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Root nodules as phosphorus sinks: Effects on growth of two high-haulm producing groundnut varieties

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ABSTRACT

Root nodules are stronger sinks for phosphorus compared to other plant parts; this emphasizes the crucial role phosphorus plays in plant growth. This study determined the influence of phosphorus on high-haulm-producing groundnut for growth and nodulation parameters in a pot experiment. The experiment was a 2 x 8 factorial design in a completely randomized design (CRD). Two groundnut varieties (NkatieSARI and Chinese) and 8 levels of phosphorus were applied with three the replicates. Plants were top-dressed with urea at 21 days after planting. Shoots were harvested twice during the growth period (R3 and R6). Results showed that shoot biomass produced at R6 was 21% and 42% higher than at R3 for both varieties. Root biomass significantly ($p = 0.045$) increased at 41.2 mg P kg⁻¹ soil compared to the control and Chinese, while NkatieSARI had an increase in root weight at ≥ 30.9 mg P kg⁻¹ soil. Phosphorus at ≥ 20.6 mg P kg⁻¹ soil significantly ($p = 0.039$) increased the number of nodules produced by NkatieSARI from an average of 37 (control) to 86.8 nodules at 51.5 mg P kg⁻¹ soil. Irrespective of the phosphorus rates, Chinese produced few but heavy nodules, while NkatieSARI produced more but light nodules.

Keywords: Effective nodules, fodder, groundnut, phosphorus, root density, shoot biomass.

INTRODUCTION

Groundnut (*Arachis hypogaeae*) is an important grain legume in the Guinea savanna agroecology of Ghana in terms of area under cultivation, accounting for over 70% of total groundnut produced in the country (Oteng-Frimpong et al., 2017). The legume offers a cheaper protein source for households, and from the agroecological perspective, groundnut contributes to the nitrogen (N) economy of soils through biological nitrogen fixation (BNF) (Mokgehle et al., 2014). In the Guinea Savanna agroecology, the sale of groundnut fetches income for the farmer households, while the haulms

serve as high-quality protein fodder for livestock (Martey et al., 2015). Oteng-Frimpong et al. (2017) reported that groundnut haulms are more palatable and richer in protein compared to stovers of cereals, which have low N, high fiber content, and poor digestibility; hence, they have low nutritive value and are used as supplementary feed for livestock.

Groundnut production for improved yield and haulm quality requires that good seeds be planted on soils with an optimal soil nutrient management system (Veeramani & Subrahmaniyan, 2011; Tekulu et al., 2020). By far, N,

P, and K are the most needed mineral nutrients in all cropping systems, due to the key roles these nutrients play in different biochemical and physiological processes of plants (Leghari et al., 2016; Dehnavard et al., 2017; Souri et al., 2019). Results from a field experiment reported by McKenzie et al. (2001) revealed that when soil N was less than 20 kg N ha⁻¹, application of starter N fertilizer improved pea yield. Conversely, the application of a higher dose of N fertilizer in legumes inhibits BNF through the development of ineffective nodules (Prasad et al., 2010). In general, a higher rate of N fertilizer negatively affects nodulation and BNF of legumes (Huang et al., 2017; Aslani & Souri, 2018; Tekulu et al., 2020).

Phosphorus (P) is also another important mineral nutrient that has different roles in plant functional metabolism, including energy transfer of legume crops during BNF (Hussain, 2017). Hence, P can promote leguminous crops to produce their own N sources, but at the time of P deficiency, rates of BNF can be negatively affected due to a reduced number of effective root nodules (Malhotra et al., 2018). Phosphorus nutrition is crucial for groundnut crops since it improves nodulation and BNF, increases residual soil nitrogen content (Yakubu et al., 2010), and significantly contributes to healthy and efficient root growth (Muhaba & Dakora, 2020). Also, phosphorus is involved in cellular energy transfer, respiration, and photosynthesis; thus, its supply at low levels can affect legume nodulation and N₂ fixation (Israel, 1987; Vance et al., 2003). Furthermore, due to the high phosphorus-sink strength of nodules, the requirement for P in nodulated legumes is higher than in non-legumes (Lovelock et al., 2006; Muhaba & Dakora, 2020). Hence, this study was conducted to assess the effects of various levels of P supply on growth and nodulation on two groundnut varieties in a pot experiment.

