass sward. *Australian Journal of Agricultural Resource*, **34**: 391-401

Beyers C.P. DE. L. and Coetzer F.J. (1971). Effect of concentration, pH and time on the properties of di- ammonium EDTA as multiple soil extractant. *Agrochemophysica*, **3**: 49-54

Bieleski R.L. and Lauchli A. 1983. *Synthesis and outlook*. In: Inorganic Plant Nutrition (Encyclopedia of Plant physiology, New Series Volume 15 B). A. Lauchli and R.L. Bieleski (Eds.) Springer-Verlag, New York pp. 745-755.

Bigg W.L. and Daniel, T.W. (1978). Effects of nitrate, ammonium and pH on the growth of conifer seedlings and their production of nitrate reductase. *Plant and Soil*, **50**:371: 385

Blacquire T. and Troelstra S.R. (1986). Nitrate reductase activity in leaves and roots of *Alnus glutinosa* (L) Gaertner. *Plant and Soil*, **95**: 301-313

Boddey R.M., Peoples M.B., Palmer B. and Dart P.J. (2000). Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling in Agroecosystems*, **57**: 235-270

Boyd D..A., Tinker P.B.H., Draycott, A.P., and Last, P. J. (1970). Nitrogen requirement of sugar beet grown on mineral soils. *Journal of Agricultural Science*, **74**: 37-46

Brady N.C. 1990. *The nature and Properties of Soils*, Tenth Edition. Macmillan Publishing CO., New York.

Bremner J.M. (1965). *Nitrogen indexes*. *In*: C.A. Black *et al*. (ed.) Methods of Soil

Analysis, Part 2. A.

Bower N., Hertel K., Oh. J. and Storey R. (1988). Nutritional evaluation of marama bean (*Tylosema esculentum*, Fabaceae): Analysis of the seed. *Economic Botany*, **42**: 533-540

Buysse J. and Merckx R. (1993). An improved colorimetric method to quantify sugar content of plant tissues. *Journal of Experimental Botany*, **44**: 1627-1629

Chaillou S., MoROT-Gudry J.F., Salsac L., Lesaint C. and Jolivet E. (1986). Compared effects of NO_3^- and NH_4^+ on growth and metabolism of French bean. *Physiologie Végetale*, **24**: 679-687

Clark G.E. and Burge G.K. (1999). Effects of nitrogen nutrition in *Sandersonia* cut flower and tuber production in a soil-less medium. *New Zealand Journal of Crop and Horticultural Science*, **44**: 145-152

Claussen W. and Lenz F. (1995). Effect of ammonium and nitrate on net photosynthesis, flower formation, growth and yield of egg plants (*Solanum melongena* L.). *Plant and Soil* **171**: 267-274

Cooke, G.W. (1975). *The Control of Soil Fertility*. London. Crosby Lockwood Ltd.

Cox, W.J. and Reisenauer H.M. (1973). Growth and ion uptake by wheat supplied with nitrogen as nitrate or ammonium or both. *Plant and Soil*, **38**: 363-380

Cramer M.D. and Lewis O.A. M. (1993). The influence of nitrate and ammonium nutrition on the growth of wheat (*Triticum aestivum*) and maize (*Zea mays*) plants. **72**(4): 359-365

Dakora F.D., Lawlor D.W. and Sibuga K.P. (1999) Assessment of symbiotic nitrogen

nutrition in marama bean (*Tylosema esculentum* L), a tuber-producing underutilized African grain legume. *Symbiosis*, **27**: 269-277

Dakora F.D. and Phillips D.A. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, **245**: 35-47

Danso S.K.A., Hadarson G. and Zapata F. (1993). Misconceptions and practical problems in the use of 15N soil enrichment techniques for estimating N_2 Fixation. *Plant and Soil*, **152**: 25-52

Davidson R. (1969). Effects of soil nutrients and moisture on root/shoot ration in *Lolium perenne* L. and *Trifolim repens* L. *Annals of Botany* **33**:571-577

De Frey H. M. (1990). 'N Biologiese Ondersoek Van *Tylosema esculentum*. Msc. Thesis. University of Pretoria

Dyer B. (1894). On the analytical determinations of probably available mineral plant-food in soil. *Journal of Chemical Society*, **65**: 115

Evans J.R. (2001). Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant and soil*, **6**: 121-126.

Evans R.D., Bloom A.J., Sukrapanna S.S. and Ehleringer .J.R. (1996). Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. Cv. T-5) grown under ammonium or nitrate nutrition. *Plant, Cell and Environment*, **19**: 1317-1323

Evans D.R. and Ehleringer J.R. (1993). Broken nitrogen cycles in arid lands: Evidence From 15N of soils. *Oecologia* **94**: 314-317

Fertilizer Society of South Africa. (1974). Manual of soil analysis methods. FSSA Publication no. 37.

