



PHOSPHORUS LEVELS IN SHOOTS OF BAMBARA GROUNDNUT IN BOTSWANA SOILS

G. M. Ramolemana , W. G. Keltjens , M Wessel & G. S. Maphanyane

To cite this article: G. M. Ramolemana , W. G. Keltjens , M Wessel & G. S. Maphanyane (2002) PHOSPHORUS LEVELS IN SHOOTS OF BAMBARA GROUNDNUT IN BOTSWANA SOILS, Journal of Plant Nutrition, 25:9, 2035-2049, DOI: [10.1081/PLN-120013293](https://doi.org/10.1081/PLN-120013293)

To link to this article: <http://dx.doi.org/10.1081/PLN-120013293>



Published online: 16 Aug 2006.



Submit your article to this journal [↗](#)



Article views: 35



View related articles [↗](#)



JOURNAL OF PLANT NUTRITION, 25(9), 2035–2049 (2002)

PHOSPHORUS LEVELS IN SHOOTS OF BAMBARA GROUNDNUT IN BOTSWANA SOILS

G. M. Ramolemana,^{1,*} W. G. Keltjens,² M. Wessel,²
and G. S. Maphanyane³

¹Botswana College of Agriculture, Private Bag 0027,
Gaborone, Botswana

²Sub-Department of Soil Science and Plant Nutrition,
Wageningen University and Research Centre, Dreijenplein
10, 6700 EC Wageningen, The Netherlands

³Department of Agricultural Research, Ministry of
Agriculture, Private Bag 0033, Gaborone, Botswana

ABSTRACT

The critical phosphorus (P) concentration and range of bambara groundnut (*Vigna subterranea*) plants were determined from a sand culture experiment at Botswana College of Agriculture. Twelve P levels (0.207–159 mg P pot⁻¹) were used for growing the plants for 78 days (early podding stage) and the shoot P concentrations determined. The critical P concentration was used for assessing the P nutritional status of bambara groundnut plants grown in farmers fields in Botswana. Plants from 10 farmers' fields were sampled at 78 days after sowing and analyzed for P. The critical shoot P concentration and range for

*Corresponding author. E-mail: gramolem@temo.bca.bw



bambara groundnut determined visually from graphs were 0.15% and 0.15–0.20%, respectively. In 80% of the farms, plants showed shoot P concentrations between 0.10% and 0.13% which correspond with moderate to severe P deficiency. Therefore, most of bambara groundnut farmers in Botswana grow the crop under limiting P conditions.

INTRODUCTION

Critical nutrient concentrations (CNC) in plants have often been used for assessing nutritional status of plants and formulation of fertilizer recommendations.^[1–3] A critical level has been defined as the nutrient concentration of the tissue associated with a 5 or 10% reduction in maximum growth due to deficiency of that nutrient.^[4] Diagnosis based on this system requires that the composition of the plant tissue be compared with the critical value determined for that species at the same stage of growth in a specific plant part.^[5] Dow and Roberts^[6] observed that it was difficult to experimentally determine a specific CNC as there is considerable variation in the points plotted in the transition zone between deficient and adequate concentrations. The critical value or range can be affected by many factors such as soil moisture supply and the concentration of other nutrients because of their internal interaction.^[5,7] Critical nutrient ranges of most essential elements have been reported for many plant species.^[3,8] Bates^[5] reported that for several species sand cultures gave the same critical concentrations as field experiments, while values obtained from nutrient solution experiments were different.

The response of bambara groundnut to P fertilization in our experiments conducted at Sebele, Botswana, in the same soil was variable, with a positive response in pot experiments and no response in the field.^[9] However, the leaf blade or shoot P concentration of bambara groundnut plants in these experiments was not affected by the treatments, even when shoot dry matter was increased. This might mean that under the experimental conditions bambara groundnut plants maintained the shoot P concentration around the critical level by converting P taken up into extra biomass.