MATERIALS AND METHODS

Experimental Site

The research was conducted at the Tamale Technical University research fields within the Guinea savanna ecology in the northern region of Ghana. Planting was done on 21st January 2023 during the dry season. The area has a unimodal rainfall of 1100 mm per annum, which occurs between May and October each year (CSIR-SARI, 2020). The soils are predominantly sandy loam, which does not retain water well.

Soil and Climatic Conditions

Before planting, soil samples were collected from holes

dug from the surface to a 30 cm depth across 10 different points chosen at random from a farmland. The soil samples were mixed, and subsamples were analyzed for chemical properties such as pH (H₂O); organic carbon (Walkley & Black, 1934); total nitrogen (Kjeldahl method); and phosphorus (P) concentration using Bray-2 (Bray & Kurtz, 1945). Analysis of K, Na, Ca, Mg, and S concentrations was also determined. The cation exchange capacity (CEC) was measured using the ammonium acetate method according to Toth & Prince (1949). Soil texture was also determined.

Experimental design and treatments

A 2 x 8 factorial pot experiment was laid out in a completely randomized design (CRD) arrangement. Plastic pots of 5L capacity measuring 22.5cm in diameter and 18.0cm in height were each filled with 10.0kg (2.0mm sieved) of dry soil. The treatments consisted of two commonly cultivated groundnut varieties (NkatieSARI and Chinese) as main plots and 8 levels of single superphosphate (SSP) fertilizer (0, 10.3, 20.6, 30.9, 41.2, 51.5, 61.8, and 72.1 mg P kg⁻¹ soil) as subplots. All plants were top dressed at 85.5 mg N pot⁻¹ with urea at 21 days after planting (DAP) and replicated three times.

Establishment of groundnut plants

Three (3) groundnut seeds were planted in each pot of the phosphorus-treated soils to obtain three seedlings per pot. Plants were watered with tap water at 700 ml to 1000 ml per pot⁻¹ on alternate days to sustain growth. All pots were kept weed-free by hand-picking when necessary.

Data collection

Sampling of shoot biomass

Shoots from each pot were separated from roots by decapitating with a secateur at the R3 growth stage (early podding). They were transferred into labeled brown paper bags, weighed to determine their fresh weight, and subsequently oven-dried at 60°C to a constant weight for dry matter yields (Unkovich et al., 2008). At the R6 growth stage (i.e., full seed), there was a second harvest of the shoot, and the above-listed procedure was repeated.

Sampling of root biomass

The decapitated roots for each treatment were carefully washed under running water to remove any attached

soils as well as minimize nodule detachment from roots. The cleaned roots were gently wiped with paper towels to dry off excess water before fresh weights were recorded. Fresh roots were then transferred into labeled brown paper bags and oven-dried at 60°C to a constant weight.

Nodule number and weight

Nodules were detached from the roots and counted for nodulation. Effective nodules were identified as pinkish in colour when cut open (Unkovich et al., 2008), and non-effective nodules per phosphorus treatment were identified and recorded. Fresh nodule weights for both effective and non-effective nodules were determined. The total number of nodules for each treatment was the sum of effective and non-effective nodules for each treatment (Ogola et al., 2012).

Data Analysis

Data collected from the pot experiment were subjected to analysis of variance using GENSTAT version 12 after the data were tested for normality. Means of treatments that were significantly different from each other were separated using a Fisher's protected least significant difference (LSD) means comparison method. Regression analysis was also done to determine the relationship among the parameters measured.

RESULTS

Soil and Climatic Conditions

The soil was sandy loam in texture with a pH of 5.62. Organic carbon was found to be 0.76%; total nitrogen was 0.08%; phosphorus was 4.15 mg/kg; potassium was measured at 0.22 cmol/kg, and the cation exchange capacity (CEC) of the soil was 4.28 cmol/kg. Average daily temperatures ranged from 29°C to 32°C during the growing season, with a minimum of 26.25°C and a maximum of 34.65°C. Relative humidity ranged from 20% in January, rising steadily to 55% in March during the 2023 dry season. There was no rainfall during the period of this experiment.