Finck, A. (1982). *Fertilizer and Fertilization: Introduction and practical guidance*

to crop fertilization. Verlag Chemie. Weinheim

Fishbeck K.A. and Philips D.A. (1981). Combined nitrogen and vegetative regrowth of symbioticall-grown alfalfa. *Agronomy Journal*, **73**: 975-978

Foth H.D. and Ellis B.G. (1997). *Soil Fertility*, Second Edition. Lewis Publishers. New York.

Friedrich, J.W. and Schrader, L.E. (1978). Sulphur deprivation and nutrient metabolism in maize seedlings. *Plant Physiology*, **61**: 900-903

Ganmore-Neumann R. and Kafkafi, U. (1980). Root temperature and percentage NO_3/ NH_4 effect on tomato plant development: growth and development. *Agronomy Journal*, **72**: 758-761

Gastal F. and Lemaire G. (2002). N uptake and distribution in crops: an agronomical and ecophysiological perspective. *Journal of Experimental Botany*, **53**: 789 - 799

Geiger M., Haake V., Ludewig, F. Sonnewald., U. and Stitt M. (1999). The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. *Plant, Cell and Environment*, **22**: 1177-1199

Geredas J., Zhu Z., Bendixen R., Ratcliffe RG. and Sttlemacher B (1997). Physiological and biological processes related ammonium toxicity in higher plants. *Zeitschrift für Pflanzenerährung und Bodenkunde*, **160**: 239-425.

Giron H.C. (1973). Atomic Absorption Newsletters. **12**:28

Goyal SS., Huffacker R.C. and Lorenz O.A. (1982). Inhibitory effects of ammoniacal nitrogen on growth of radish plants. II. Investigation of the possible causes of

ammonium toxicity to radish plants and its reversal by nitrate. *Journal of American Society of Horticultural Science*, **107**: 130-135.

Greenway H. and Gibbs J. (2003). Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Functional Plant Biology*, **30**: 999-1036

Greidanus T., Peterson L.A. and Dana M.N. (1972). Essentiality of ammonium for cranberry nutrition. *Journal of the American Society for Horticultural Science*, **97**: 272-277

Handley L.L. and Raven. J.A. (1992). The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell and Environment*, **15**: 965-985

Haynes R. J., and Goh K. M. (1978). Ammonium and nitrate nutrition of higher plants. Biological Review, **53**: 465-510

Heaton T.H.E., Spiro B., Madeline S. and Robertson C. (1997). Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. *Oecologia*, **109**:600-607

Hewitt E.J. (1966). Sand and water culture methods used in the study of plant nutrition 2nd revised edition, Commonwealth Bureau of Horticultural and Plantation Crops, East Malling Technical Communication No. 22, Commonwealth agricultural Bureau, Farnham Royal, England.

Hiscox and Israelstam G.F. (1979). A method for the extraction of chlorophyll from leaf tissues without maceration. *Canadian Journal of Botany*, **57**: 1332-1334.

Hobbie E.A., Macko S.A. and Williams M. (2000). Correlation between foliar $\delta^{15}N$ and nitrogen concentration may indicate plant-mycorrhizal interactions. *Oecologia*, **122**: 273-283

Högberg P. (1997). ^{15}N natural abundance in soil-plant systems. The New Phytologist, **137** (2): 179-203

Ignacimuthu S and Babu C.R. (1987). *Vigna radiata* var. sublobata (Fabaceae): Economicallyuseful wild relative of Urd and Mung beans. *Economic Botany*, **41**: 418-422

Ilies P. and Mavinic D.S. (2001). A pre- and post-denitrification system treating a very high ammonia landfill leachate: Effect of pH change on process performance. *Environmental Technology*, **22** (3): 289-300

Ingemarsson B., Oscarson P., af Ugglas M. and Larsson C.M. (1987a). Nitrogen utilization in *Lemna*. II. Studies of Nitrate Uptake using $^{13}NO_3^-$. *Plant Physiology*, **85**: 860-864.

Jaworski E.G. (1971). Nitrate reductase assay in intact plant tissues. *Biochemical and Biophysical Research Communications*, **43**: 1274 – 1279

Jobagy E.G. and Jackson R.B. (2001). The distribution of soil nutrients in with depth: golobal patterns and the imprint of plants. *Biogeochemistry*, **53**: 51-77

Jones J.B., Wolf, and H.A. Mills. (1991). Plant analysis handbook. Micro-Macro publishing, Inc. Athens, GA.

Jovaní M., Barberá. and Farré R. (2001). Review: Effect of some components of milk- and soy-based infant Formulas on mineral Bioavailability. *Food Science and Technology International* **7**(3): 191-198

Jungk A. (1977). Wirkung von Ammonium- und Nitrat-Stickstoff aus das Wachstum und die Zusammensetzung von Pflanzen. *Landwirtschaftliche Forschung*, Kongresβand. Teil II, 18-26.