In Botswana, traditional farmers who grow bambara groundnut do not apply fertilizer or manure to the crop.^[10] However, soil moisture status seems to be the first limiting factor for crop production in Botswana.^[11] Limited soil moisture availability can inhibit plant growth both directly due to water stress, and indirectly as a result of limited nutrient availability, the latter specifically with phosphorus. Most of the soils under cultivation in Botswana are also reported to have low soil P availability which limit crop production.^[12] Specially for bambara groundnut, information is lacking on the soil and plant P nutritional status in the

**P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT****2037**

traditional farms of Botswana, and whether P fertilization is necessary under those conditions. Therefore, a sand culture experiment was conducted to determine the critical P range for bambara groundnut for use in assessing the P nutritional status of plants in our experiments^[9] and from the farmers fields in Botswana. A critical level was defined according to Epstein.^[4]

MATERIALS AND METHODS**Sand Culture**

The experiment was conducted in a greenhouse at Botswana College of Agriculture—Sebele (24°33'S; 25°54'E; 994 m) for a period of 78 days (early podding) from August 20 to November 5 1997. The average monthly temperatures in the greenhouse ranged from 27.5 to 32.8°C during the day and 15.0 to 20.5°C at night.

Coarse riverbed sand, washed with deionized water, was used for growing the plants. The main chemical characteristics of the sand were: pH (CaCl₂) 7.4;^[16] P Bray 3.8 mg P kg⁻¹; organic carbon 0.02 (wt.%); CEC 1.00 [cmol (+) kg⁻¹]; exchangeable cations [cmol (+) kg⁻¹]: Ca 1.35, magnesium 0.30, K 0.04 and sodium 0.04.^[17] The 12 P treatments were 0.207, 0.414, 0.828, 1.656, 3.312, 6.625, 13.25, 26.5, 53.0, 79.5, 106 and 159 mg P pot⁻¹ for the whole growing period. Phosphorus (NaH₂PO₄ dissolved in water) was added to the pots at 12 different rates according to the relative addition rate technique (RAR)^[13]. With this technique P was added during the entire growth period in accordance with the plants actual growth rate, the latter varying from maximum to severely limited. Patterns of maximum growth rate and corresponding P uptake vs. time were obtained by constructing a standard curve based on growth and shoot P concentrations of a previous greenhouse pot experiment.^[9] Phosphorus was added weekly to the pots at different rates, increasing with time according to the growth rate of the plants, varying from low to high on the bases of intended growth and corresponding P requirement.

The experiment was arranged in a completely randomized design with treatments replicated four times. Three seeds of bambara groundnut landrace “Diphiri Cream” were sown in pots (five liter) containing eight kg of sand and thinned to one seedling 13 days after sowing (DAS). A minus P nutrient solution was prepared with deionized water and applied weekly starting from sowing. The nutrient solution used was of the following composition (mmol L⁻¹): 3.75 NH₄NO₃; 1.25 K₂SO₄; 1.00 MgSO₄; and trace elements (umol L⁻¹): 82 iron (Fe-EDTA); 46 boron (H₃BO₃); 9 manganese (MnSO₄·H₂O); 0.3 copper (CuSO₄·5H₂O); 0.8 zinc (ZnSO₄·7H₂O) and 0.01 molybdenum (MoO₃). The nutrient solution was applied according to the RAR technique^[13] based on



the N requirement of bambara groundnut determined from a standard growth curve and shoot nitrogen (N) concentrations from a previous experiment (as for P above). Contrary to P, all the pots received the same amount of nutrient solution, the amount increasing with time according to the growth rate of the plants. The smaller quantities of nutrient solution at early stages of growth were added once a week while the larger quantities at later growth stages were distributed throughout the week. The pH of the nutrient solution was maintained between 5.7 and 6.0. Water stress was prevented by applying deionized water daily to each pot in addition to the nutrient solution.

