Nutrient deficiencies in lesser yam (*Dioscorea esculenta*) characterized using constant–water table sand culture

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Summary

Nutritional deficiencies in *Dioscorea esculenta* (Lour.) Burk were studied using a novel culture system, applying a constant water table in acid-washed sand, and a demand-driven nutrient supply schedule. This system provided a stable growth environment and was highly efficient with respect to resources and labor. Yam plants (cv. Balbal) were propagated from 30 g tuber head setts and grown for 12 or 20 weeks, with nutrients supplied in the water reservoir to meet demand according to weekly leaf counts. Deficiencies of nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur were induced by reducing supply of the relevant nutrient to one tenth of normal. Deficiencies of iron, boron, manganese, zinc, copper, and molybdenum were induced by omitting the relevant nutrient from the culture medium. After 12 weeks, leaf blades of the main stem were sampled from four positions (immature, young expanded, mid, and old) weighed and analyzed for nutrient concentration, and dry weight of plant parts was recorded. Significant growth reduction was achieved for each deficiency except Fe, Zn, Cu, and Mo, which nonetheless developed some foliar symptoms. Effects on nutrient concentrations in leaves are reported, providing concentrations indicative of adequate and deficient status. *Dioscorea esculenta* was found to be particularly sensitive to Mn deficiency, although symptom presentation was atypical. Unusually low translocation of phloem-mobile nutrients was also observed, paralleling reported observations on *D. alata*.

1 Introduction

Dioscorea esculenta (Lour.) Burk. is the least studied of the major staple yam species, although it is widely cultivated in southern Asia and the Pacific and is the dominant or co-dominant staple food in parts of India and Papua New Guinea. *Bourke* and *Vlassak* (2004) estimate annual production in Papua New Guinea exceeds 180,000 tons. The crop is almost always grown in low-input systems, where soil fertility is a major limitation to yield. In most areas, cropping intensity has increased in recent years due to land pressure. In Papua New Guinea, farmers reported shortened fallows and progressive yield decline, but symptoms of nutritional stress were not recognized by farmers (O'Sullivan and Ernest, unpublished). In several coastal areas with high-pH soils, symptoms of apparent micronutrient deficiencies have increased in severity as soil organic matter has declined. Identifying the major limiting nutrients in each situation is central to designing improved soil management practices, but has been hampered by a lack of diagnostic information on this crop.

This study aimed to provide information on the visible symptoms of deficiencies of all nutrients for which field deficiencies are expected, as well as on leaf-tissue nutrient concentrations associated with healthy and deficient plants. It was part of a larger study on diagnosis and correction of nutritional disorders of yams in the Pacific. The experimental system was constrained by the limited distilled water and laboratory resources of the research station in Papua New Guinea and the inability to transfer planting material to better-resourced facilities in Australia due to the lack of phytosanitary information on this species, which is propagated vegetatively and has not been successfully tissue-cultured. A novel sand-culture system, using a bottom-watered (constant water table) closed-pot technique (adapted from *Hunter*, 1981) was adopted to provide a stable root environment with minimum consumption of distilled water, low maintenance requirement, and fine control of nutrient supply. The results are discussed in relation to symptoms described for *Dioscorea rotundata* Poir. (*Gaztambide* and *Cibes*, 1975) and *D. alata* L. (*O'Sullivan* and *Jenner*, 2006).

2 Materials and methods

The experiment was conducted in a well-ventilated polycarbonate greenhouse at the Sir Alkan Tololo Research Centre of the National Agricultural Research Centre, near Lae, Papua New Guinea (latitude 6°40′ S, longitude 146°54′ E), between January and May 2003. Mean minimum and maximum temperatures during the experimental period were 23.9°C and 32.2°C, respectively. Temperatures in the greenhouse were similar to ambient, and light transmittance was approximately 85%, giving a mean daytime light incidence of 5.4 MJ m^{-2}.

Fine silica sand, from coastal dunes near Brisbane, Australia, was selected as having extremely low natural nutrient levels and supporting high growth rates comparable to those achieved with a high-quality potting medium in preliminary tests. Sand was acid-washed in five 100 L batches using one

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volume of 1 M HCl and rinsed with 10×300 L of deionized water. Organic debris was removed during siphoning of each rinse. The washed sand maintained a pH of 4.8 and was adjusted to pH 6.8 using 0.1 mmol NaOH per L of sand before drying and shipping to Papua New Guinea.

Plant pots consisting of 10 L domestic plastic buckets were fitted with a cylindrical plastic well, placed vertically on a piece of fiberglass capillary mat, before filling the pot with 9 kg of slightly moist sand. Moist sand packed less densely than fully dry sand, improving aeration and root penetration. In pots for the "minus B" treatment, 10 g of borate-specific resin (Amberlite IRA 743) were mixed with the sand, as water purification was *via* a glass still and some B contamination of water was anticipated. Plastic plates were cut to cover the surface of the sand as an evaporation barrier, with openings for the well and planting hole. The upper surface of plates was painted white and the outside of pots covered with aluminum foil, to minimize heat absorption. Pots were arranged in four blocks of 14 and treatments randomly assigned within blocks. Each block contained two treatments receiving all nutrients (control) and one treatment deficient in each of N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Cu, and Mo. Doubling the number of controls in nutrient-omission experiments is the most efficient way of increasing degrees of freedom for statistical analysis, in which each omission treatment is compared with the control (*Asher* et al., 2002).
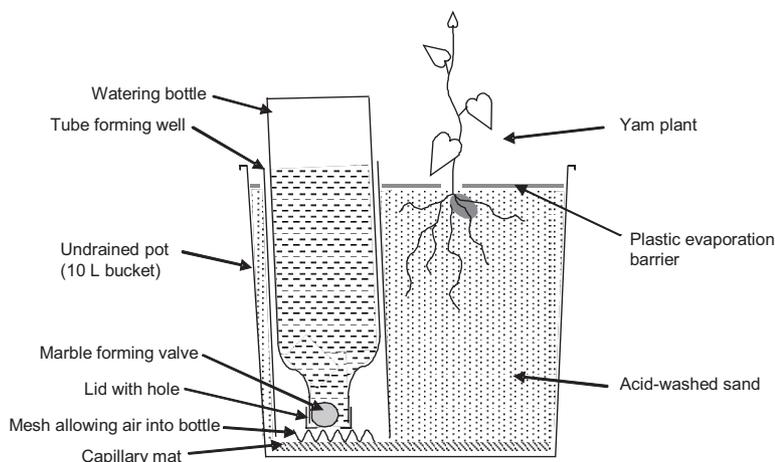
Water and nutrients were supplied to each pot *via* a 750 mL plastic screw-cap bottle upturned in the well. A 14 mm hole was cut in the lid of each bottle and a 15 mm glass marble placed inside to act as a valve, minimizing solution escape during removal and replacement of bottles. When in position, the bottle top sat on a small square of coarse nylon mesh on the capillary mat, pushing up the marble and allowing exchange of air and solution to maintain a constant water table of a few millimeters depth at the base of the pot. The exposed basal part of the bottle was painted with aluminum paint to eliminate light. The plant culture system is illustrated in Fig. 1.