Effect of P on groundnut shoot biomass yield

Shoot dry matter production associated with various rates of phosphorus in the first and second sampling for Chinese and NkatieSARI varieties are shown in Tables 1 and 2, respectively. It is evident from the results that the mean shoot biomass produced in the second harvest was

21% and 42% higher than production in the first harvest in Chinese and NkatieSARI, respectively. NkatieSARI had significantly higher ($p < 0.05$) shoot dry matter yields at phosphorus rates of $P \geq 20.6 \text{ mg kg}^{-1}$ soil compared to the control treatment in both first and second harvests (Table 2). However, such differences in dry shoot weight in Chinese were observed only in the second harvest at P rates above 41.2 mg kg^{-1} soil (Table 1). Consequently, both groundnut varieties produced significantly more biomass in the second harvest (R6) compared to the first harvest (R3).

Influence of phosphorus on root biomass accumulation

Results presented in Table 3 below show that root dry matter yield for Chinese significantly ($p=0.045$) increased to $39.9 \text{ gm plant}^{-1}$ at $41.2 \text{ mg P kg}^{-1}$ compared to the control treatment, which had a mean weight of $24.6 \text{ gm plant}^{-1}$. On the other hand, NkatieSARI root weight was also significantly increased at $30.9 \text{ mg P kg}^{-1}$ soil. Addition of phosphorus in excess of these rates did not translate into any gain in weight except at $72.1 \text{ mg P kg}^{-1}$ soil, which resulted in NkatieSARI root biomass being increased by about 84%, while Chinese had root weight increased by 20% (Table 3). Comparison of Chinese and NkatieSARI dry root biomasses, across phosphorus rates, revealed that NkatieSARI roots were about 15% heavier than Chinese roots, with more pronounced variations being observed at lower rates of fertilization.

Influence of phosphorus on nodulation

The effect of phosphorus application on nodulation varied with the rate applied in both groundnut varieties, although the number of nodules in Chinese did not respond significantly to phosphorus application (Table 4). The Chinese variety produced a mean of 31.3 nodules pot⁻¹ at the control treatment. However, the number of nodules increased to about 71 nodules in pot⁻¹ at $72.1 \text{ mg P kg}^{-1}$ soil (Table 4). Application of phosphorus at $P \geq 20.6 \text{ mg P kg}^{-1}$ soil significantly ($p = 0.039$) increased the number of nodules produced by NkatieSARI. The number of nodules increased from an average of 37 (control) to 86.8 nodules pot⁻¹ at $51.5 \text{ mg P kg}^{-1}$ soil (Table 4). Fresh nodule weight was not significantly affected by phosphorus application in NkatieSARI (Table 4), while Chinese produced significantly lighter nodules at 41.2 and $51.5 \text{ mg P kg}^{-1}$ soil (Table 4). Even though Chinese produced few but relatively heavy nodules, nodules associated with NkatieSARI were large in number and relatively light in weight, irrespective of the phosphorus rates.

Table 1. Influence of P on shoot biomass accumulation (growth) of Chinese groundnut variety.

P Treatment (mg kg ⁻¹)	First harvest @ R3 (g pot ⁻¹)	Second harvest @ R6 (g pot ⁻¹)	Cumulative
0	22.2	32.6	54.8
10.3	31.1	39.7	70.7
20.6	36.6	40.1	76.7
30.9	34.5	41.5	76.0
41.2	36.3	45.6	81.9
51.5	37.9	41.0	78.8
61.8	38.9	43.8	82.8
72.1	35.8	45.4	81.2
Mean	34.1 ^b	41.2 ^a	37.7
LSD	NS	7.5	23.9
CV (%)	5.5	7.0	8.0

Means followed with the same letter are not significantly different.

Table 2. Influence of P on shoot biomass accumulation (growth) of NkatieSARI groundnut variety.

P Treatment (mg kg ⁻¹)	First harvest @ R3 (g pot ⁻¹)	Second Harvest @ R6 (g pot ⁻¹)	Cumulative
0	25.1	29.5	54.6
10.3	45.7	51.0	96.7
20.6	54.5	69.2	123.7
30.9	53.6	77.0	130.5
41.2	51.6	70.0	121.7
51.5	58.9	81.5	140.4
61.8	50.1	77.1	127.2
72.1	45.1	88.7	133.8
Mean	48.1 ^b	68.0 ^a	58.0
LSD	21.3	31.2	46.2
CV (%)	7.8	5.6	9.7

Means followed with the same letter are not significantly different.