Keegan A. and von Staden J. (1981). Marama bean, *Tylosema esculentum*, a plant worthy of cultivation. *South African Journal of science*, **77**:387

Kim D., Matsuda O. and Yamamoto T. (1997). Nitrification, denitrification and nitrate reductase rates in the sediments of Hiroshima Bay, Japan. *Journal of Oceanography*, **53**: 317-324

Kirkby E.A. (1968). Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen and carbohydrate metabolism of white mustard plants grown in dilute nutrient solution. *Soil Science*, **105**: 133-141

Kirkby E.A. and Hughes A.D. (1970). Some aspects of ammonium nutrition in plant metabolism. In: Kirkby E.A. ed. Nitrogen Nutrition of the Plant. Leeds: The Waverley Press, 69-76.

Kirkby E.A., and Mengel K. (1967) ionic balance in different tissues of tomato plant in relation to nitrate, urea, or ammonium nutrition. *Plant Physiolog,y* **42**: 6-14

Koba K., Tokuchi N., Yoshioka T., Hobbie E.A. and Iwatsubo G. (1998). Natural abundance of ^{15}N in a forest soil. *Soil Science Society of America Journal*, **62**: 778-781

Kohl D.H., and Shearer, G. (1980). Isotopic fractionation associated with symbiotic N_2 fixation and uptake of NO_3^- by plants. *Plant Physiology*, **66**; 51-56

Lasa B., Frechilla S., Lamfus C and Aparicio-Tejo P.M. (2001). The sensitivity to

ammonium nutrition is related to nitrogen accumulation, *Scientia Horticulturae*, **91**:143-152

Lewis O.A. M., James D. M. and Hewitt E. J, (1982). Nitrogen assimilation in barley (*Hordeum vulgare* L. cv. Mazurka) in response to nitrate and ammonium nutrition. *Annals of Botany*, **49**:39-50.

MacDowall F.D.H. (1983). Kinetics of first-cutting regrowth of alfalfa plants and nitrogenase activity in a controlled environment with or without added nitrate. *Canadian Journal of Botany*, **61**:2405-2409

Marschner H. (1995). *Mineral nutrition in higher plants*. Second Edition. Academic Press, London.

Matsumoto H. and Tamura K. (1981). Respiratory stress in cucumber roots treated with ammonium or nitrate nitrogen. *Plant and Soil*, **60**: 195-204

Meuriot F., Avice J.C., Decau M.L., Simon J.C., Laine P., Volence J.J. and Oury A. Accumulation of N reserves and vegetative storage protein (VSP) in taproots of non-nodulated alfalfa (*Medicago sativa* L.) are affected by mineral N availability. *Plant Science*, **165**: 709-718

Michelsen A., Schimidt IK., Jonasson S., Quarmby C. and Sleep D. (996). Leaf 15N abundance of subarctic plants provides field evidence that ericoid, ectomyrrhizal and non- and arbuscular mycorrhizal species asses different sources of soil nitrogen. *Oecologia*, **105**: 53-63

Millard P. (1996). Ecophysiology of the internal cycling of nitrogen for tree growth. *Z. Planzenernahr*. Bodenk, **159**: 1-10

Muofhe M.L. and Dakora F.D. (1999a). Root phenolic accumulation and loss of

autoregulation of root nodule formation in Mbabara groundnut (*Vigna subteranea*) following boron nutrition and cotyledon excision. *Australian Journal of Botany*, **26**: 435-441

Muofhe M.L. and Dakora F.D. (1999b). Symbiotic response of the African tea legume *Aspalanthus linearis* (Rooibos Tea) to nutrient supply under glasshouse conditions. *Symbiosis,* **27**: 279-292

Muofhe M.L. and Dakora F.D. (1999c). Nitrogen nutrition in nodulated field plants of the shrub tea legume *Aspalanthus linearis* assessed using ^{15}N natural abundance. *Plant and Soil*, **209**: 181-186

National Academy of Sciences. (1979). *Tropical legumes: resources for the future.* National Research Council, Washington D C.

Noctor G., Veljovic-Jovanovic S., Driscoll S., Novitskaya L. and Foyer C.H. (2002). Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? *Annals of Botany*, **89**: 841-850

Oscarson P., Ingemarsson B., af Ugglas M. and Larsson C.M. (1987). Short term studies of NO_3^- uptake in *Pisum* using $^{13}NO_3^-$. *Planta*, **170**: 550-555

Pate J.S., Atkins C.A., White S.T, Rainbird RM. and Woo K.C. (1980). Nitrogen nutrition and xylem transport of nitrogen in ureide-producing grain legumes. *Plant Physiology*, **65**: 961-965

Phillips S.B., Warren D.A. K., and Mullins G.L. (2004). Nitrogen management for white potato production. *Crop, Soil and Environmental Sciences*. Publication No. 438-012

Postel S.L. (2000). Entering an Era of Water Scarcity. *Ecological Applications*, **10**: 941-948 [ISI]

Powell A.M. (1987). Marama bean (*Tylosema esculentum*, Fabaceae): Seed crop in Texas. *Economic Botany*, **41**: 216-220

Raab T.K. and Terry N. (1994). Nitrogen source regulation of growth and photosynthesis in Beta Vulgaris L. *Plant Physiology*, **105**: 1159-1166.