Nutrient stock solutions were made up to provide sufficient nutrients for growth of one new leaf in 4 mL of solution, based on previous whole-plant nutrient analysis of apparently healthy plants. Separate nutrient solutions were prepared for each treatment. In the "All nutrients" treatment, concentrations in the stock solution were (mM) 169 N, 7.65 P, 75 K, 73 Ca, 6.5 Mg, 4.9 S, 0.141 Fe, 0.439 B, 0.288 Mn, 0.060 Zn, 0.009 Cu, 0.0017 Mo, 0.00013 Ni, 17 Na. In deficient treatments, macronutrients were added at 0.1 times the normal rate, and micronutrients were omitted. Cations and anions were supplied as their chloride and sodium salts, respectively, except a combination of ammonium nitrate and calcium nitrate supplied N and Ca, to avoid excessive Na and Cl concentrations. In the "minus N" treatment, calcium chloride replaced calcium nitrate, and in "minus Ca", potassium nitrate replaced both calcium nitrate and potassium chloride. Stock solutions were adjusted to pH 5.7 and when diluted had a pH of 6.3.

Initial wetting up was achieved by adding required nutrients to the watering bottle, filling with distilled water and upturning into the pot well. When the bottle was emptied through capillary uptake by the sand, it was refilled with distilled water until the water-uptake capacity of the sand had been reached. The uptake capacity of each pot was 2.3 L water. Nutrients added were 80 mL of the treatment-specific nutrient solution, together with additional amounts of magnesium chloride (except in "minus Mg"), sodium sulfate (except in "minus S"), and FeNaEDTA (except in "minus Fe") to achieve soil solution concentrations considered adequate for optimal plant growth. Concentrations after wetting up were calculated to be (μM) 5966 N, 270 P, 2635 K, 2570 Ca, 617 Mg, 440 S, 18 Fe, 15 B, 10 Mn, 2.1 Zn, 0.3 Cu, 0.06 Mo, 0.005 Ni, 1141 Na.

Small whole tubers of *D. esculenta* (Lour.) Burk. cv. Balbal were sprouted in sawdust. Tubers were trimmed to approximately 30 g weight, roots washed free of all sprouting medium and rinsed in distilled water before transplanting to the sand culture. Due to variability in sprout development, plants were blocked according to initial vine height (maximum 48 cm). Leaf number was recorded initially and weekly thereafter, and nutrients supplied into the watering bottle at 4 mL per additional leaf. Thus, the initial nutrient concentrations were approximately maintained throughout the experiment. Solution was maintained in the bottles at all times, refilling

Figure 1: Diagrammatic representation of the constant–water table plant culture system.

Labels: Watering bottle; Tube forming well; Undrained pot (10 L bucket); Marble forming valve; Lid with hole; Mesh allowing air into bottle; Capillary mat; Yam plant; Plastic evaporation barrier; Acid-washed sand.

with distilled water between nutrient additions when necessary. Vines were trained up vertical strings attached to overhead wires at 2 m height.

At 12 weeks, replicates 1–3 were harvested, recording vine length, leaf number, and dry weights of vines (including leaf samples), tubers, and roots (including stolons). Replicate 4 was maintained for a further 8 weeks to continue development of deficiency symptoms. Quantitative data from this replicate are not included in analyses presented. Leaf blades were sampled from the main vine at the 5th–6th node from youngest open leaf (immature leaves), the 7th–8th node (young expanded leaves and the proposed index tissue), the middle two nodes, and 5th–6th from the base of the vine (old leaves). Leaf tissue was analyzed by ICP-AES following 5:1 nitric-to-perchloric acid digestion (P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo) or following dry ashing (B). Concentrations of Cu and Mo were checked by additional analysis by ICP-MS. Nitrogen was analyzed using a Leco CNS combustion analyzer ("All nutrients", "minus N", and "minus Mo" only). Data were analyzed using the General Linear Means procedure (SAS), and least squares means are quoted. For leaf nutrient concentrations, statistical procedures were performed on log-transformed data to normalize residuals.

3 Results and discussion

3.1 Growth

Plants in control treatments ("All nutrients") grew well showing no signs of stress at 12 weeks. Nutrient-omission treatments significantly reduced dry-matter production in all treatments except "minus Fe", "minus Zn", "minus Cu", and "minus Mo" (Fig. 2). The plants in "minus B" grew very slowly, and two of the four replicates died. While this was initially attributed to very severe B deficiency, subsequent experiments using the Amberlite IRA 743 resin to regulate B supply to *D. alata* revealed that the resin itself appears to be toxic to yam.
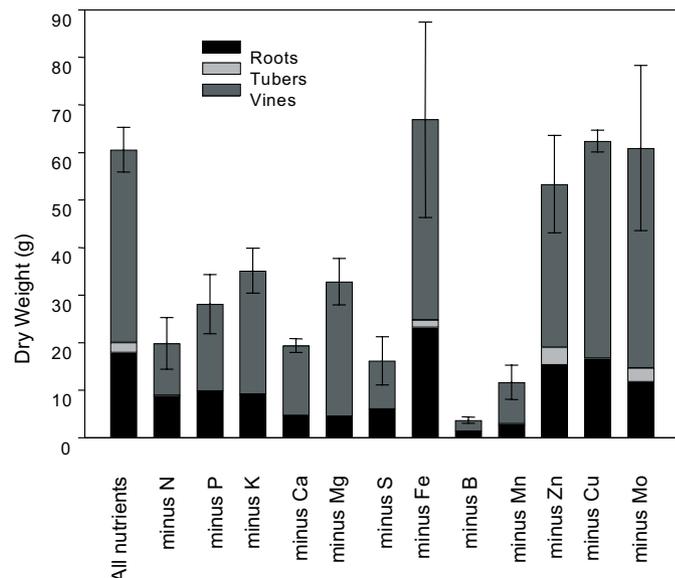
Variation in growth parameters of the vine are summarized in Fig. 3. For each parameter, the mean of control plants $\pm$ LSD$_{(p=0.05)}$ for the parameter were as follows: vine length 9.53±3.82 m, node number 123±66.8, average internode length 79.1±22.6 mm, branchiness 46.5±25.3%, top-to-root ratio 2.30±1.75.