Comparison of effective and ineffective nodules as influenced by P fertilization

Both groundnut varieties produced ineffective and effective nodules (pinkish nodules), suggesting that effective nodules could be active in fixing nitrogen. Application of P at rates $P \geq 41.2$ mg P kg⁻¹ soil did not affect the number of nodules in Chinese (Figure 1A). But the number of active nodules obtained in the control treatment was significantly ($p \leq 0.05$) lower compared to the ones obtained at 20.6 and 30.9 mg P kg⁻¹ soil in Chinese. Application of P at 30.6 mg P kg⁻¹ soil not only increased the number of effective nodules (6 nodules per plant) but also induced the highest number (13 nodules per plant) of total nodules produced in Chinese. The

NkatieSARI groundnut variety had a significant ($p \leq 0.05$) increase in total nodules at a P rate ≥ 30.9 mg P kg⁻¹ soil, except at 41.2 mg P kg⁻¹ soil (Figure 1B). A significantly high number of effective nodules were produced by NkatieSARI at 30.9, 51.5, and 60.8 mg P kg⁻¹ soil application rates of fertilization compared to the control treatment. The number of effective nodules produced by either Chinese or NkatieSARI generally followed a trend similar to that of total nodules produced.

Relationships between nodule number and root biomass

Nodules and root biomass revealed a positive relationship

Table 3. Influence of phosphorus on groundnut root biomass accumulation.

P Treatment (mg kg ⁻¹)	Chinese (gm ⁻¹ plant)	NkatieSARI (gm ⁻¹ plant)	Mean
0	24.6	19.1	21.8
10.3	28.9	32.5	30.7
20.6	30.4	41.5	36.0
30.9	33.3	43.9	38.6
41.2	39.9	43.1	41.5
51.5	34.6	37.4	36.0
61.8	29.6	35.2	32.4
72.1	29.7	34.6	32.1
Mean	31.4	35.9	33.6
LSD	NS	11.4	7.5
CV (%)	7.1	5.0	6.9
SE	3.4	3.8	3.1
P level	0.07	0.05	0.006

Means followed with the same letter are not significantly different.

Table 4. Influence of P on number of nodules and fresh nodule weight of two groundnut varieties.

P Treatment (mg kg ⁻¹)	Chinese	NkatieSARI	Chinese	NkatieSARI
	Total number of nodules nodules ⁻¹ pot		Fresh nodule weight (g ⁻¹ nodule)	
0	31.3	37.0	0.7	0.5
10.3	45.7	33.0	0.8	0.5
20.6	46.0	52.3	0.7	0.4
30.9	62.0	51.8	0.7	0.4
41.2	61.0	68.0	0.6	0.5
51.5	55.3	86.8	0.6	0.3
61.8	50.3	70.6	0.7	0.4
72.1	70.7	73.6	0.6	0.5
Mean	53.5b	60.0a	0.7a	0.4b
LSD	NS	NS	0.14	0.16
CV (%)	9.2	6.6	5.4	8.8

Means followed with the same letter are not significantly different.

at low rates of phosphorus application. (Figures 2A and B). The number of nodules consistently increased with root weight and peaked at 41.2 mg P kg⁻¹ soil. Further increase in phosphorus was less beneficial, although at 72.1 mg P kg⁻¹ soil, phosphorus application outstandingly increased the number of nodules in Chinese by about 80%. The highest number of nodules was obtained at 41.2 mg P kg⁻¹ soil, which represents a significant increase of 112% compared to the control treatment (Figure 2B). The trend line showing the relationship

between nodule fresh weight and total number of nodules showed a negative correlation, with critical phosphorus rate being obtained at 55 and 46 mg P kg⁻¹ soil for Chinese and NkatieSARI, respectively (Figures 3A and B). However, the response of root weight to phosphorus was positive; phosphorus had no influence on nodule weight (Figures 4A and B). Interestingly, nodule weight and root biomass exhibited a trend similar to that observed in the nodule density and shoot biomass relationship. The number of nodules increased as root

