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Full Length Article

Application of Rock Phosphate Levels Influenced the Root Growth, N and P uptake in Bambara Groundnut (*Vigna subterranea*) Landraces

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Abstract

Bambara groundnut production (*Vigna subterranea* L. Verdc.) is constrained by inherent low soil phosphorus (P) content and high cost of water-soluble P fertilizers. A screenhouse study on the root growth, N and P uptake of Bambara groundnut was conducted to evaluate the effect of rock phosphate application at University of Nottingham Malaysia Campus. Treatments consisted of factorial combination of two Bambara groundnut landraces (Kangiwa brown and Kaaro) and four rock phosphate (0, 20, 40 and 60 kg P_2O_5 ha^{-1}) levels. The results revealed that Kaaro landrace was higher than Kangiwa brown in root length, volume, surface area, length $volume^{-1}$, root dry weight, plant dry weight and N and P content. Application of rock phosphate at 40 kg P_2O_5 ha^{-1} was optimum in root volume, surface area and root dry weight. However, plant dry weight as well as N, P and ash contents were optimum at 20 kg P_2O_5 ha^{-1}. Thus, rock phosphate could serve as an alternative source of P for Bambara groundnut production in the study area. Application of 20 kg P_2O_5 ha^{-1} (0.28 g kg^{-1} of soil) was optimum for the N and P uptake of the crop. Overall Kaaro landrace performed better than Kangiwa brown in the study area. © 2019 Friends Science Publishers

Keywords: Alternative P source; Nutrients uptake; Root characteristics; Underutilized legume

Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc.), is an underutilized pulse legume of tropical African origin. The crop is cultivated for its rich grain which serves as a source of food in its production regions and as such a potential crop for improving food security (Mayes *et al.*, 2011). The crop is cultivated for its seeds, which contain 16–25% protein, 42–60% carbohydrate, 5–6% lipid, 4.8% fiber and 3.4% ash (Mwale *et al.*, 2007). The crop is reported to produce adequate yield under varying climatic conditions ranging from semi-arid to sub-humid tropical areas (Musa *et al.*, 2016).

Despite the importance of the crop, its production is constrained by inherent low soil phosphorus status of most of the production regions and high cost of water-soluble inorganic P fertilizers that are beyond the reach of most tropical African farmers (Agyin-Birikorang *et al.*, 2007; Singh and Satyanarayana, 2011; Mew, 2016). Additionally, water soluble P fertilizers are sometimes ineffective due to high P fixation of these soils (Campos *et al.*, 2016) and little information is available on the response of legumes such as Bambara groundnut to alternative sources of P such as rock phosphate (RP). Tropical acidic soils could enhance the dissolution of P from Ca-bound RP fertilizers due to lower soil pH and inherent low soil P status (Agyin-Birikorang *et al.*, 2007).

Root characteristics such as length, volume and surface area are important for moisture uptake and nutrients absorption (Desnos, 2008). Root characteristics play a significant role in nutrient deficient soils and drought prone areas where roots need to go deep into the soil for moisture uptake and explore large volume of soil for nutrients (Hill *et al.*, 2006). Root characteristics are also important in the acquisition of immobile nutrients such as P (Desnos, 2008).

Hence, studies on the root growth of the crop at RP levels could provide an insight in to its P nutrition which could be used to address the P constraint and enhance the production of the crop (Hill *et al.*, 2006; Sokoto and Singh, 2008; Musa *et al.*, 2012; Zou *et al.*, 2018). Thus, the experiment was conducted to study the root growth, N and P uptake of the crop as influenced by RP levels at pod development stage.

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Materials and Methods

Experimental Site

A screenhouse experiment was conducted at The University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia. The experimental area is located on latitude $2^o57^'$N and longitude $101^o51^'$E at an altitude of 560 m above sea level. The climate of the area is Tropical sub-humid with mean annual rainfall of 3000 mm. The result of the soil analysis at the site revealed that the soil was strongly acidic (pH $_{(water)}$ 4.20), low in C (0.48%), N (0.05%), P (2.3 mg kg^{-1}), K (0.10 cmol kg^{-1} soil) and CEC (4.5 cmol kg^{-1} soil). The soil was clayey in texture (Table 1).