Despite a significant total-dry-weight reduction, "minus Mg" did not show significant reduction in vine length, as a result of increased top-to-root ratio, largely achieved by lengthened internodes—an etiolation response commonly documented in Mg-deficient plants and consistent with observations of *D. rotundata* by *Gaztambide* and *Cibes* (1975). "Minus Zn" and "minus Fe", which did not show significant dry-weight reduction, did have significantly reduced internode length, compensated by an increase in node number, particularly through increased branching.

Tuber development was variable among replicates, as the harvest was during the period of tuber initiation. Many plants had developing stolons but no tubers, while others had several small tubers. Thus treatment effects on tuber yield were not determined.

3.2 Leaf nutrient concentrations

The leaves of actively growing *D. esculenta* vines remain very small and densely hairy for some distance from the vine tip, with substantial expansion and maturity beginning around six nodes back from the tip (Fig. 6a). Consequently, the index tissue sample selected for *D. alata* (blades of the 5th and 6th leaves) was considered too immature on *D. esculenta.* Blades of 7th and 8th leaves were generally well expanded, and although they were not of maximum size, older leaves at specific nodes are difficult to sample in the field due to entanglement of the vine. Therefore, in this study blades of 7th and 8th leaves were taken as tentative index leaves, and compared with younger leaves at nodes 5 and 6, mid leaves half-way down the vine, and older leaves being the 5th and 6th from

Figure 2: Dry weight of *D. esculenta* plants and its distribution among roots, tubers, and vines 12 weeks after transplanting to sand culture, with full nutrient supply and with supply deficient in one of 12 elements per treatment. Error bars are the standard error of the mean total weight of three replicate plants.
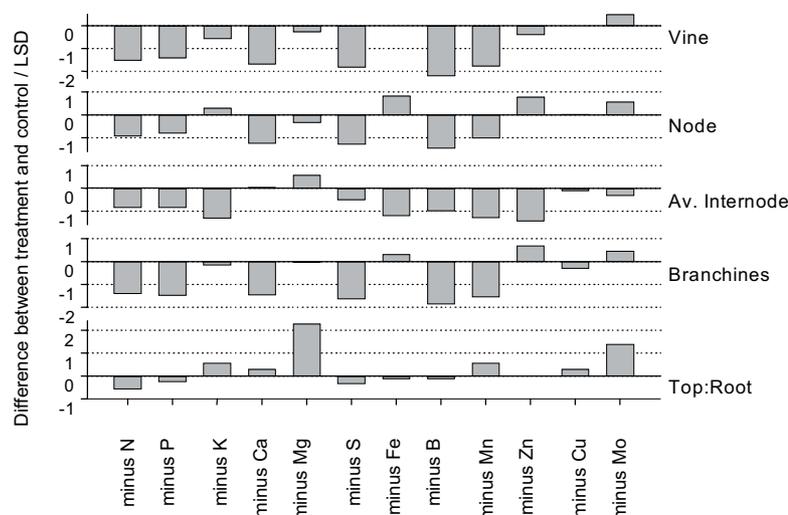
Figure 3: Effects of nutrient-omission treatments on various growth parameters of *D. esculenta* vines 12 weeks after transplanting to sand culture. Values are expressed as the difference between the deficient treatment and control "All nutrients" treatment, as multiples of the $LSD_{p=0.05}$ for the parameter. Values greater than ±1 are considered significant effects of the treatment. Branchiness is the length of branches as a proportion of total vine length.

the base. The oldest leaves present were not sampled, as these may have formed before the imposition of treatments.

Figure 4 summarizes the nutrient concentrations in the four leaf samples, from control "All nutrients" treatment and from the deficient treatment for each element. As was found in *D. alata* (*O'Sullivan* and *Jenner*, 2006), the concentrations of N and P tended to decline with leaf age in healthy plants, but the decline was less pronounced in deficient plants. Most species show the reverse trend, with leaf N and P concentration relatively stable in healthy plants but declining with age in deficient plants due to remobilization from the older leaves. The decline in healthy plants may result from dilution due to continued vacuolar expansion, lignification, or starch deposition. The smaller decline in deficient plants suggests poor remobilization capacity for these nutrients. However, in contrast to the *D. alata* study, K showed a greater decline in older leaves of deficient plants than healthy plants.

With the exception of Mn, micronutrients tended to be elevated in immature leaves and also to accumulate in oldest

leaves, with mid leaves having lowest concentrations. The same pattern was evident for Mg and S. These patterns were mirrored at lower levels in deficient plants, except for B and Mg where lowest concentrations were in the oldest leaves indicating either remobilization or diversion from the transpiration stream.

The effects of deficiency treatments on elemental profiles of index leaves are shown in Tab. 1 and Fig. 5. In Fig. 5, deviations from the "All nutrients" treatment are expressed as multiples of the $LSD_{p=0.05}$, based on analysis of variance of the log-transformed data. All deficiency treatments significantly reduced the concentration of the withheld nutrient in index leaves, except "minus K", "minus Fe", "minus Cu", and "minus Mo". In "minus Mo", it is noteworthy that N concentration was significantly elevated, consistent with accumulation of nitrate due to insufficient nitrate reductase, which contains Mo as a cofactor. For "minus K", the reduction in K concentration was significant in mid and old leaves. "Minus K" plants had elevated concentrations of Ca and Mg, while "minus S", and to a lesser extent "minus P", showed elevation of cations generally. The low concentrations of P and Zn in "minus Ca" may

Table 1: Effects of nutrient-omission treatments on relative yield (total dry matter as % of treatment "All nutrients") and on nutrient composition of leaves from the 7th and 8th nodes from first open leaf, of *D. esculenta* cv. Balbal sampled 12 weeks after transplanting to sand culture. Data are the mean of three replicates. Concentration of the test element for each treatment is given in bold text. Statistical separations are shown in Fig. 5.