At final harvest (78 DAS), leaf number was recorded and plants separated into shoots, roots, nodules and pods. The leaf blades were separated from the petioles to determine the total leaf area per plant. The leaf area per plant was determined by stacking 20 fully matured leaves and cutting through them with a one centimeter diameter cork borer. The weight of the 20 leaf blade discs and total weight of the leaf blades per plant were taken. A factor relating weight of the leaf blade discs to their area was determined and used to calculate the total leaf area per plant [leaf area per plant = total leaf weight per plant \times (area of the 20 leaf blade discs (15.71 cm²) divided by weight of the discs)]. The plant parts were oven-dried at 70°C for 24 hours and weighed. The dried leaf blades were ground in a plant grinder with a two mm sieve and subsequently analyzed for P as described by Ramolemana.^[9] The data were subjected to ANOVA using SAS^[14] statistical program and differences between means were tested for significance with the least significant difference (LSD_{0.05}). The critical P concentration was determined from the relation between shoot dry matter production and shoot P concentration and defined as the concentration giving 90% of its maximum shoot dry matter.

Shoot Phosphorus from Farms

For this study, ten farmers growing cream seeded bambara groundnut landraces were randomly selected from the Kgatleng District in Eastern Botswana, within a radius of 60 km from Botswana College of Agriculture—Sebele (24°33'S; 25°54'E, 994 m). The farmers were selected by extension workers on the basis of their interest and many years of experience of growing the crop. The farmers were requested to grow the crop as usual and keep records of planting date and yield of pods at harvest. Where the farmer did not keep a record of the planting date, it was estimated with information given by the farmer. The farmers were visited at least once a month during the cropping period to monitor the farming operations and to collect information on the cropping activities. The nearest weather station to the farms was at Sebele and the rainfall data from this station was used to generally describe the climatic conditions in the survey area.



P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT

2039

Table 1. Some Chemical Characteristics of Soils from Ten Different Bambara Groundnut Farms

Farm No. and Name	pH	P-Bray (mg kg ⁻¹)	Org. Carbon (wt. %)	CEC (cmol (+) kg ⁻¹)	Exchangeable Cations (cmol (+) kg ⁻¹)			
					Ca	Mg	K	Na
1. Masule	5.06	3.33	0.17	3.96	1.49	0.74	0.33	0.04
2. Lekorwe	4.22	9.08	0.22	280	0.52	0.32	0.33	0.04
3. Mosekiemang	4.62	2.77	0.19	2.08	0.87	0.32	0.16	0.04
4. Motswasele	4.43	2.97	0.13	2.08	0.42	0.28	0.20	0.04
5. Thamage	4.50	2.88	0.16	2.04	0.92	0.24	0.20	0.04
6. Gare	4.67	3.33	0.25	2.12	0.70	0.26	0.16	0.04
7. Masupu	4.91	4.10	0.26	2.44	1.01	0.42	0.29	0.04
8. Ntlhwasane	3.95	3.78	0.26	1.60	0.11	0.15	0.12	0.04
9. Tlhase	4.11	7.18	0.19	1.96	0.60	0.28	0.20	0.04
10. Maotsela	5.50	4.27	0.13	2.68	1.20	0.51	0.29	0.04
Average	4.6	4.4	0.20	2.4	0.78	0.35	0.23	0.04



Planting in the farms was done between 8 November and 20 December 1996 and the area under bambara groundnut per farm varied between 0.08 and 2.17 ha. The main criterion determining the date of planting was the availability of rains. The area under bambara groundnut was determined with a measuring tape. Three farmers (30%) had broadcast the seed and ploughed to cover it with soil, while the rest planted in rows with either a tractor or an animal drawn planter after ploughing. None of the farmers applied fertilizer during the survey period, but one farmer (No. 2 in Table 1) applied P fertilizer in the previous season while another (No. 9 in Table 1) about eight years previously. In all the farms, the crop was a pure stand planted on the flat.

Plant and soil samples from each farm were taken around 78 days after sowing (DAS), which was 50% podding stage.^[15] The sampling period was estimated from the planting date as provided by the farmers. A sample of six plants 10 m apart was taken from the middle of the area under bambara groundnut starting five meters from the edge. The soil sample collected per farm was a composite of 24 subsamples taken with an auger (20 mm diameter and 30 cm long) to a depth of 20 cm. At each of the spots where the six plants were sampled, four soil subsamples were taken. Three soil subsamples were taken at a radius of 50 cm around each plant selected for sampling, with the fourth on the spot where the plant was grown.