Treatments and Experimental Design

Treatments consisted of factorial combination of two Bambara groundnut landraces (Kangiwa brown and Kaaro) and four RP levels [0, 20, 40 and 60 kg P_2O_5 ha^{-1} which were equivalent to 0, 555.6, 1111.1 and 1666.7 kg ha^{-1} of Christmas Island Rock Phosphate (32% P_2O_5, 3.6% solubility using 2% Neutral Ammonium Citrate) applied at 0.28, 0.56 and 0.84 g kg^{-1} of soil] making a total of eight treatment combinations. The experimental layout was completely randomized design replicated thrice (Fig. 4).

Cultural Practices

Prior to sowing, PVC pipes of 0.2 m in diameter were cut at 0.75 m height and in each pipe, 18.5 kg air-dried soil was thoroughly mixed with perlite at the soil:perlite ratio of 3:1 (v/v). Seeds of the two Bambara groundnut landraces (Kangiwa brown and Kaaro) were sown in PVC pipes arranged at 0.5 m x 0.2 m. Two seeds per pipe were planted at a depth of 20–30 mm using dibbling method and at two weeks after sowing the plants were thinned to one plant/pipe. Weeds were manually controlled throughout the trial. Nitrogen (N) and potassium (K) fertilizers were applied at the rate of 20 and 40 kg N and K_2O ha^{-1}, respectively using Urea (46% N) and Muriate of potash (60% K_2O). Phosphorus was applied as per treatment using Christmas Island Rock Phosphate (32% P_2O_5, 3.6% solubility using 2% Neutral Ammonium Citrate). All the P_2O_5 levels were computed using the NAC solubility of 3.6%. The fertilizers were incorporated before sowing using soil weight basis of 2 000 000 kg soil ha^{-1}. Fungicide (Mancozeb 80% w/w at the rate of 2.5 kg ha^{-1}) and insecticide (Cypermethrin 5.5% at the rate of 800 mL ha^{-1}) were sprayed three times each at two weeks interval during the growing period. The experiment was maintained under daily irrigation as the perlite included in the mixture ensures good drainage and to facilitate the dissolution of the applied RP. The research was terminated at 8 weeks after sowing (*i.e.*, at pod development stage). For each pot, the shoots were severed from the roots using a knife and immediately taken to the oven for drying. The oven temperature was maintained at 70ºC for 72 h.

Data Collection and Analysis

Data were collected on root length, root volume, root diameter, root surface area, root length volume^{-1}, root dry weight, plant dry weight, N concentration, N content, P concentration, P content and Ash.

Plant samples were grounded, sieved using 80-mesh (0.2 mm) and the % N, P and Ash were determined at Applied Agricultural Resources Laboratories Sdn Bhd. Malaysia. The nitrogen content was determined using Micro Kjeldahl digestion techniques as suggested by Unkovich *et al*. (2008). Nitrogen (N) content was determined as the product of % N and plant dry weight as described by Mohale *et al.* (2014):

$$N\ content\ = plant\ dry\ weight\ \times\ \%\ plant\ N$$

The roots were carefully washed, floated on water and scanned using Flatbed Scanner, Epson Expression 11000XL, Seiko, Japan with WinRhizo Pro version 2013 (Regent Instruments, Canada) software (Fig. 5).

The data were subjected to analysis of variance (ANOVA) procedure for completely randomized design (CRD) in GenStat® 16th edition. Means were separated using Tukey's test.

Results

Root Length, Volume and Diameter

The root length and volume were significantly (P < 0.05) higher in Kaaro landrace than Kangiwa brown landrace. However, despite the differences in the root length and volume of the landraces, the root diameter of the landraces was not significantly (P > 0.05) different (Table 2).

The effect of applied RP on the root volume of the landraces was significant (P < 0.05). Application of RP at the rate of 40–60 kg P_2O_5 ha^{-1} recorded higher root volume than the treatments which received application of 0–20 kg P_2O_5 ha^{-1}. However, despite the differences in root volume observed as a result of P application, the root length and diameter of the landraces were not influenced by the RP levels (Table 2).