| Treatment	N	P	K	Ca	Mg	S	Fe	B	Mn	Zn	Cu	Mo	Na	Rel. Yield
	%	%	%	%	%	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
All nutrients	2.23	0.26	2.46	1.03	0.17	0.18	156	64	76	8.4	3.5	1.5	153	100
minus N	**1.19**	0.24	2.94	1.27	0.15	0.11	106	89	97	7.7	4.5	1.6	72	33
minus P	n.a.[a]	**0.10**	3.03	1.56	0.23	0.22	185	62	160	12.5	8.1	1.3	142	49
minus K	n.a.	0.38	**1.81**	2.78	0.32	0.22	157	103	104	11.1	2.4	1.3	128	59
minus Ca	n.a.	0.11	3.64	**0.27**	0.25	0.14	158	29	52	3.7	2.1	0.7	40	37
minus Mg	n.a.	0.30	3.34	1.06	**0.09**	0.18	139	32	66	8.5	6.4	1.1	86	57
minus S	n.a.	0.36	4.10	2.22	0.28	**0.05**	178	71	204	15.9	7.2	1.2	99	29
minus Fe	n.a.	0.26	2.78	0.95	0.18	0.18	**99**	47	58	6.7	2.4	1.3	153	104
minus B	n.a.	0.41	3.61	0.59	0.20	0.17	446	**10**	8	25.9	5.7	0.8	325	6
minus Mn	n.a.	0.40	4.13	0.92	0.22	0.24	198	85	**6**	9.7	7.0	1.4	173	19
minus Zn	n.a.	0.27	2.40	2.27	0.22	0.19	133	77	143	**5.2**	3.4	1.5	71	90
minus Cu	n.a.	0.31	2.48	0.98	0.24	0.20	194	63	57	9.2	**2.6**	1.8	159	104
minus Mo	2.95	0.20	2.72	1.27	0.20	0.16	102	38	82	7.9	2.4	**0.8**	82	108

[a] n.a. = not analyzed.
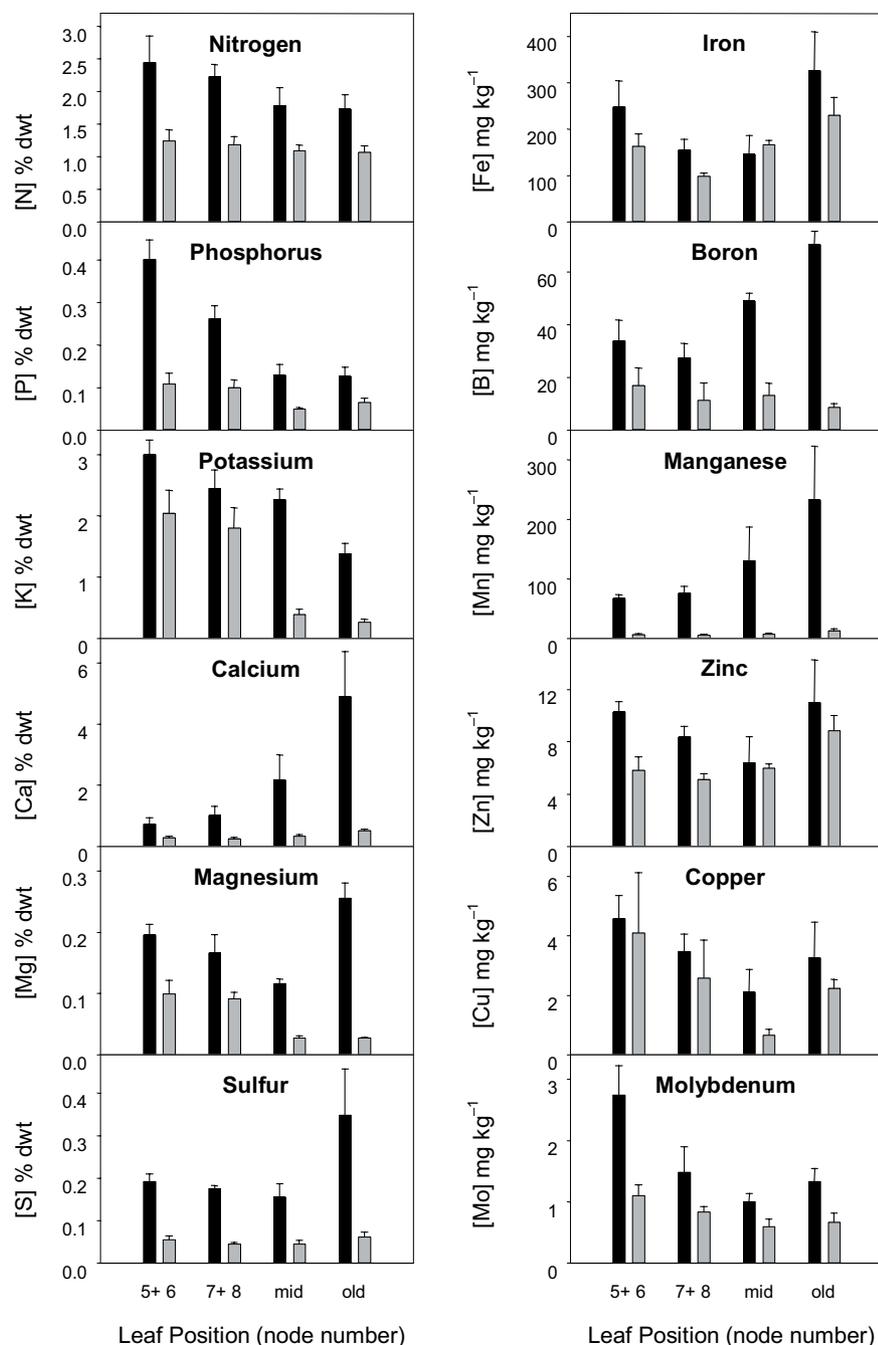
Figure 4: Concentration of nutrient elements in leaf blades of *D. esculenta* from plants supplied with all nutrients (black) and plants deprived of the reported element (gray). Macronutrient-deficient treatments received one tenth of the normal supply level, while micronutrients were omitted from their deficient treatment. Error bars are standard error of the mean of three replicates.

be attributable to reduced root surface area, particularly growth of root hairs and fine roots. "Minus N" was low in S, most likely reflecting a drop in demand for S due to deficiency of the N-containing precursors for S-metabolites, particularly amino acids. The "minus B" plants had elevated concentrations of Fe and Zn, but very low Mn.