The plant shoots were oven-dried at 70°C for 24 hours and weighed. The plants were then ground to make one composite sample and subsequently analyzed for N, P, and potassium as described by Ramolemana.^[9] The soil samples were air dried for two weeks and analyzed for mineral nutrients (Table 1).^[16,17]

Pod production estimates from the area under bambara groundnut in each farm, as obtained from the farmers, were converted to yields per hectare. Data on plant densities of bambara groundnut in the farms involved in the study were not collected. Correlations of parameters measured from both plant and soil samples were determined using the SAS^[14] statistical program.

RESULTS

Sand Culture

Plant growth and dry matter production: From Fig. 1a–c it can be seen that P rates of 26.5 to 159 mg P pot⁻¹ significantly increased number of leaves, leaf area and shoot DM per plant, relative to the lowest P rate. Beyond a supply of 106 mg P pot⁻¹ further increases were no longer significant. Between P rates 0.207 and 26.5 mg P pot⁻¹, the plants showed some P deficiency symptoms, which were stunted growth, chlorotic blotches and brownish spots on older



P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT

2041

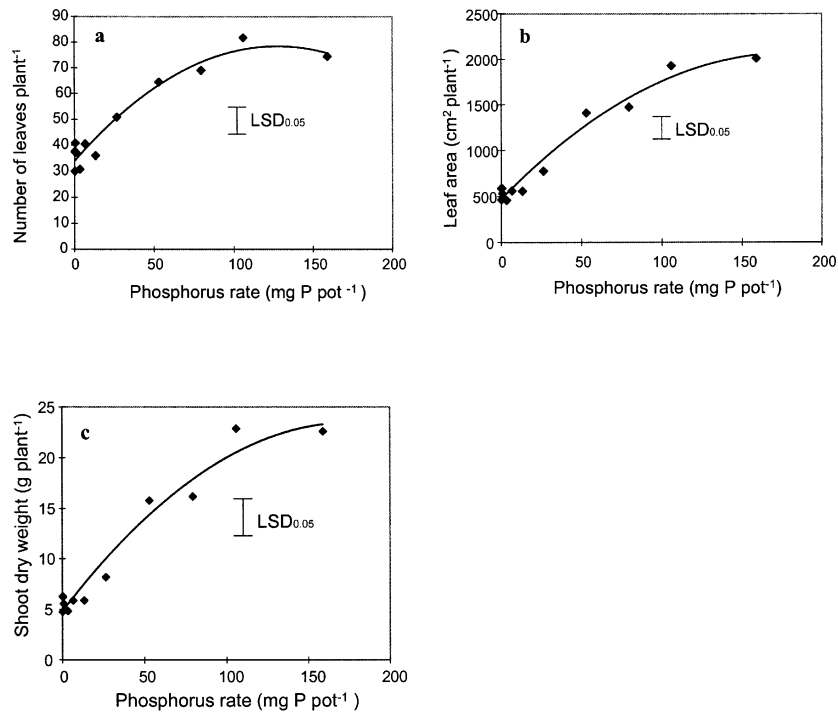


Figure 1. Effect of level of phosphorus application on the number of leaves (a), total leaf area (b), and shoot dry weight (c) of “Diphiri Cream” plants at 78 days after sowing.

leaves, and as growth progressed, the chlorotic areas on leaf edges became necrotic. The P deficiency symptoms (purplish to brownish spots) were only on older leaves, while later maturing leaves had no symptoms.

Traces of P deficiency were also noticed on plants given 53.0 mg P pot⁻¹, but none at P rates of 79.5 to 159 mg P pot⁻¹. The P effect observed on shoot growth was similar for root and nodule growth and pod development; the number of days to flowering and podding was not affected by P rate.

Shoot phosphorus concentration: Shoot P concentration significantly increased with increased level of P fertilization and varied between 0.07 and 0.20% (Fig. 2). The increases in P concentration were significant at P additions higher than 53 mg P pot⁻¹. In agreement with the observed visual P deficiency symptoms at P rates between 0.207 and 26.5 mg P pot⁻¹, shoot P concentrations within this range were low and varied between 0.07 and 0.09%.