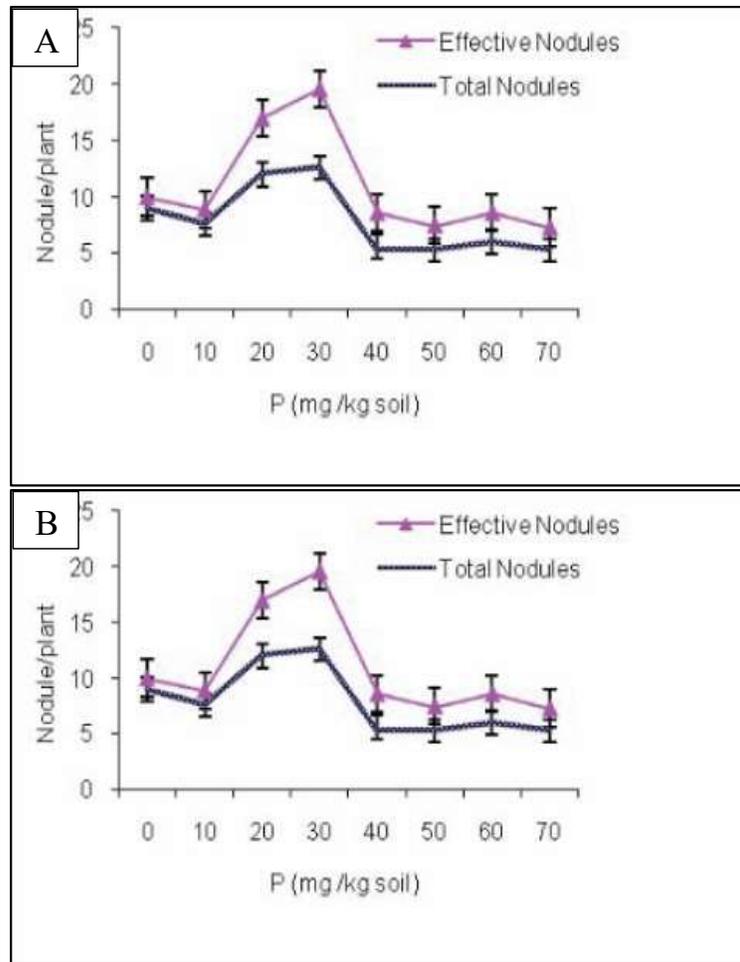


Figure 1. Influence of varying rates of phosphorus on nodulation of (A) Chinese and (B) NkatieSARI groundnut varieties.