Root Weight, Surface Area and Length Volume^{-1}

Kaaro landrace was higher root weight, surface area and length volume^{-1} than Kangiwa brown landrace (Table 3). Application of RP significantly (P < 0.05) increased the root weight and surface area of the crop being optimum at the application of 40 kg P_2O_5 ha^{-1} for root weight and surface area. However, despite the increase in the root weight and surface area of the crop as a result of RP application, the root

Table 1: Physico-chemical properties of the soil used during the experiment at the University of Nottingham, Malaysia Campus

Chemical Properties	Values	Physical properties	Values
pH (H_20)	4.20	Sand (g kg^{-1})	430
P (mg kg^{-1})	2.3	Silt (g kg^{-1})	110
Carbon (%)	0.48	Clay (g kg^{-1})	460
Total N (%)	0.05	Soil texture	Clay
Exchangeable cations (cmol kg^{-1} soil)			
K	0.10		
Ca	1.16		
Mg	0.12		
Na	0.09		
Al	0.67		
CEC (cmol kg^{-1})	4.5		

Table 2: Root length, volume and diameter of Bambara groundnut landraces as influenced by rock phosphate (RP) levels in the Screenhouse, The University of Nottingham, Malaysia Campus

Treatment	Root length (cm)	Root volume (cm^3)	Root diameter (mm)
Landraces (L)			
Kangiwa brown	357b	0.17b	0.26
Kaaro	466a	0.29a	0.32
SEM	32.4	0.016	0.023
P values	0.031	<0.001	0.091
RP (kg P_2O_5 ha^{-1}) (g RP kg $soil^{-1}$)			
0 (0.00)	365	0.19b	0.25
20 (0.28)	391	0.20b	0.29
40 (0.56)	468	0.25ab	0.29
60 (0.84)	423	0.27a	0.32
SEM	45.8	0.022	0.033
P values	0.449	0.051	0.563
Interaction (P values)			
L X RP	0.333	0.219	0.716

Within each treatment group, means in a column followed by same letter(s) are not significantly different using Tukey's test. SEM= Standard error of means, P values= probability values

Table 3: Root surface area, length $volume^{-1}$ and root dry weight of Bambara groundnut landraces as influenced by rock phosphate (RP) levels in the Screenhouse, The University of Nottingham, Malaysia Campus

Treatment	Root surface area (cm^2)	Root length $volume^{-1}$ (cm m^{-3})	Root dry weight (g $plant^{-1}$)
Landraces (L)			
Kangiwa brown	27.4b	17245b	0.6b
Kaaro	44.2a	22352a	1.1a
SEM	1.98	1737	<.001
P values	<0.001	0.057	0.002
RP levels (kg P_2O_5 ha^{-1}) (g kg $soil^{-1}$)			
0 (0.00)	27.4b	17008	0.6b
20 (0.28)	31.2b	19560	0.8b
40 (0.56)	40.6a	21473	1.0a
60 (0.84)	43.9a	21155	1.0a
SEM	2.79	2457	0.064
P values	0.003	0.573	<.001
Interaction (P values)			
L X RP	0.185	0.586	0.632

Within each treatment group, means in a column followed by same letter(s) are not significantly different using Tukey's test. SEM= Standard error of means, P values= probability values

length $volume^{-1}$ of the landraces was not significantly influenced by the application of RP on the crop (Table 3).

Table 4: Plant dry weight, N and P concentration of Bambara groundnut landraces as influenced by rock phosphate (RP) levels in the Screenhouse, The University of Nottingham, Malaysia Campus

Treatment	Plant dry weight (g $plant^{-1}$)	Nitrogen concentration (%)	Phosphorus concentration (%)
Landraces (L)			
Kangiwa brown	9.07b	4.47	0.213
Kaaro	12.40a	4.26	0.205
SEM	0.862	0.141	0.008
P values	0.005	0.312	0.539
RP levels (kg P_2O_5 ha^{-1}) (g kg $soil^{-1}$)			
0 (0.00)	6.35b	5.13a	0.144b
20 (0.28)	10.95ab	4.39b	0.233a
40 (0.56)	12.62a	4.08b	0.237a
60 (0.84)	13.02a	3.87b	0.222a
SEM	1.219	0.199	0.012
P values	0.015	0.002	<.001
Interaction (P values)			
L X RP	0.311	0.822	0.987

Within each treatment group, means in a column followed by same letter(s) are not significantly different using Tukey's test. SEM= Standard error of means, P values= probability values

Plant Dry Weight, Nitrogen and Phosphorus Contents

Kaaro landrace recorded higher plant dry weight than Kangiwa brown landrace. However, despite the difference between the landraces in plant dry weight, the N and P concentrations of the crop were not significantly *(P* > 0.05) different between the landraces (Table 4).