Nutrient calibration curves relating shoot P concentrations to shoot DM, number of leaves and leaf area per plant are shown in Fig. 3a–c. The critical shoot

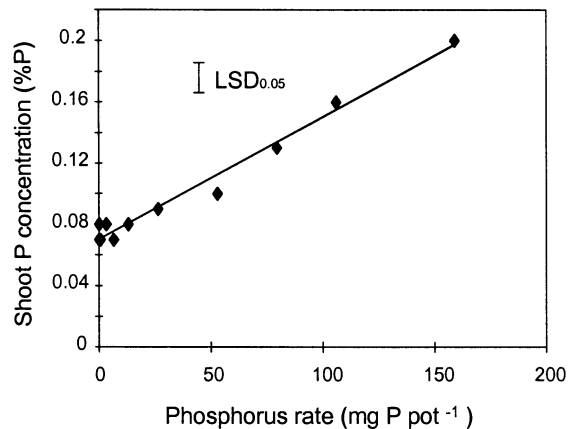


Figure 2. Effect of the level of phosphorus application on shoot phosphorus concentration of “Diphiri Cream” plants at 78 days after sowing.

P concentrations associated with a 10% reduction in maximum shoot DM, number of leaves plant⁻¹ and leaf area plant⁻¹, determined visually from the graphs in Fig. 3a–c, were essentially the same for the three parameters being 0.15%, 0.15% and 0.16%, respectively. The critical shoot P range for the three parameters ranged between 0.15 and 0.20%.

Shoot Phosphorus Status from Farms

Soil phosphorus status: The soil P levels (P-Bray) of the ten farms ranged from 2.8 to 9.1 mg P kg⁻¹ with the highest at farm No. 2, the farm where P fertilizer was applied the previous season (Table 1). On average, P-Bray of the fields was 4.4 mg P kg⁻¹ soil. The difference between other soil characteristics were small (Table 1). The pH levels of the soils were on average 4.6 and rather low.

Shoot nutrient status: The shoot P concentrations ranged from 0.10 to 0.16% with the highest level again on farm No. 2, the farm where P fertilizer was applied the previous season (Table 2). Total P in the plants (represented by the shoot P content in mg P plant⁻¹) varied with a factor of four. The variation in shoot P content was however due to variation in shoot DM (Table 2). Variations in shoot N and K concentrations were observed, but the lowest levels measured being respectively 2.14 and 1.56% are adequate, as compared to means of 2.70 and 1.13% at the same growth stage from a previous field experiment.^[9]