Rufty T.W.; Raper C.D. and Huber S.C. (1984). Alteration in internal partitioning of carbon in soybean plants in response to nitrogen stress. *Canadian Journal of Botany*, **62**:501-508

Sanchez E.E., Righetti T.L., Sugar D. and Lombard P.B. (1991). Recycling of nitrogen in field-grown 'Comice' pears. *Journal of Horticultural Science*, **66**: 479-486

Schrader L.E (1978). *Uptake, accumulation, assimilation and transport of nitrogen in higher plants*. In. Nitrogen in the environment (D.R. Nielsen. and J.G. MacDonalds, eds.), **2**:101-141. Academic Press, New York.

Schortemeyer, M. and Stamp P., Feil, B. (1997). Ammonium tolerance and carbohydrate tolerance on carbohydrate status in maize cultivars. *Annals of Botany*, **79**: 25-30

Shearer G. and Kohl D.H. (1986). N_2-fixation in field settings: estimates based on natural ^{15}N abundance. *Australian Journal of Plant Physiology*, **13**: 699-756

Shearer G., Kohl D.H. and Harper J.E. (1980). Distribution of 15N among plant parts of nodulating and nonnodulating isolines of soybeans. *Plant Physiolog,y* **66**: 57-60

Smethurst P.J. (2000). Soil solution and other soil analyses as indicators of nutrient supply. *Forest Ecology and Management*, **138** (1-3): 397-411

Sprent J.I. (1980). Root nodule anatomy, type of export product and evolutionary origin in some Leguminoseae. *Plant Cell and Environment*, **3**: 35-43

Staswick P.E. (1994). Storage proteins of vegetative plant tissues. *Annual Review of Plant Physiology and Plant Molecular Biology*, **45**: 303-322

Tisdale S.A., Nelson W.L., Beaton J.D., John L. and Alvin J.L. (1993). *Soil fertility and fertilizers*, Fifth Edition. Macmillan Publishing Company. New York.

Trielweiler F.J. and Lindsay W.L. (1969). EDTA- Ammonium carbonate soil test for Zinc. *Soil Science Society of American Proceedings*, **33**: 49 – 54

Unkovich M.J., Pate J.S., Lefroy E.C., Arthur D.J. (2000). Nitrogen isotope fractionation in the fodder tree legume tagasaste (Chamaescytisus proliferus) and assessment of N_2 fixation inputs in deep sandy soils of Western Australia. *Australian Journal of Plant Physiology*, **27**: 921-929

Vasilieva G.G., Mironova A. and Glyanko A.K. (2001).The low above-zero temperature effect in the zone of roots on nitrate reductase activity in pea organs in the process of vegetating. *Turkish Journal of Botany*, **25**: 255-260.

Walkley A. and Black I.A. (1934). An estimation of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, **37**: 29-38

WallaceW. (1986). Distribution of nitrate assimilation between the root and shoot of legumes and a comparison with wheat. *Plant Physiology*, **66**: 630 - 636

Walvoord M.A, Plumer M.A., Sonestrom D.A., Evans R.D., Hartsough, P.C., Newman B.D. and Striegl R.G. (2003). A reservoir of nitrate beneath desert soils. *Science*, **302**: 1021-1024

Watt J.M. and Breyer-Brandwijk. (1962). The medicinal and poisonous plants of Southern and Eastern Africa. 2nd edition. E. & S. Livingstone Ltd., Edinburgh.

Weinbaum S.A., Klein I., Broadbent F.E., Micke W.C. and Muraoka T.T. (1984). Effects of time of nitrogen application and soil texture on the availability of isotopically labelled fertilizer nitrogen to reproductive and vegetative tissue of mature almond trees. *Journal of the American Society for Horticultural Science*. **109**:526-529

Wheeler, C.T., Tilak, Scrimgeour, Hooker J.E., and, Handley L.L. (2000). Effects of symbiosis with *Frankia* and arbuscular mycorrhizal fungus on the natural abundance of ^{15}N in four species of *Casuarina*. *Journal Experimental Botany*, **51**: 287-297.