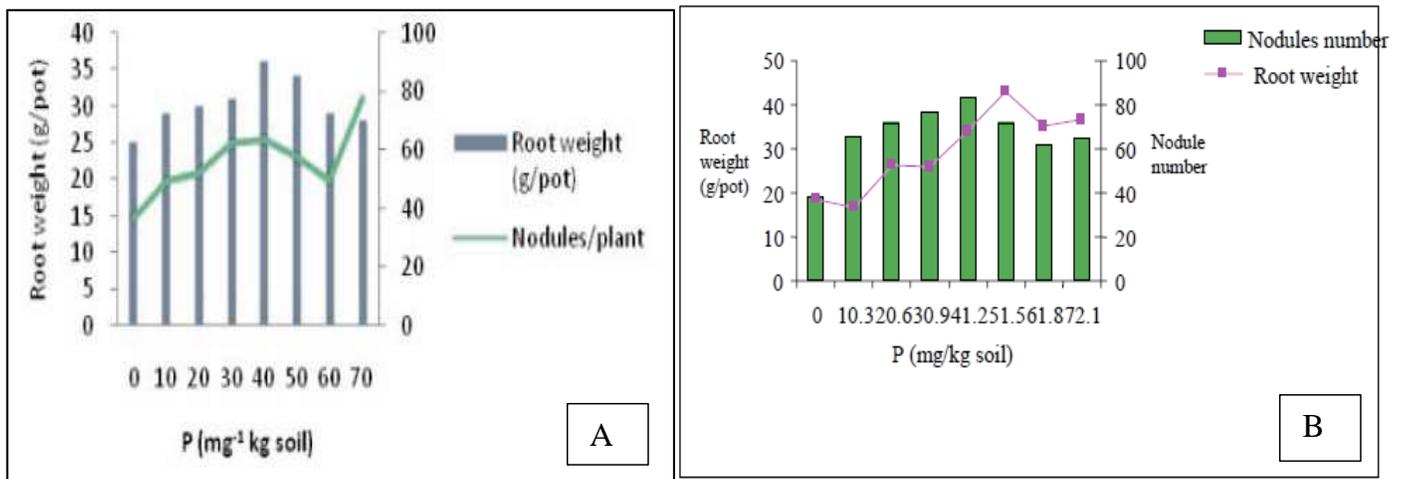


Figure 2. Relationship between root biomass and nodule number as affected by phosphorus on (A) Chinese and (B) NkatieSARI groundnut varieties.

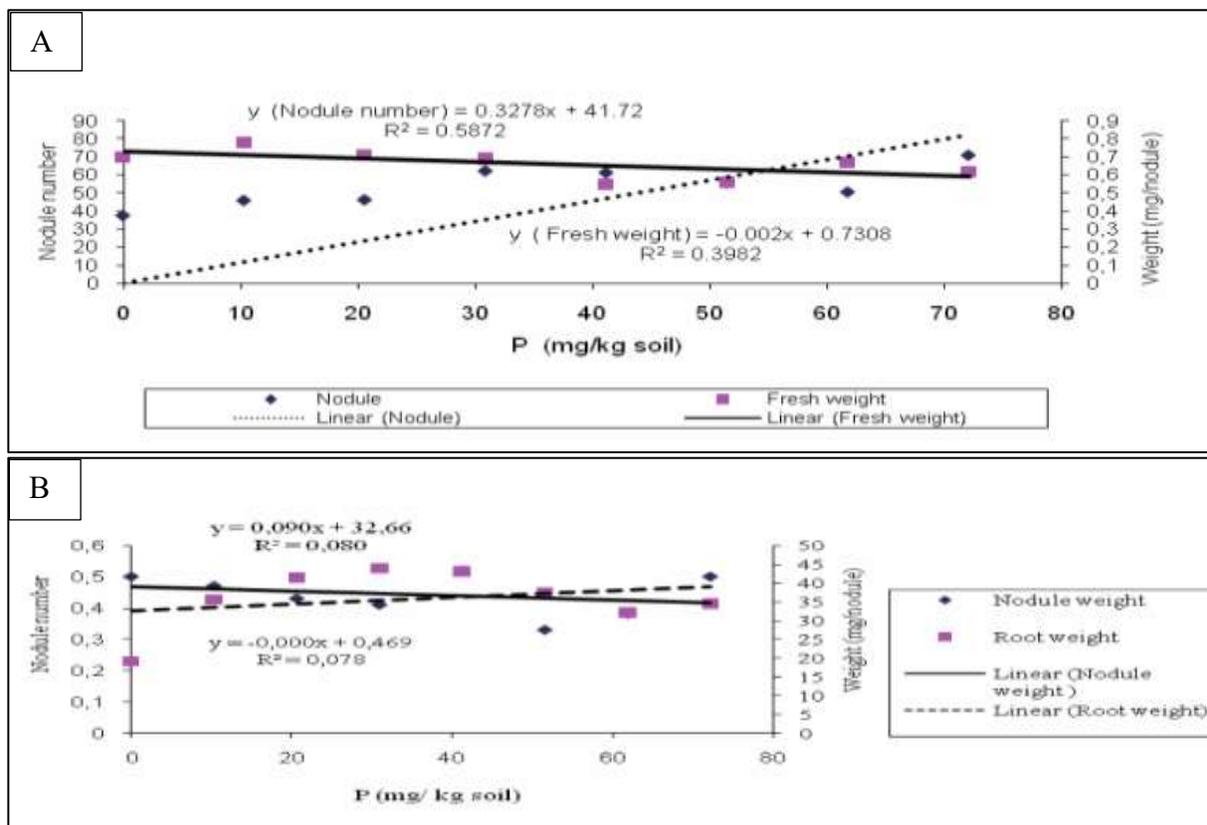


Figure 3. Relationship between number of nodules and fresh nodule weight of (A) Chinese and (B) NkatieSARI groundnut varieties as affected by phosphorus.

biomass increased and curved downwards as root weight decreased.