P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT

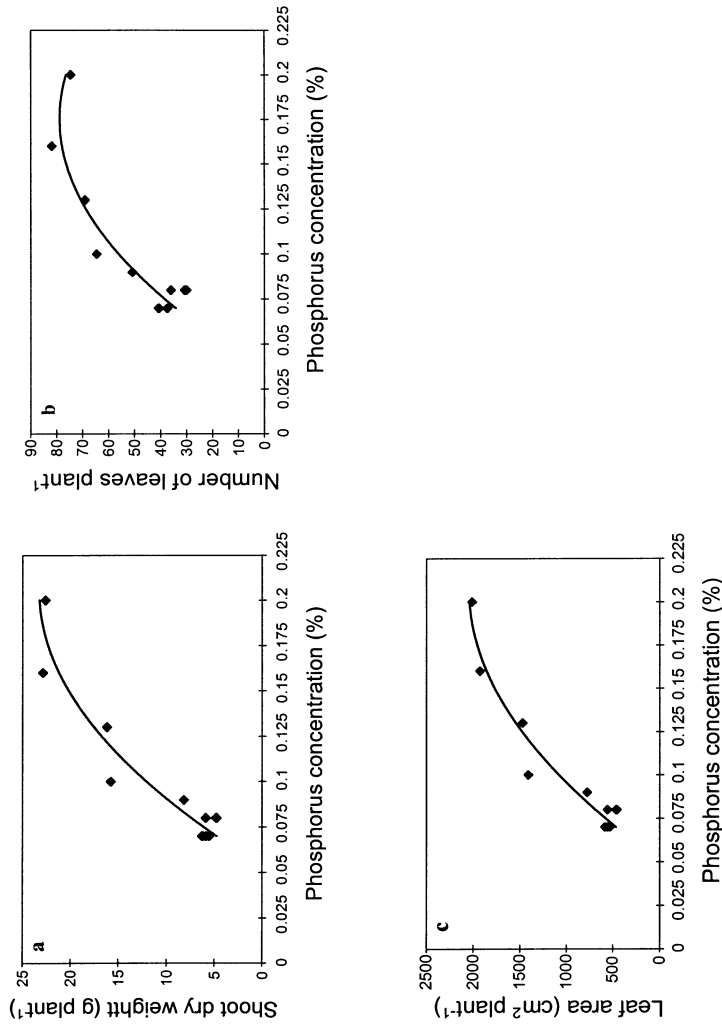


Figure 3. Relationship between shoot phosphorus concentration and shoot dry weight (a), number of leaves (b), and leaf area (c) of “Diphiri Cream” plants at 78 days after sowing.

Table 2. Mineral Nutrient Status of Plant Samples Collected at 78 Days After Sowing and Pod Yield Estimates at Final Harvest from Ten Different Bambara Groundnut Farms

Farm No. and Name	Shoot DM (g Plant ⁻¹)	Shoot P (%)	Shoot N (%)	Shoot K (%)	Total P (mg Plant ⁻¹) ²	Pod Yield (kg ha ⁻¹)
1. Masule	76.3	0.12	2.35	1.88	92	875
2. Lekorwe	23.8	0.16	2.60	2.26	38	778
3. Moseklemang	54.8	0.15	2.33	2.25	82	806
4. Motswasele	54.6	0.11	2.14	2.19	60	759
5. Thamage ¹	19.1	0.12	2.30	2.11	23	n.a.
6. Gare	28.9	0.11	2.35	1.64	32	635
7. Masupu	47.5	0.13	2.60	1.96	62	875
8. Ntlhwasane	29.4	0.11	2.62	1.78	32	673
9. Tlhase	36.7	0.10	2.38	1.56	37	740
10. Maotsetla	25.2	0.13	2.09	1.85	33	700
Average	39.6	0.12	2.38	1.95	49	760

¹The crop was later destroyed by porcupines and ground squirrels.

²Shoot P content.





P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT

2045

Estimated pod yields varied between 635 and 875 kg ha⁻¹ (Table 2). This variation was much less than the observed variation in shoot DM plant⁻¹.

No correlations were found between soil P levels and shoot DM and shoot P concentration. Correlations between shoot P content and shoot DM, shoot DM and pod yield, soil organic matter content and shoot N concentration were significant.

DISCUSSION

Sand Culture

The critical shoot P concentration and range for bambara groundnut determined visually from the graphs were 0.15% and 0.15–0.20%, respectively. Stunted growth seemed to be the major symptom of P deficiency of bambara groundnut and was observed in plants with shoot P concentrations less than 0.10%. This demonstrates the important role of P in growth and development of bambara groundnut.

The critical level of a nutrient means that at that level that element is still slightly limiting growth.^[18] A shoot P concentration of about 0.15% seemed to be critical for bambara groundnut and therefore an indication that plants that produce biomass with that P level in their shoots are at least marginally supplied with P. In our field experiment, at 78 DAS, shoot P concentrations were around the critical P level, being 0.15 and 0.14% for rainfed and irrigated treatments, respectively.^[9] The plants in our pot experiments were also suffering from P deficiency with shoot P concentrations of 0.11–0.13%.^[9]

The critical shoot P level of bambara groundnut determined in the sand culture experiment has demonstrated that the P status of bambara groundnut plants in all our previous experiments (pot and field) was marginal. This seemed to be due not only to a low soil P status, but to a combination of low soil P and low soil moisture content, probably strengthened by a relatively poor root development of bambara groundnut.^[9]

Shoot P Status from Farms: The average soil P content of 4.4 mg P kg⁻¹ found in the ten bambara groundnut farms in Botswana is generally considered to be low, compared to a minimum of P-Bray of 10 mg P kg⁻¹ required for sorghum.^[12] Shoot P concentrations were also low (Table 2). Only in two fields did shoot P concentrations reach the level of 0.15 and 0.16% which corresponds with the critical shoot P level for bambara groundnut. In all other fields, plants showed shoot P concentrations between 0.10 and 0.13% which correspond with moderate to severe P deficiency. This means that in all those fields P supply was suboptimal.