Potassium concentration was elevated over the control in most of the treatments showing significant growth depression. A similar pattern was recorded for *D. rotundata* by *Gaztambide* and *Cibes* (1975), but they sampled a composite of all leaf blades, from senescent vines near crop maturity, hence the very low K concentration recorded in their control plants. In the current study, this pattern might suggest that

the K supply to the control treatment may not have been optimal, and the concentrations from 3.0% to 4.1% (Tab. 1), seen in those treatments whose limited growth was unable to deplete the K supply, may be more indicative of optimal levels. Studies with *D. alata* (O'Sullivan, unpublished) indicated a critical K concentration around 3.3% and limited luxury uptake of K. However, *Vander Zaag* et al. (1980) recorded a K concentration range of 2.3%–3.1% among cultivars of *D. esculenta* and 3.1%–3.4% among *D. alata* grown under the same conditions. Their sample, consisting of recently mature leaves at 4 months, was similar to our index leaves, except for the inclusion of petioles which may have elevated K concentrations slightly. This would suggest that the critical K concentration for *D. esculenta* is likely to be

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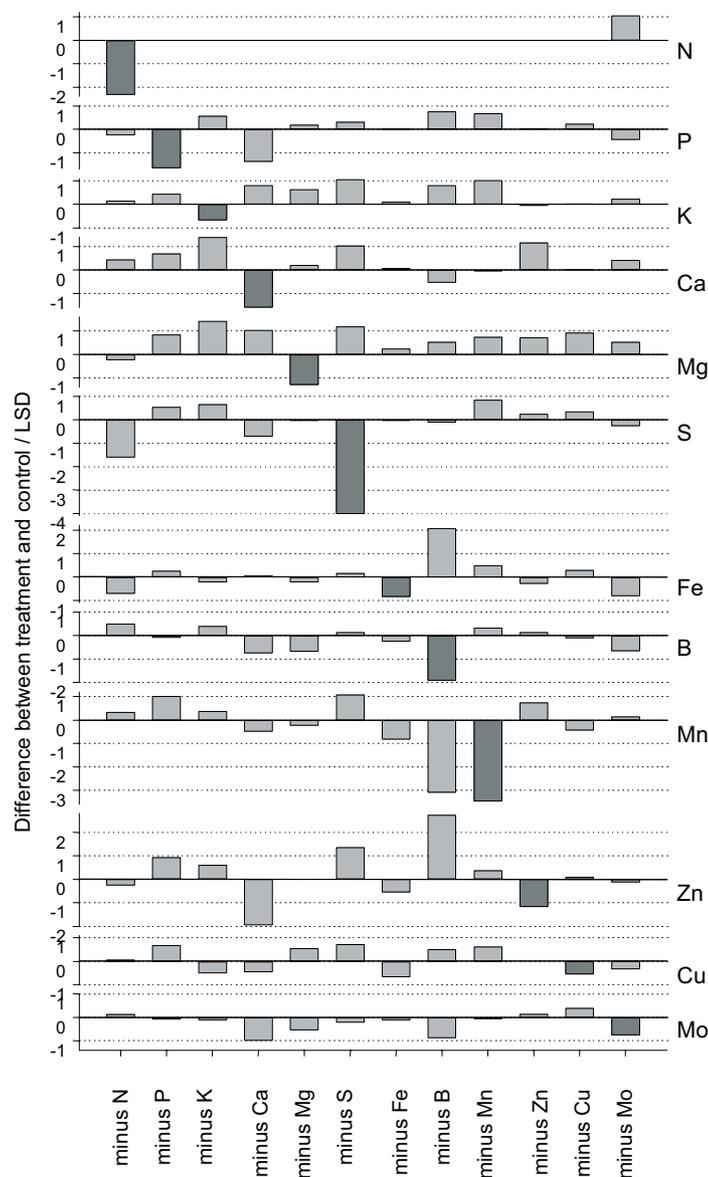
Figure 5: Effects of nutrient-omission treatments on concentrations of nutrients in leaf blades from the 7th and 8th youngest leaves (proposed index leaves) of *D. esculenta*, 12 weeks after transplanting to sand culture. Dark shading indicates the withheld element. Statistics are derived from log-transformed data. Values are expressed as the difference between the deficient treatment and control "All nutrients" treatment, as multiples of the LSD$_{p=0.05}$ for the parameter. Values greater than ±1 are considered significant effects of the treatment. Nitrogen was analyzed only in "All nutrients", "minus N", and "minus Mo".

below 3.0%. *Obigbesan* and *Agboola* (1978) also recorded higher foliar K in *D. alata* compared with *D. rotundata* and *D. cayenensis*, and we suggest this was related to the relatively low dry-matter content (high water content) they recorded in the *D. alata* foliage. These observations support a conclusion that the K concentrations around 2.5% recorded in "All nutrients" plants approximate an adequate level for *D. esculenta*.

3.3 Visible symptoms

Control ("All nutrients") plants generally had an even mid to dark green color, with actively growing tips, as evidenced by the considerable length of vine bearing expanding leaves above the first mature leaves (Fig. 6a).

Nitrogen-deficient plants had thin, generally unbranched vines bearing small, light green, soft-textured leaves. Tissue adjacent to veins was slightly greener on younger leaves

(Fig. 6b). Stems and petioles tended to turn red on mature parts of the vine in this cultivar. Older leaves developed small, irregularly shaped, red-brown lesions scattered in interveinal areas and most concentrated near the margins (Fig. 6c). Occasionally, larger, spreading lesions developed. Older leaves were paler than young leaves in only one replicate, and premature senescence of oldest leaves was not observed.

Sulfur deficiency also induced small, light green leaves, most uniform in color but some darkening near veins. Leaves were slightly stiffened and shiny, contrasting with N-deficient plants. Young mature leaves developed scattered brown spots which appeared bruised (water-soaked) and stained, rather than fully necrotic. They were surrounded by a yellow halo (Fig. 6d). On more mature leaves, these became larger and more irregular in shape, and some areas developed spreading necrosis (Fig. 6e). Mid leaves were most affected, with oldest leaves showing fewer lesions. Some mid leaves

developed brown sections along the main veins on the underside of the leaf and a dark staining of minor veins near the margin (Fig. 6f). Similar browning of veins has been recorded in S-deficient plants of *D. rotundata* (*Gaztambide* and *Cibes*, 1975) and *D. alata* (*O'Sullivan* and *Jenner*, 2006).

Younger leaves of P-deficient plants were dark green but smaller than normal and distinctly stiffened. They tended to show puckering along the main veins and mild cupping, indicating uneven expansion (Fig. 6g). Some young mature leaves developed red-brown lesions at the margin (Fig. 6h). The incidence of P-deficiency symptoms on younger, rather than older, leaves was also noted in *D. alata* (*O'Sullivan* and *Jenner*, 2006) and concurs with leaf analyses indicating lack of remobilization of P (Fig. 4).

Potassium deficiency induced only mild symptoms, in the form of a mild interveinal chlorosis, most distinct on mid leaves (Fig. 6i). The minor veins retained a green margin, finely dividing the chlorotic area. This is similar to the response seen in *D. alata* (*O'Sullivan* and *Jenner*, 2006). *Gaztambide* and *Cibes* (1975) documented extensive development of necrosis and shedding of leaves in K-deficient *D. rotundata.* The timing of symptom development is unclear in their account, but they indicate that plants appeared normal for some time, and once initiated, symptom development was rapid, affecting all foliage. It seems likely that, since these plants had a harvest index around 50% of controls, symptom development was after tuber initiation and in response to sink demand from the developing tubers. The current study was terminated at an earlier stage.