DISCUSSIONS

Effect of phosphorus on shoot biomass

Consistently, this study observed a significant increase in shoot biomass enhanced by phosphorus fertilization at higher rates of application. Consequently, the greater shoot biomass accumulation at higher P rates translated into the enhanced root growth and increased nutrient uptake. The findings of this study revealed that both Chinese and NkatieSARI had accumulated biomass in response to their growth and physiological development. Expectedly, mature plants produced higher haulms/pasturage dry matter yields than the young ones. At the R6 stage of sampling, the groundnut plants had matured and had roots fully established in the pot, resulting in optimizing nutrient uptake, thereby accelerating the accumulation of shoot biomass. These observations are consistent with previous findings by

Carlsson & Huss-Danell (2003) and Ogola et al. (2012). Kabir et al. (2013) also reported that application of N beyond 40 kg N ha⁻¹ resulted in a significant decline in total herbage dry matter yield, while application of P at 23 and 46 kg P₂O₅ ha⁻¹ markedly induced an increase in pasturage dry matter yield. Similarly, as observed in this study, the study by Kabir et al. (2013) also observed no gain in shoot dry matter yield at P rates above 46 kg P₂O₅ ha⁻¹.

Influence of phosphorus on root biomass

Phosphorus fertilization at 41.2 mg P kg⁻¹ soil significantly increased Chinese and NkatieSARI root biomass by 62 and 131%, respectively. The increase in biomass was more marked among the NkatieSARI treatment, which suggests that NkatieSARI was more responsive to phosphorus application than Chinese. In a study that quantified nitrogen fixation by eight tropical forage legumes at two levels of P and K supply, Carlsson & Huss-Danell (2003) found that phosphorus was positively correlated with biomass production of forage