Significant ($P < 0.05$) effect of RP application on plant dry weight, N and P concentration was observed during the trial. Application of RP (20–60 kg P_2O_5 ha^{-1}) significantly increased the plant dry weight and P concentration of the crop compared to control (0 kg P_2O_5 ha^{-1}). However, no significant difference among the RP levels of 20–60 kg P_2O_5 ha^{-1} was observed in both the plant dry weight and P concentration of the crop. For the N concentration, application of RP (20–60 kg P_2O_5 ha^{-1}) significantly ($P >$ 0.05) decreased the N concentration of the crop compared to control (Table 4).

N, P and Ash Contents

Significant ($P < 0.05$) interaction between the landraces and RP levels on the N content of the crop was observed in the trial. At 0 kg P_2O_5 ha^{-1}, the N content of the crop was not significantly different between the landraces. However, application of RP (20–60 kg P_2O_5 ha^{-1}) significantly ($P <$ 0.05) increased the N content of both the landraces and was higher in Kaaro landrace than Kangiwa brown landrace. The highest N content was observed at the application of 20 kg P_2O_5 ha^{-1} on Kaaro landrace which was not significantly different from the N content recorded at the application of 40 kg P_2O_5 ha^{-1} on the same landrace (Fig. 1).

Significant ($P < 0.05$) interaction between the landraces and RP levels on P and ash content of the crop was observed in the trial. At 0 kg P_2O_5 ha^{-1}, the P and ash

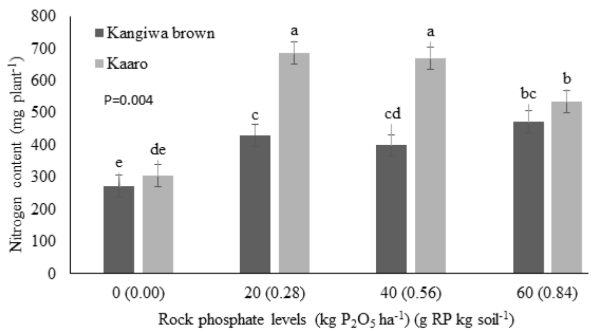

Fig. 1: Nitrogen content of Bambara groundnut landraces as influenced by rock phosphate (RP) levels in the Screenhouse, The University of Nottingham, Malaysia Campus. Error bars are SEM values. Bars with the same letter(s) are not significantly different using Tukey's test

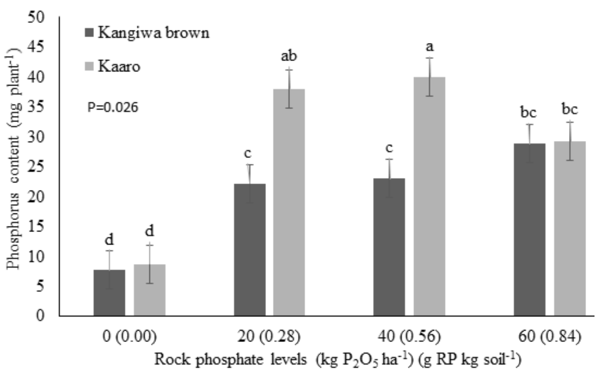

Fig. 2: Phosphorus content of Bambara groundnut landraces as influenced by rock phosphate (RP) levels in the Screenhouse, The University of Nottingham, Malaysia Campus. Error bars are SEM values. Bars with the same letter(s) are not significantly different using Tukey's test