Magnesium-deficient plants were generally pale, with greener veins contrasting on younger leaves but becoming indistinct on older leaves (Fig. 6j). Some mature leaves developed initially small interveinal lesions well away from the margin, which varied from very pale to dark red-brown (Fig. 6k). Older leaves often developed a diffuse staining on the upper surface, spreading in a fragmented pattern from the petiole attachment (Fig. 6l).

Leaves of Ca-deficient plants were generally of normal size and color, but shoot tips were either inactive or had very low activity at the time of harvest, resulting in quite mature leaves just below the tip. Axillary shoots often developed but produced only one or two leaves before becoming inactive (Fig. 6m). Root systems were stunted with many brown roots and short, thick branches.

Symptoms of Fe deficiency were mild and developed late in the experimental period. Young to mid leaves developed a distinct light green interveinal chlorosis (Fig. 7a). On two repli-
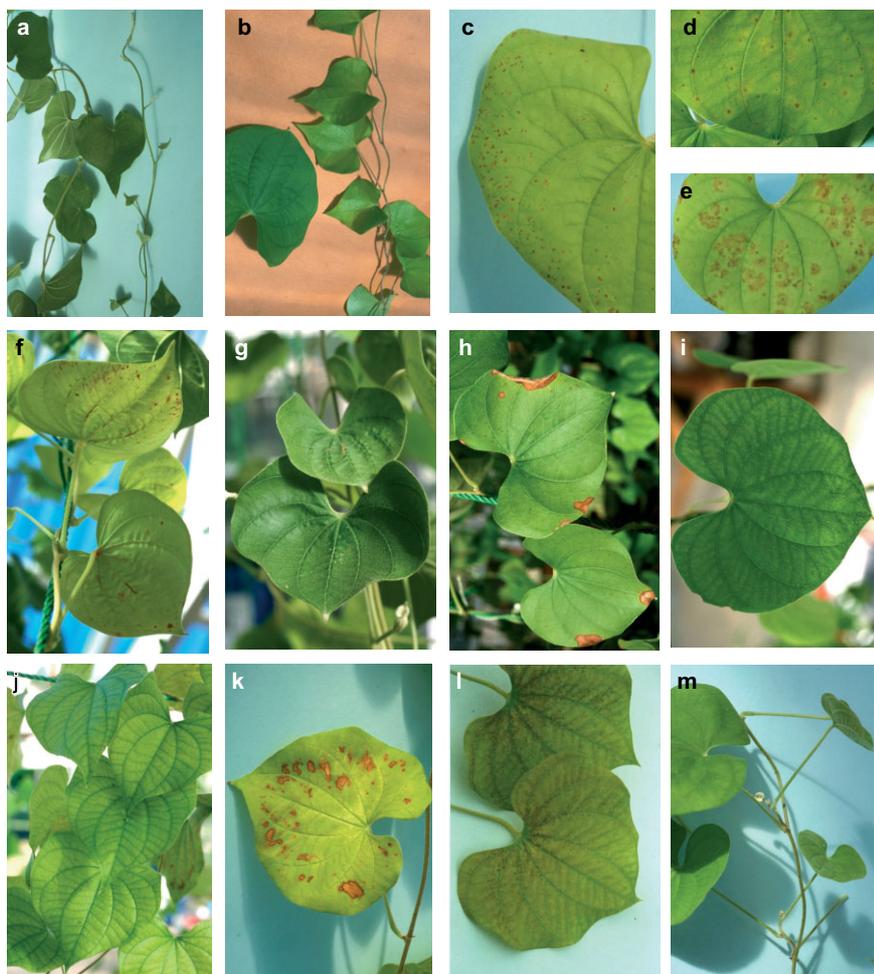
Figure 6: a) A vine tip and a mature section of vine from a healthy plant typical of the "All nutrient" treatment. b) Small, light green leaves of the "minus N" treatment compared with control leaf. c) Small necrotic spots on an older leaf of a N-deficient plant. d) Brown spots with a yellow halo on a young mature leaf deficient in S. e) Brown stained areas of irregular shape on a mature leaf of "minus S". f) Necrosis along main veins and browning of minor veins near the margin on the lower surface of "minus S" leaves. g) Young mature leaves on a P-deficient plant showing puckering along veins and upward cupping. h) Necrotic lesions at the margin of mature leaves on a P-deficient plant. i) Finely divided interveinal chlorosis on a middle leaf of a K-deficient plant. j) Young mature leaves showing distinct interveinal chlorosis in response to Mg deficiency. k) Mid leaves of a Mg-deficient plant showing interveinal lesions. l) Staining on the upper surface of an older leaf of "minus Mg". m) Axillary shoots on a Ca-deficient plant baring one or two mature leaves and an inactive tip.
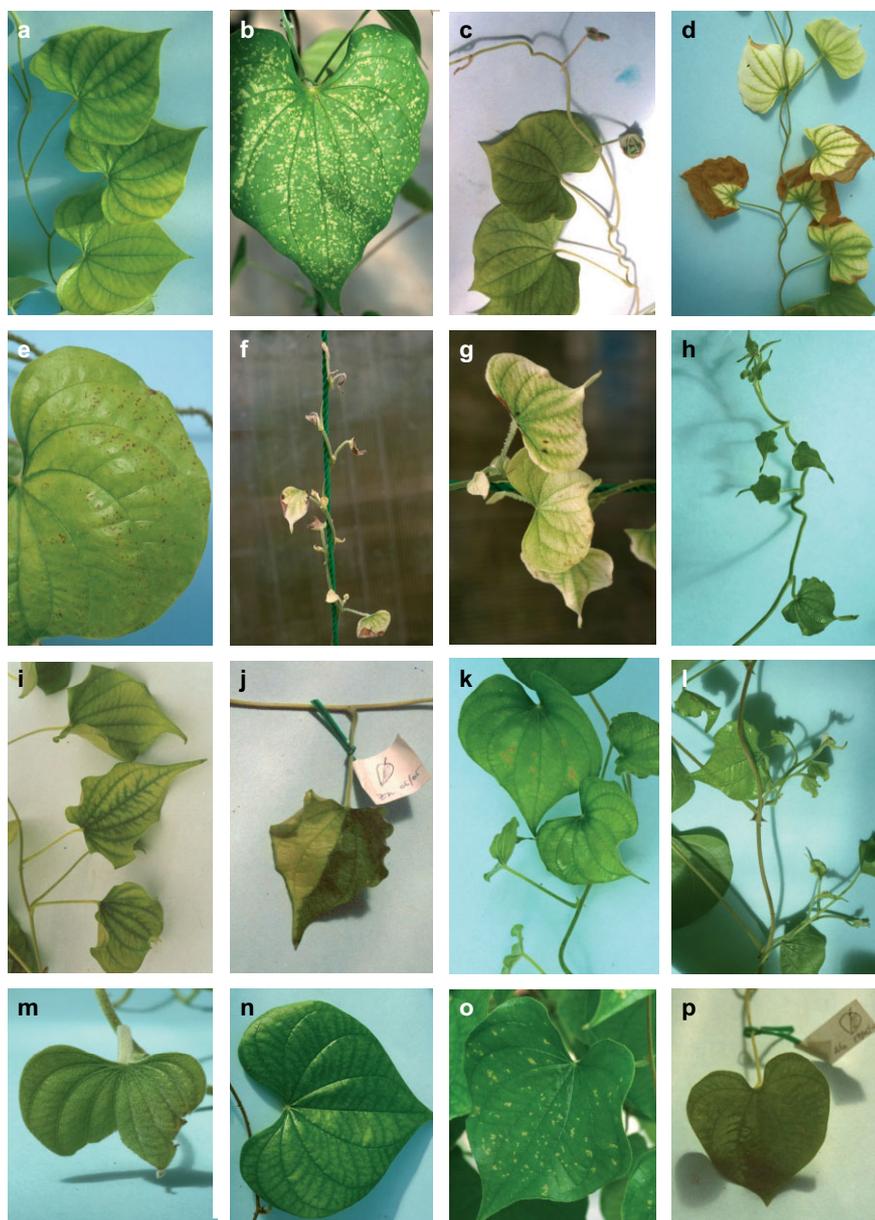
Figure 7: a) Interveinal chlorosis on young mature leaves in response to Fe deficiency. b) Small pale lesions on the upper surface of a lower leaf of "minus Fe". c) Necrosis and shriveling of young leaves of "minus Fe". d) Bleaching of young leaves and spreading necrosis due to Mn deficiency. e) Small dark lesions on an older leaf of an Mn-deficient plant. f) Bleaching and necrosis of the tip and young leaves of a severely stunted "minus B" plant. g) Short, broad leaves with twisted tips on a "minus B" plant. h) Vine tip of a Zn-deficient plant showing short internodes and small elongated leaves with wavy margins. i) Young mature leaves showing strong interveinal chlorosis at an advanced stage of Zn deficiency. j) Chlorosis reversed on half a Zn-deficient leaf one week after painting with 1% zinc sulfate solution. k) Interveinal chlorosis finely divided by green minor veins and interveinal necrotic lesions on young mature leaves of a Zn-deficient plant. l) Axillary shoots showing small, pale, and twisted leaves due to Zn deficiency. m) A young leaf incompletely formed, with missing tip and ragged margin, on a "minus Cu" plant. n) Mild but sharply delineated interveinal chlorosis on a mid leaf of "minus Mo". o) Pale, irregular shaped interveinal lesions on mid leaves of "minus Mo". p) Regreening on an older leaf of "minus Mo" one week after painting the surface with 0.5% sodium molybdate.