Yoneyama T. (1996). Characterization of natural ^{15}N abudance of soils. In: Boutton T.W. and Yamasaki S. eds. *Mass Spectrometry of Soils.* New York. Marcell Dekker, 205-223

Appendices

Appendix 1. Modified Hoagland N-Free Nutrient Solution (Hewitt, 1966)

Reagents	mol.wt. (g/mol)	Stock solution (g/l)	Full strength (ml/60 l)	1/2 strength (ml/60 l)	1/4 strength (ml/60 l)	1/8 strength (ml/60 l)	1/16 strength (ml/60 l)	1/32 strength (ml/60 l)
$MgSO_4.7H_2O$	246.48	246.48	128	64	32	16	8	4
$CaCl_2$	110.9	111.0	128	64	32	16	8	4
K_2SO_4	174.27	87.14	128	64	32	16	8	4
KH_2PO_4	136.09	68.0	64	32	16	8	4	2
K_2HPO_4	174.18	87.1	64	32	16	8	4	2
$MnCl_2.4H_2O$	197.91	0.724	64	32	16	8	4	2
$ZNCl_2$.	136.28	0.11	64	32	16	8	4	2
$CuCl_2.2H_2O$	170.48	0.07	64	32	16	8	4	2
$Na_2MoO_4.2H_2O$	241.05	0.025	64	32	16	8	4	2
$CoCl_2.6H_2O$	237.95	0.06	64	32	16	8	4	2
H_3BO_3	61.83	5.72	64	32	16	8	4	2
Sequestrene (138 Fe) Fe chelate			256	128	64	32	16	8

Appendix 2 A. Soil pH levels, soil organic matter (SOM) content and total % N in profiles of soils sampled from different sites in Namibia. Values (Mean ± SE, n = 3-4) with the same letter within a column under each parameter are not significantly different ($P < 0.05$).

Site	Soil depth (cm)	pH	%N	SOM (%)
Marama bean soil				
Sandveld Site 1	0 – 40	5.83 ± 0.15a	0.04 ± 0.001a	0.41 ± 0.05a
	40 – 80	5.47 ± 0.61a	0.04 ± 0.001a	0.34 ± 0.04a
	80 - 120	7.33 ± 0.38a	0.04 ± 0.001a	0.38 ± 0.03a
Sandveld Site 2	0 – 40	5.18 ± 0.15a	0.03 ± 0.002a	0.39 ± 0.06a
	40 – 80	4.98 ± 0.17a	0.03 ± 0.003a	0.37 ± 0.06a
	80 - 120	5.08 ± 0.41a	0.03 ± 0.002a	0.35 ± 0.03a
Sandveld Site 3	0 – 40	6.37 ± 0.40a	0.04 ± 0.004a	0.47 ± 0.03a
	40 – 80	6.80 ± 0.98a	0.04 ± 0.004a	0.43 ± 0.06a
	80 - 120	7.53 ± 0.55a	0.04 ± 0.005a	0.41 ± 0.03a
Buitepos	0 – 40	6.23 ± 1.08a	0.03 ± 0.002a	0.31 ± 0.03a
	40 – 80	5.40 ± 1.10a	0.03 ± 0.003a	0.28 ± 0.06a
	80 - 120	5.65 ± 0.99a	0.03 ±0.002a	0.33 ± 0.07a
Non-marama bean soil				
Sandveld Site 4	0 – 40	6.60 ± 0.56a	0.05 ± 0.005a	0.57 ± 0.07a
	40 – 80	6.93 ± 0.67a	0.04 ± 0.001a	0.53 ± 0.15a
	80 - 120	7.83 ± 0.10a	0.04 ± 0.005a	0.66 ± 0.05a

Appendix 2 B. Soil pH levels, soil organic matter (SOM) content and total %N in profile of soil sampled from different sites in Botswana. Values (Mean ± SE, n = 3-4) with the same letter within a column under each parameter are not significantly different ($P < 0.05$).

Site	Soil depth (cm)	pH	%N	SOM (%)
Marama bean soil				
Charleshill	0 – 40	5.57 ± 0.12a	0.02 ± 0.002a	nd
	40 – 80	5.63 ± 0.15a	0.02 ± 0.002a	nd
	80 - 120	5.60 ± 0.06a	0.02 ± 0.00a	nd
Chobokwane	0 – 40	5.67 ± 0.29a	0.02 ± 0.001a	nd
	40 – 80	4.77 ± 0.27a	0.02 ± 0.001a	nd
	80 - 120	4.70 ± 0.31a	0.02 ± 0.001a	nd
Ghanzi	0 – 40	7.40 ± 0.38a	0.04 ± 0.002	nd
	40 – 80	7.40 ± 0.40a	0.05 ± 0.002a	nd
	80 - 120	nd	nd	nd
Groote Laagte Site 1	0 – 40	6.30 ± 0.25a	0.04 ± 0.007a	nd
	40 – 80	6.03 ± 0.20a	0.03 ± 0.007a	nd
	80 - 120	5.97 ± 0.12a	0.04 ± 0.007a	nd
Makgobokgobo Site 1	0 – 40	5.15 ± 0.03a	0.05 ± 0.001a	0.32 ± 0.05a
	40 – 80	5.28 ± 0.05a	0.05 ± 0.001a	0.29 ± 0.03a
	80 - 120	5.30 ± 0.09a	0.05 ± 0.002a	0.28 ± 0.05a
Xanagas	0 – 40	6.40 ± 0.34a	0.04 ± 0.003a	nd
	40 – 80	5.83 ± 0.12a	0.04 ± 0.002a	nd
	80 - 120	5.83 ± 0.43a	0.03 ± 0.002a	nd
Xhoga	0 – 40	5.67 ± 0.23a	0.05 ± 0.004a	nd
	40 – 80	5.77 ± 0.38a	0.04 ± 0.001a	nd
	80 - 120	5.90 ± 0.31a	0.04 ± 0.002a	nd
Non-marama bean soil				
Groote Laagte Site 2	0 – 40	5.23 ± 0.27a	0.04 ± 0.001a	nd
	40 – 80	4.70 ± 0.03a	0.03 ± 0.002a	nd
	80 - 120	4.73 ± 0.24a	0.03 ± 0.002a	nd
Makgobokgobo Site 2	0 – 40	4.03 ± 0.03a	0.03 ± 0.002a	0.07 ± 0.004a
	40 – 80	4.03 ± 0.03a	0.04 ± 0.002a	0.05 ± 0.003a
	80 - 120	4.07 ± 0.07a	0.03 ± 0.001a	0.03 ± 0.002a