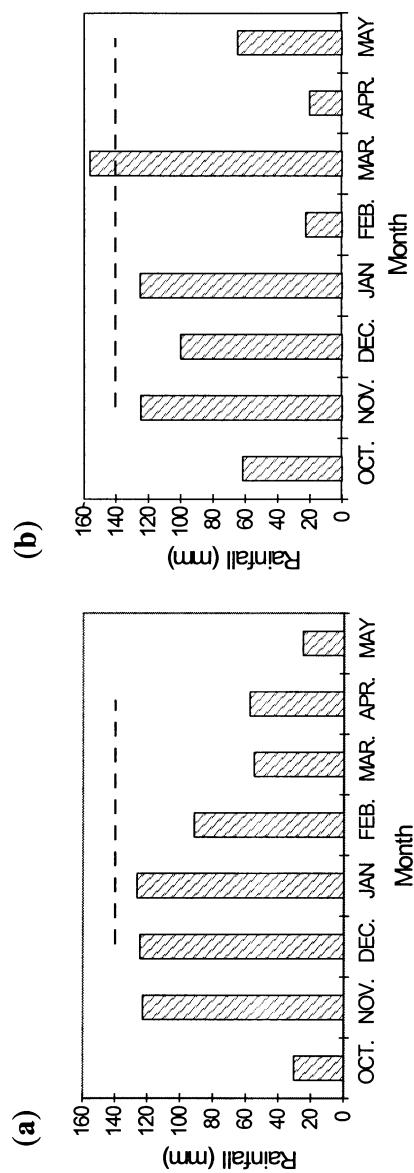


Figure 4. Rainfall distribution during the 1995/96 (a) and 1996/97 (b) planting seasons as recorded at Sebele Botswana meteorology station (---- Bambara groundnut growth period).



P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT

2047

The shoot P content of rainfed plants from the field experiment^[9] was 109 mg P plant⁻¹, being about twice as high as the average of plants grown on the traditional farms investigated in this study (Table 2). Plant growth and shoot P content of only one farm (No. 1) was similar to that of our rainfed treatment with a shoot dry weight of 76.3 g plant⁻¹. Probably during our field experiment (1995/96) the rainfall distribution was better than in the 1996/97 growing season, when the survey was carried out (Fig. 4). Differences can not be explained on the basis of differences in total rainfall between the years 1995/96 and 1996/97, being respectively 633 and 676 mm. Since shoot P content was directly related to shoot dry matter weight (growth), the latter probably acting as a sink for P uptake, differences in shoot P content between our field experiment and most of the farm-grown plants may also be due to differences in cultivation technique. Plant density, weed control and crop protection in our experimental plots were probably better than in farmers' fields.

Finally, whether the low seed yields as presented in Table 2 are a direct result of a too low P content of the soil is questionable. Probably without adequate soil moisture P availability was very low leading to low P uptake and P deficiencies. A direct water stress can also be involved. Besides P deficiency, also a low plant density contributes to the low yields in farmers fields.^[10] Therefore, yield increases can only be achieved when soil moisture is adequate in combination with increased plant density, and subsequently followed by P fertilization.

CONCLUSIONS

The internal shoot P concentrations levels in bambara groundnut plants, measured in our previous experiments and in traditional farms of Botswana, were not adequate according to the critical shoot P level determined from our sand culture. The soil P status of bambara groundnut fields of traditional farmers can be described as low. Therefore, P nutrition of bambara groundnut should be recognized as a yield limiting factor in Botswana soils.