cates, mid leaves became stippled with numerous small whitish marks (Fig. 7b). At the later harvest of replicate 4, at 5 months after transplanting, chlorosis was more widespread, and young leaves developed necrotic margins and shriveled before full expansion (Fig. 7c).

The severity of Mn deficiency induced in this experiment was consistent with the response of *D. alata* (*O'Sullivan* and *Jenner*, 2006). Young leaves became pale, almost white, with main veins retaining a green margin (Fig. 7d). Necrosis spread on affected leaves as they reached full expansion. A diffuse-edged interveinal chlorosis extended to mid leaves. Some older leaves had small dark brown spots in interveinal strips (Fig. 7e). While such spots are characteristic of Mn deficiency in *D. alata* and other crops, the associated interveinal pitting or lesions on young leaves usually symptomatic of Mn deficiency were not evident on *D. esculenta.*

The symptoms of Mn deficiency in *D. esculenta* are commonly seen on high-pH soils developed on coral platforms in Papua New Guinea, but have previously been interpreted as Fe deficiency. *Johnston* (1996) first identified Mn deficiency as the likely cause through positive response to leaf painting and compared the symptoms on several root crops in the same location. *Dioscorea esculenta* was the most severely affected, with milder symptoms consistent with Mn deficiency observed on *D. alata*, taro (*Colocasia esculenta*), tannia (*Xanthosoma sagittifolium*), sweet potato (*Ipomoea batatas*), and cassava (*Manihot esculenta*). It would seem that *D. esculenta* is unusually sensitive to low Mn supply.

As mentioned above, the "minus B" treatment was severely stunted, but this may have been caused by a toxic response to the borate-specific resin. Leaf analysis confirmed low concentrations of B, and also of Mn. Leaves were very small and pale

with green veins, and necrotic lesions spread from the margins of younger leaves (Fig. 7f). From the evidence of leaf analyses, the chlorosis may have been due to Mn deficiency, induced as a result of root necrosis. Some mature leaves were short and broad in shape, with a narrow and twisted tip (Fig. 7g). This symptom is consistent with B deficiency in *D. alata.*

Symptoms of Zn deficiency became evident late in the experiment. Young leaves developed wavy margins and elongated, twisted tips (Fig. 7h). They were soft-textured and shiny and developed a mild interveinal mottle as they expanded. As severity increased, young leaves developed near-white interveinal chlorosis (Fig. 7i) or became uniformly pale. This chlorosis was reversible by painting the leaf surface with zinc sulfate solution (Fig. 7j). Mid leaves also showed a mild interveinal chlorosis, with green retained on minor veins finely dividing the chlorotic tissue. Occasionally, light brown lesions formed in the interveinal zones (Fig. 7k). Axillary shoots produced very small, pale and twisted leaves (Fig. 7l).

Plants in the "minus Cu" and "minus Mo" treatments grew well with little sign of stress. Two replicates of "minus Cu" developed a mild interveinal chlorotic mottle on mid leaves, and one plant produced several deformed or incomplete leaf blades on two vines before the shoot tips became inactive (Fig. 7m). "Minus Mo" plants had a mild, finely divided interveinal chlorosis on most mature leaves (Fig. 7n), and some mid leaves developed small, irregular-shaped, pale lesions on the upper surface (Fig. 7o). Leaf painting with sodium molybdate caused regreening (Fig. 7p).