Appendix 2 C. Macronutrients levels in marama bean and non-marama bean soils sampled at different soil depths from different locations in Namibia. Values (Mean ± SE, n = 3-4) followed by the same letter within a column under each site are not significantly different ($P < 0.05$).

		Macronutrient levels			
Site	Soil depth (cm)	P (mg/kg)	Ca (me %)*	Mg (me %)	K (mg/kg)
Marama bean soil					
Sandveld Site 1	0 – 40	4.33 ± 0.25a	1.56 ± 0.02ab	0.50 ± 0.01a	94.5 ± 2.1b
	40 – 80	3.33 ± 0.25a	0.82 ± 0.02a	0.38 ± 0.01a	48.0 ± 7.1a
	80 - 120	3.00 ± 0.50a	2.81 ± 0.00b	1.10 ± 0.50a	92.0 ± 2.7b
Sandveld Site 2	0 – 40	2.00 ± 0.00a	0.70 ± 0.03a	0.29 ± 0.02a	57.0 ± 1.0a
	40 – 80	2.25 ± 0.30a	0.59 ± 0.06a	0.25 ± 0.01a	56.8 ± 4.1a
	80 - 120	2.33 ± 1.15a	0.57 ± 0.05a	0.23 ± 0.02a	56.0 ± 3.46a
Sandveld Site 3	0 – 40	3.33 ± 0.25ab	3.11 ± 1.21a	0.67 ± 0.08a	107 ± 2.8a
	40 – 80	2.33 ± 0.25a	3.00 ± 0.30a	0.77 ± 0.11a	96.3 ± 6.7a
	80 - 120	4.00 ± 0.65b	3.20 ± 0.31a	3.34 ± 0.13b	98.5 ± 2.1a
Buitepos	0 – 40	3.00 ± 0.60a	1.56 ± 1.17b	0.28 ± 0.16a	62.0 ± 7.1a
	40 – 80	2.50 ± 0.24a	0.69 ± 0.42a	0.30 ± 0.17a	65.8 ± 6.3a
	80 - 120	2.50 ± 0.25a	0.72 ± 0.15a	0.34 ± 0.14a	70.5 ± 8.2a
Non-marama bean soil					
Sandveld Site 4	0 – 40	2.25 ± 0.45a	2.72 ± 0.32a	0.86 ± 0.09a	162 ± 5.7b
	40 – 80	3.50 ± 0.35a	3.29 ±0.37a	1.07 ±0.07a	66.7 ± 3.6a
	80 - 120	2.33 ± 0.75a	4.28 ±0.50a	2.84 ± 0.27b	161 ± 6.2b

* To convert me% of calcium to mg/kg multiply the me% by 200, for magnesium multiply by 150.

Appendix 2 D. Macronutrients levels in marama bean and non-marama bean soils sampled at different soil depths from different locations in Botswana. Values (Mean ± SE) followed by the same letter within a column under each site are not significantly different (P ≤0.05).