ACKNOWLEDGMENTS

We thank Botswana College of Agriculture and Wageningen University and Research Centre for the financial and technical support. Prof. Karikari is also thanked for the review of the manuscript.



REFERENCES

1. Melsted, S.W.; Motto, H.L.; Peck, T.R. Critical Plant Nutrient Composition Values Useful in Interpreting Plant Analysis Data. *Agron. J.* **1969**, *61*, 17–20.
2. Lockman, R.B. Mineral Composition of Grain Sorghum Plant Samples. III. Suggested Nutrient Sufficiency Limits at Various Stages of Growth. *Commun. Soil Sci. Plant Anal.* **1972**, *3*, 295–306.
3. Sumner, M.E. Interpretation of Foliar Analyses for Diagnostic Purposes. *Agron. J.* **1979**, *71*, 343–348.
4. Epstein, E. *Mineral Nutrition of Plants: Principles and Perspectives*; John Wiley and Sons: New York, 1972; 412 pp.
5. Bates, T.E. Factors Affecting Critical Nutrient Concentrations in Plants and their Evaluation: A Review. *Soil Sci.* **1971**, *112* (2), 116–130.
6. Dow, A.I.; Roberts, S. Proposal: Critical Nutrient Ranges for Crop Diagnosis. *Agron. J.* **1982**, *74*, 401–403.
7. Dumenil, L. Nitrogen and Phosphorus Composition of Corn Leaves and Corn Yields in Relation to Critical Levels and Nutrient Balance. *Soil Sci. Soc. Am. Proc.* **1961**, *25*, 295–298.
8. Reuter, D.J.; Robinson, J.B. *Plant Analysis: An Interpretation Manual*; Inkata Press: Melbourne, Sydney, Australia, 1986.
9. Ramolemana, G.M. *The Phosphorus and Nitrogen Nutrition of Bambara Groundnut (Vigna subterranea (L.) Verdec.) in Botswana Soils. An Exploratory Study*; Wageningen University and Research Centre Liaison Office: Wageningen, The Netherlands, 1999; Trop. Resour. Mgt. Papers No. 26, 89 pp.
10. Brink, M.; Collinson, S.T.; Wigglesworth, D.J. Characteristics of Bambara Groundnut (*Vigna subterranea*) Cultivation in Botswana. In *Proceedings of the International Bambara Groundnut Symposium*, University of Nottingham, UK, July 23–25, 1996; University of Nottingham: UK, 1997.
11. Sims, D. *Agro-Climatological Information, Crop Requirements and Agricultural Zones for Botswana*; Land Utilization Division, Ministry of Agriculture: Gaborone, Botswana, 1981.
12. Beynon, S. The Response of Sorghum to Phosphate Fertilizer in Botswana. In *The Bulletin of Agricultural Research in Botswana No. 9*; Ministry of Agriculture: Gaborone, Botswana, 1991; 67–78.
13. Ingestad, T.; Lund, A.B. Review Article: Theory and Techniques for Steady State Mineral Nutrition and Growth of Plants. *Scandinavian J. For. Res.* **1986**, *1*, 439–453.
14. SAS. *Statistical Analysis Systems User's Guide*; SAS Institute: Cary, NC, 1985.



P LEVELS IN SHOOTS OF BAMBARA GROUNDNUT

2049

15. Ramolemana, G.M.; Maphanyane, G.S.; Wessel, M. Response of Bambara Groundnut to Phosphorus With and Without Irrigation. In Proceedings of the International Bambara Groundnut Symposium, University of Nottingham, UK, July 23–25, 1996; University of Nottingham: UK, 1997.
16. Clark, W.M. *The Determination of Hydrogen Ions*, 3rd Ed.; Williams and Wilkins Co.: Baltimore, MD, 1923.
17. Thomas, G.W. Exchangeable Cations. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd Ed.; Black, C.A., Evans, D.D., White, J.L., Eds.; American Society of Agronomy: Madison, WI, 1982.
18. Smith, P.F. Mineral Analysis of Plant Tissues. *Annu. Rev. Plant Physiol.* **1962**, *13*, 81–108.