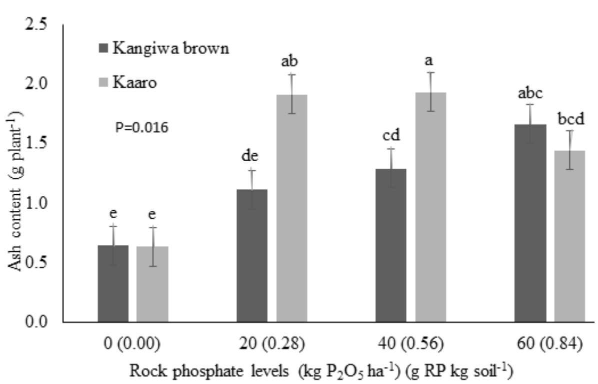

Fig. 3: Ash content of Bambara groundnut landraces as influenced by rock phosphate (RP) levels in the Screenhouse, The University of Nottingham, Malaysia Campus. Error bars are SEM values. Bars with the same letter(s) are not significantly different using Tukey's test

contents of the two landraces were both lower and not significantly different between the landraces. However, application of RP increased the P and ash contents of the crop, which were higher in Kaaro landrace at the application rate of 20 and 40 kg P_2O_5 ha^{-1} than Kangiwa brown landrace. The two landraces were similar in P and ash contents at the RP application rate of 60 kg P_2O_5 ha^{-1} (Fig. 2 and 3).

Fig. 4: Section view of the experimental area showing arrangement of the PVC pipes used during the experiment in the Screenhouse, The University of Nottingham, Malaysia Campus

Fig. 5: A picture showing the roots of the two landraces grown in the Screenhouse, The University of Nottingham, Malaysia Campus

Discussion

This study revealed the differences between the landraces in root growth and N and P content and the potentials of using rock phosphate in addressing the P constraint that limits crop production. The differences in the landraces observed in this trial could be because of the differences in their genetic make-up and to the differences in the adaptation of the landraces to the study environment. The remarkable response of the landraces to RP application could be attributed to the lower soil P status of the experimental site, the high P requirement of the crop and the lower soil pH which favour's the dissolution of the applied rock phosphate in the study area. These attributes were earlier suggested as potentialities for using RP as alternative source of P in crop production (Agyin-Birikorang *et al.*, 2007).

The higher root length, volume, weight and surface area recorded by Kaaro landrace could be due to its higher shoot dry weight. The higher root weight, volume and surface area recorded where RP was applied could be attributed to the role of phosphorus in root growth and development and hence, the more exploration of soil for nutrients (Jones and Ljung, 2012). P deficiency in plants has been reported to restrict plant performance in root growth and shoot dry content (Desnos, 2008; Zhou *et al.*, 2018).

The higher plant dry weight observed in Kaaro landrace could be because of its higher root growth than Kangiwa brown landrace. Jones and Ljung (2012) reported the superiority of high root biomass in exploring large soil volume for P and other nutrients. The higher N content recorded by Kaaro landrace could be because of its higher plant dry weight since the N concentration of the landraces was not significantly different (4.3–4.5%). The higher plant dry weight and N content observed in the treatments that received RP application compared to control could be attributed to the role of P in improving plant physiological processes including photosynthesis. Phosphorus is reported to play significant role in most of the plant physiological processes including photosynthesis and energy metabolism (Bouain *et al.*, 2014; Leggett *et al.*, 2015).

The higher P and ash content observed in Kaaro landrace compared to Kangiwa brown could be attributed to its higher root and plant dry weight since no significant difference in the P concentration of the landraces was observed. Hill *et al.* (2006) associated higher rooting density with the ability to explore large soil volume for P and other nutrients. The higher P concentration, P content and ash recorded in the treatments where RP was applied could be due to increased supply of P by the RP which resulted in increased root surface area of the crop and hence the more the exploration of large volume of soil for nutrients. High root surface area has been reported as an advantage in exploring large volume of soil for P and other nutrients (Desnos, 2008; Zhao et al., 2017).

Conclusion

The crop responded positively to rock phosphate application in the study area. Application of rock phosphate at the rate of 40 kg P_2O_5 ha^{-1} was optimum for root growth of the crop, whereas plant dry weight, P concentration and nutrients contents were maximized at the application of 20 kg P_2O_5 ha^{-1}. Kaaro landrace performed better than Kangiwa brown landrace in root growth and nutrients uptake. Thus, rock phosphate could serve as an alternative source of P for Bambara groundnut production in the study area. Application of 20 kg P_2O_5 ha^{-1} (0.28 g kg^{-1} of soil) was optimum for the N and P uptake of the crop. Overall Kaaro landrace performed better than Kangiwa brown in the study area.

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