Site	Soil depth (cm)	Macronutrient levels			
		P (mg/kg)	Ca (me %)	Mg (me %)	K (mg/kg)
Marama bean soil					
Charleshill	0 – 40	2.00 ± 0.00b	1.26 ± 0.07a	0.44 ± 0.01a	78.0 ± 2.52a
	40 – 80	1.00 ± 0.00a	1.41 ± 0.09a	0.47 ± 0.01a	79.7 ± 2.67a
	80 - 120	1.00 ± 0.00a	1.46 ± 0.04a	0.47 ± 0.00a	84.3 ± 4.84a
Chobokwane	0 – 40	2.00 ± 0.00b	1.10 ± 0.18a	0.60 ± 0.04a	71.0 ± 5.51a
	40 – 80	1.33 ± 0.33ab	0.73 ± 0.10a	0.55 ± 0.04a	66.0 ±3.05a
	80 - 120	1.00 ± 0.00a	0.67 ± 0.14a	0.55 ± 0.04a	58.0 ± 6.56a
Ghanzi	0 – 40	7.67 ± 1.76a	4.64 ± 1.06a	0.54 ± 0.12a	68.3 ± 4.10a
	40 – 80	6.33 ± 2.40a	6.70 ± 2.76a	0.56 ±0.14a	60.1 ± 3.06a
	80 - 120	nd	nd	nd	nd
Groote Laagte Site 1	0 – 40	2.00 ± 0.00a	1.66 ± 0.05a	1.64 ± 0.10a	80.3 ± 1.07a
	40 – 80	1.33 ± 0.33a	1.39 ± 0.07a	1.83 ± 0.11a	66.3 ± 4.80a
	80 - 120	1.67 ± 0.33a	1.40 ± 0.07a	1.90 ±0.17a	69.3 ± 5.70a
Makgobokgobo Site 1	0 – 40	1.00 ± 0.00a	1.36 ± 0.07a	0.60 ± 0.05a	80.7 ± 1.33a
	40 – 80	1.000 ± 0.00a	1.44 ± 0.07a	0.66 ± 0.03a	73.8 ± 9.16a
	80 - 120	1.00 ± 0.00a	1.58 ± 0.11 a	0.77 ± 0.04a	67.8 ± 11.9a
Xanagas	0 – 40	4.67 ± 0.33b	1.71 ± 0.32a	0.47 ± 0.03a	80.7 ± 1.45a
	40 – 80	1.67 ± 0.33a	1.33 ± 0.04a	0.50 ± 0.09a	67.0 ± 2.08a
	80 - 120	1.67 ± 0.33a	1.67 ± 0.45a	0.69 ± 0.10a	73.0 ± 2.08b
Xhoga	0 – 40	2.67 ± 0.33b	3.79 ± 0.50a	0.83 ± 0.14a	114 ± 17.0b
	40 – 80	1.67 ± 0.33a	4.24 ± 0.72a	0.81 ± 0.12a	74.0 ± 5.68a
	80 - 120	2.00 ± 0.28ab	4.12 ± 0.13a	0.80 ± 0.18a	69.3 ± 5.92b
Non-marama bean soil					
Groote Laagte Site 2	0 – 40	1.00 ± 0.00a	0.85 ± 0.08a	0.58 ± 0.04a	50.0 ± 6.56a
	40 – 80	1.33 ± 0.33a	0.56 ± 0.07a	0.56 ± 0.07a	50.0 ± 7.00a
	80 - 120	1.00 ± 0.00a	0.75 ± 0.13a	0.54 ± 0.04a	43.7 ± 5.36a
Makgobokgobo Site 2	0 – 40	1.67 ± 0.33a	0.16 ± 0.01a	0.30 ± 0.05a	49.0 ± 14.5a
	40 – 80	1.00 ± 0.00a	0.16 ± 0.01a	0.31 ± 0.04a	39.7 ± 1.86a
	80 - 120	1.00 ± 0.00a	0.15 ± 0.01a	0.34 ± 0.05a	39.3 ± 4.48a

Appendix 2. E Micronutrients levels in marama bean and non-marama bean soils sampled at different soil depths from different locations in Namibia. Values (Mean ± SE, n = 3-4) followed by the same letter within a column under each site are not significantly different (P ≤0.05).

		Micronutrient levels			
Site	Soil depth (cm)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Al (mg/kg)
Marama bean soil					
Sandveld Site 1	0 – 40	0.38 ± 0.04a	0.99 ± 0.15a	62.9 ± 6.0b	18.6 ± 0.40a
	40 – 80	0.42 ± 0.01a	0.83 ± 0.17a	33.8 ± 5.1a	15.5 ± 0.45a
	80 - 120	1.04 ± 0.00b	2.72 ± 0.21b	45.3 ± 4.5a	21.5 ± 1.15a
Sandveld Site 2	0 – 40	0.21 ± 0.08a	1.27 ± 0.35b	34.5 ± 3.5a	12.4 ± 0.44a
	40 – 80	0.13 ± 0.03a	0.56 ± 0.16a	27.4 ± 3.3a	12.1 ± 0.70 a
	80 - 120	0.16 ± 0.02a	0.78 ± 0.12ab	24.2 ± 3.3a	14.4 ± 2.33a
Sandveld Site 3	0 – 40	0.26 ± 0.00a	0.46 ± 0.05a	60.4 ± 4.5c	26.3 ± 2.12b
	40 – 80	0.75 ± 0.08b	0.85 ± 0.16b	42.9 ± 1.0b	26.3 ± 0.89b
	80 - 120	0.87 ± 0.20b	1.59 ± 0.00c	7.19 ± 0.32a	10.1 ± 0.41a
Buitepos	0 – 40	0.25 ± 0.06a	0.82 ± 0.48a	20.5 ± 3.1a	13.7 ± 1.52a
	40 – 80	0.15 ± 0.05a	0.54 ± 0.09a	20.8 ± 2.2a	11.4 ± 1.25a
	80 - 120	0.17 ± 0.03a	0.53 ± 0.28a	14.7 ± 1.2a	11.5 ± 0.89a
Non-marama bean soil					
Sandveld Site 4	0 – 40	1.05 ± 0.04a	1.05 ± 0.19a	75.1 ± 8.1c	31.7 ± 1.52b
	40 – 80	1.29 ± 0.17 a	1.15 ± 0.54a	50.2 ± 6.3b	32.0 ± 4.31b
	80 - 120	1.29 ± 0.23a	1.23 ± 0.45a	7.52 ± 1.3 a	5.62 ± 0.93a