3.4 Remobilization of phloem-mobile nutrients in yam

This study and an earlier study with *D. alata* (*O'Sullivan* and *Jenner*, 2006) indicate that these species either have a low remobilization capability for usually mobile macronutrients, particularly N and P, or a low sink strength of the vine tips. *Kabeerathumma* et al. (1991) recorded a marked decline in foliar K concentrations between the 5th and 7th months, in well-fertilized *D. esculenta*, *D. alata*, and *D. rotundata*, suggesting that some remobilization to the tubers does occur. Nitrogen and P declined to a lesser extent and generally later than K. *Sobulo* (1972a) and *Irizarry* and *Rivera* (1985) in *D. rotundata* and *Irizarry* et al. (1995) in *D. alata* reported similar trends, while *Obigbesan* and *Agboola* (1978) found no decline in P for *D. alata*, *D. rotundata*, and *D. cayenensis.*

Apparently due to the lack of remobilization within the vine, both *D. esculenta* and *D. alata* (*O'Sullivan* and *Jenner*, 2006) expressed symptoms of acute P deficiency in young, rather than old leaves. In *D. rotundata*, *Gaztambide* and *Cibes* (1975) recorded only a reduction in leaf size and general stunting in P-deficient plants, but did not note senescence of older leaves. It is noteworthy that symptoms have not been identified in association with P deficiency in the field (*Vander Zaag* et al., 1980) and few fertilizer trials with yams have recorded responses to P (*Vander Zaag*, 1980; *Obigbesan*, 1981), although *Halavatau* (2002) consistently obtained P responses on the highly P-fixing soils of Tonga. Given the

atypical presentation of P deficiency in yams, it is possible that symptoms present were not recognized. However, it is also apparent that yams have a low external P requirement. Reported superphosphate responses tended to be limited to low rates of P application (*Obigbesan*, 1981) and were most likely where yield, and hence P demand, was very high. *Vander Zaag* et al. (1980) estimated that the external P requirement for yams (*D. esculenta*, *D. alata*, and *D. rotundata*) was between 0.005 and 0.02 ppm in the soil solution (0.16–0.65 μM), with an apparent relationship between external requirement and yield potential such that only high-yielding crops responded to additional P above 0.005 ppm. They noted that, at low external P concentrations, the relative yield of three *D. esculenta* cultivars was correlated with the extent of mycorrhizal infection. However, this relationship was not reflected in the leaf P concentrations, where the highest-yielding cultivar had the lowest foliar P concentration, suggesting tolerance of low tissue P as a mechanism for resistance to P deficiency, rather than enhanced P access through increased mycorrhizal association.

3.5 Significance of leaf-tissue selection for diagnostic analysis

Earlier reports on composition of yam leaves have varied in the time and method of sampling. *Sobulo* (1972a), *Gaztambide* and *Cibes* (1975), *Irizarry* and *Rivera* (1985), and *Irizarry* et al. (1995) analyzed all leaf blades, while *Sobulo* (1972b), *Obigbesan* and *Agboola* (1978), and *Vander Zaag* et al. (1980) sampled young mature leaves. For the purpose of diagnostic sampling, obtaining a representative sample of all leaves on the plant is not practical. Our data suggest that leaf position is likely to be a source of considerable variability, and a well-defined index tissue will be essential to make valid comparisons with reference critical concentrations. Counting nodes from a shoot tip allows a highly reproducible sampling regime. However, yam leaves on actively growing vines are not fully expanded for some distance from the tip, and the vine becomes difficult to trace. For *D. alata* and *D. rotundata*, we have found leaf blades at the 5th and 6th nodes are sufficiently mature to serve as an index sample, but on *D. esculenta*, which bears only one leaf at each node, leaves at this position were generally expanding rapidly and in transition to mature form. The 7th and 8th nodes provide leaves of mature habit if not full expansion, while older nodes become difficult to sample in the field due to entanglement of the vines. The immature leaves of *D. esculenta* are also densely hairy, which can hinder representative sampling of the dried tissue for analysis. We recommend the leaves are crushed rather than ground before analysis, as grinding allows the hairs to separate and clump.

Even using a precisely defined sampling regime, the absolute age and physiological maturity of leaves at the specified position may be influenced by other factors influencing growth rate, such as temperature, light, water availability, and the aspect of the leaves on the canopy. The robustness of the information provided on adequate and deficient nutrient concentrations should be verified through comparison with field data from a range of production environments.

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3.6 Evaluation of the culture system

The sand-culture system used in this experiment was successful in inducing deficiencies of a wide range of nutrient elements and was highly efficient with respect to time and resources, compared with the flush-through system used by *Gatzambide* and *Cibes* (1975). The constant water table ensured constant soil moisture and eliminated water stress, with minimum use of distilled water. The demand-driven nutrient-addition procedure ensured concentrations of the treatment nutrients were relatively stable in the root environment throughout the experiment and differed little among treatments regardless of growth rate. However, due to the inclusion of Na^+ and Cl^- as counter-ions at levels exceeding plant uptake, the accumulation of salinity would potentially cause harm to plants in longer-term experiments and was possibly the cause of a mild interveinal chlorosis observed on control plants of the 4th replicate, which were maintained to 5 months. There is scope to adjust nutrient schedules to reduce Na and Cl inputs, but this would require more complex substitutions to maintain counter-ions when particular salts are omitted. The method chosen was intended to minimize experimenter error, given that the Papua New Guinea personnel were unfamiliar with the chemistry. It served our purpose well, but offers opportunity for refinement.

4 Conclusion

This paper reports the first published study of nutritional deficiencies in *D. esculenta.* In a culture system using accessible materials and requiring relatively little purified water, chemical salts, and labor, symptoms indicative of deficiency were successfully induced for each of the 12 nutrients studied. Effects on nutrient concentrations in leaves are reported, providing concentrations indicative of adequate and deficient status, which may be useful in field studies of this species.

Effects of nutrient deficiencies on nontreatment nutrient concentrations are rarely reported due to the size of the data sets and difficulty in representation. We have devised a concise format in which this very useful data can be readily interpreted (see Fig. 5).

The study supported earlier observations of the high sensitivity to Mn deficiency exhibited in yams (*O'Sullivan* and *Jenner*, 2006), and in *D. esculenta* in particular (*Johnston*, 1996). Further evidence was also obtained of the unusually low translocation of phloem-mobile nutrients within yam vines, supporting the observations of *O'Sullivan* and *Jenner* (2006). The absence of typical older-leaf symptoms for N, K, and particularly P deficiency suggests that their field occurrence may be underrecognized in this crop.

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