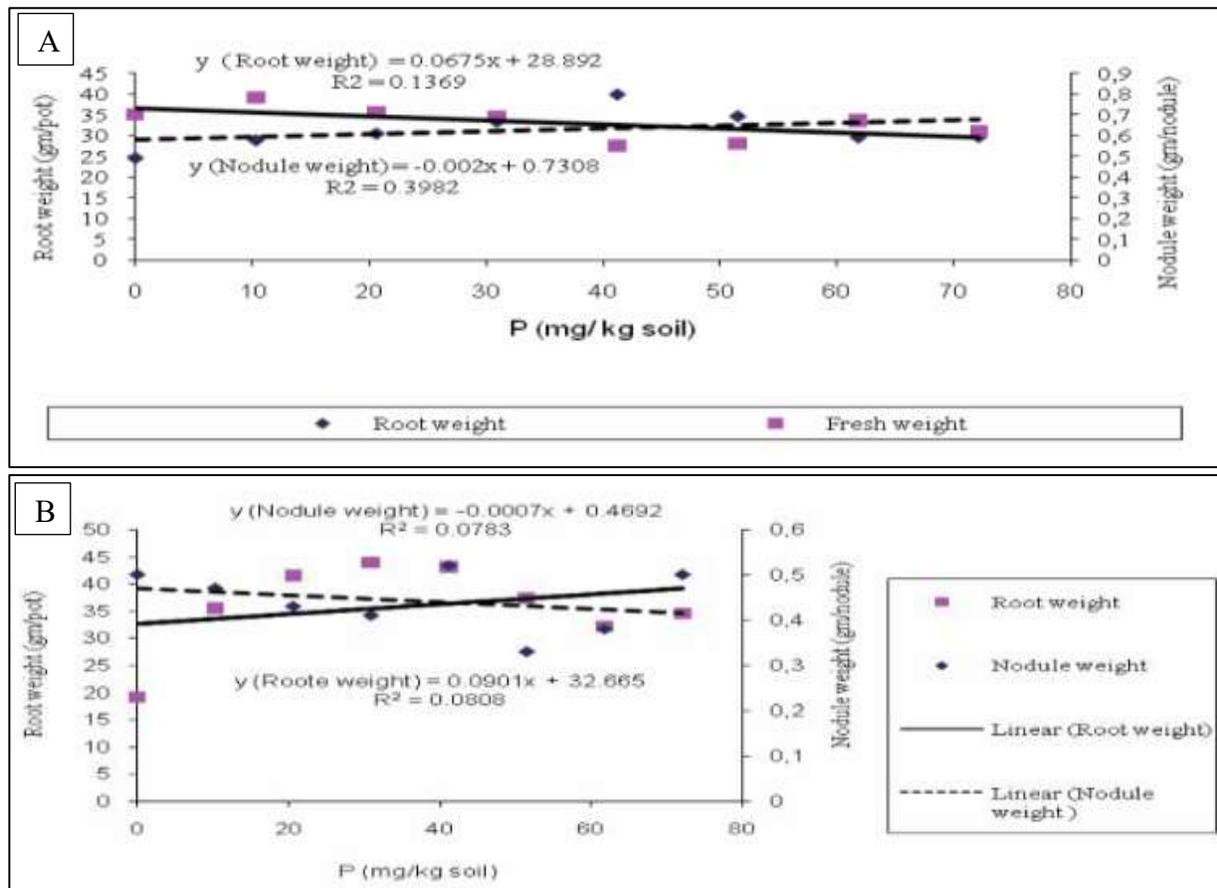


Figure 4. Relationship between fresh nodule weight and root biomass as affected by phosphorus among (A) Chinese and (B) NkatieSARI groundnut varieties.

groundnut in a phosphorus-deficient soil, while severe phosphorus stress reduced its root length by 50%. Lack of response by root biomass to phosphorus application above 51.5 mg P kg⁻¹ soil was probably caused by high P concentration (associated with the application rates) in the soil. This may have induced deficiency and/or triggered the uptake of other nutrients at a cost to root growth.

Groundnut nodulation, relationship between nodule number and nodule weight

Legumes require effective rhizobia strains and adequate soil phosphorus to fully exploit their nitrogen fixation potential (Kebede, 2021; Goyal et al., 2021). The results of this study revealed that phosphorus fertilization induced the production of not only large number but also small-sized and lighter nodules as well. This observation was evidenced among NkatieSARI treatments. Although a significant number of the total nodules produced by

each variety were pinkish in colour (when cut open), indicating the possibility of nitrogen fixation. The proportion of effective nodules was 59% and 64% of total nodules in Chinese and NkatieSARI treatments, respectively. Interestingly, treatments that produced a high number of effective nodules in Chinese also produced lighter nodules, while treatments that produced few nodules had relatively heavy nodules. This observation is suggestive that nodule production by Chinese was inversely related to nodule fresh weight. This inference may be of interest to measure and compare the biologically fixed nitrogen associated with the relatively few but big nodules in the Chinese groundnut variety and the small but large in number, produced by NkatieSARI. The inverse relationship between the nodule number and nodule mass was apparent and more pronounced in NkatieSARI than in Chinese. Fertilizing the groundnut varieties with phosphorus at higher rates enhanced production of small and light nodules as well as induced emergence of primary and lateral roots on which most nodules were

attached. This relationship was strengthened as far as root dry mass benefited from phosphorus application, particularly in the NkatieSARI treatments.

CONCLUSION

In conclusion, a consistent increase in shoot biomass was enhanced by phosphorus application, an indication that shoot biomass accumulation observed at optimum P rates translated into enhanced root growth, which increased nutrient uptake. This study also revealed that mature plants produced higher herbage dry matter yields compared to the young plants. Phosphorus fertilization rates at and above 41.2 mg kg⁻¹ soil evidently increased biomass more in NkatieSARI compared to Chinese. That observation suggests that NkatieSARI was more responsive to phosphorus application than Chinese.

This study underscores the critical role of phosphorus in optimizing nodulation and biomass production in groundnut varieties, with notable variety variation in response patterns. The findings contribute valuable insights for nutrient management strategies aimed at improving groundnut productivity and fodder quality, particularly in phosphorus-deficient soil, particularly in sub-Saharan regions. Field trials across diverse agroecological zones are recommended to validate and expand on these pot-based results, enhancing their applicability to sustainable groundnut production systems.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest regarding the publication of this article.

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