Appendix 2 F. Micronutrients levels in marama bean and non-marama bean soils sampled at different soil depths from different locations in Botswana. Values (Mean ± SE, n = 3-4) followed by the same letter within a column under each site are not significantly different (P ≤0.05).

		Micronutrient levels			
Site	Soil depth (cm)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Al (mg/kg)
Marama bean soil					
Charleshill	0 – 40	0.31 ± 0.01a	0.76 ± 0.17b	23.1 ± 0.9a	0.31 ± 0.01a
	40 – 80	0.29 ± 0.04a	0.39 ± 0.01a	25.3 ± 1.0a	0.31 ± 0.00a
	80 - 120	0.31 ± 0.03a	0.48 ± 0.11ab	24.6 ± 1.2a	0.34 ± 0.03a
Chobokwane	0 – 40	0.23 ± 0.04a	0.76 ± 0.24ab	9.96 ± 1.4b	0.28 ± 0.01a
	40 – 80	0.18 ± 0.02a	1.13 ± 0.54b	7.48 ± 2.1ab	0.33 ± 0.04a
	80 - 120	0.19 ± 0.00a	0.66 ± 0.15a	5.64 ± 1.6a	0.39 ± 0.07a
Ghanzi	0 – 40	0.82 ± 0.14a	1.02 ± 0.35a	17.9 ± 3.7a	0.36 ± 0.01a
	40 – 80	0.89 ± 0.15a	0.70 ± 0.07a	22.2 ± 4.4a	0.38 ± 0.01a
	80 - 120	nd	nd	nd	nd
Groote Laagte Site 1	0 – 40	0.27 ± 0.12a	1.19 ± 0.35a	19.4 ± 1.7a	0.30 ± 0.00a
	40 – 80	0.26 ± 0.09a	1.20 ± 0.50a	18.7 ± 1.0a	0.30 ± 0.01a
	80 - 120	0.25 ± 0.02a	0.81 ± 0.27a	17.9 ± 0.8a	0.30 ± 0.01a
Makgobokgobo Site 1	0 – 40	0.40 ± 0.04a	0.71 ± 0.18a	32.2 ± 2.6a	0.57 ± 0.07a
	40 – 80	0.44 ± 0.03a	1.53 ± 0.61b	32.4 ± 1.7a	0.53 ± 0.04a
	80 - 120	0.42 ± 0.03a	1.24 ± 0.49ab	30.1 ± 2.3a	0.54 ± 0.06a
Xanagas	0 – 40	0.39 ± 0.02 a	0.63 ± 0.07a	17.3 ± 1.5a	0.43 ± 0.02a
	40 – 80	0.34 ± 0.04a	0.49 ± 0.03a	19.9 ± 1.2a	0.42 ± 0.01a
	80 - 120	0.40 ± 0.03a	0.42 ± 0.05a	20.1 ± 2.3a	0.32 ± 0.10a
Xhoga	0 – 40	0.77 ± 0.15a	1.10 ± 0.58a	64.8 ± 19.5a	0.43 ± 0.02a
	40 – 80	0.71 ± 0.09a	1.29 ± 0.05a	69.1 ± 15.5a	0.42 ± 0.01a
	80 - 120	0.86 ± 0.11a	0.77 ± 0.02	60.0 ± 14.0a	0.32 ± 0.10a
Non-marama bean soil					
Groote laagte Site 2	0 – 40	0.15 ± 0.04a	0.62 ± 0.09a	8.52 ± 2.17a	0.30 ± 0.01a
	40 – 80	0.13 ± 0.01a	0.80 ± 0.24a	6.67 ± 1.42a	0.40 ± 0.07a
	80 - 120	0.15 ± 0.03a	0.57 ± 0.07a	5.43 ± 1.70a	0.24 ± 0.11a
Makgobokgobo Site 2	0 – 40	0.37 ± 0.08a	0.81 ± 0.48b	8.63 ±1.50a	1.90 ±0.68a
	40 – 80	0.40 ± 0.02a	0.45 ± 0.09ab	7.73 ± 0.55a	1.99 ± 0.22a
	80 - 120	0.33 ± 0.05a	0.27 ± 0.07a	6.21 ± 1.49a	2.07 ± 0.22a