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Nodulation and N_2-fixation by Grain Legumes as Affected by Boron Fertilizer in Sudano-sahelian Zone of North-eastern Nigeria

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Abstract: Grain legumes can fix a substantial amount N in the tropics. However, low boron content in the Sudano - Sahelian soils of Northeastern Nigeria may limit the formation of nodules by legumes and affect their N_2 fixing potential. A two year field experiments were conducted at the Department of Soil Science Teaching and Research Farm University of Maiduguri to evaluate the influence of boron (B) on N_2 fixation by groundnut, (Arachis hypogeae L.) , cowpea (Vigna unguiculata (L.) Walp.) and bambara groundnut (Vigna subterranean L.Verdc.). The legume crops and a sorghum variety were applied 0, 0.5 and 1 Kg Bha^{-1} and grown for 50 days, after which they were harvested and the amount of N fixed was determined. B fertilization significantly increased the amount of N fixed by the crops. Application of 0.5 $KgBha^{-1}$ increased N fixation in cowpea, groundnut and bambara groundnut by 89, 126 and 5% respectively, over the control. However, application of 1kgB/ha significantly reduced nitrogen fixation in the plants. There was significant ($P<0.05$) difference among the crops in the amount of N fixed. Groundnut fixed 31.79N/ha, Cowpea (27.98N/ha) and bambara groundnut fixed (17.85N/ha). Cultivation of Kolji Kanuri groundnut variety with application of 0.5 $KgBha^{-1}$ would improve the soil N status.

Key words: Nodulation · N_2-fixation · Boron · Grain legumes · Sudano-sahelian

INTRODUCTION

Plant growth in soils throughout the world is often restricted by the supply of available N and as a result, it is N supply other than the supply of any other nutrient in the soil that limits world crop production [1]. In Sub-Saharan Africa, grain yield is declining in recent years [2]. This is partly due to low N fertility of many savannah soils especially in northeastern Nigeria where in addition to N, P, B and Mo deficiencies are reported [3-7]. With the removal of subsidies on fertilizers by the government, chemical fertilizers became unaffordable to the smallholders who are the majority in the region. Consequently, most of the N required for crop production comes largely from a judicious management of biological nitrogen fixation in traditional cropping systems [8]. Biological N fixation is a cheaper means of improving soil fertility and productivity.

The amount of N fixed by grain legumes in the tropics can be quite substantial. Giller [9] reported the N_2-fixing potential of cowpea, groundnut and soybean as 9 - 201; 21-206 and 55-188 kgN/ha/year, respectively. Studies conducted in three soils in Ghana [10] showed that bambara groundnut fixed N to the tune to about 97 - 106 kg/ha. Studies in humid regions of Western Nigeria [11] have shown that cowpea can fix 66-120 kgN//ha in 57 days. It was reported by Yusuf *et al.* [12] that cowpea fixed 16-34 kgN/ha and soybean fixed between 41-50 kgN/ha in northern Guinea savanna zone of Nigeria. Extensive studies by Dakora [13] revealed that the amount of N fixed by groundnut in Guinea Savanna range from 32 to 134 kgN/ha/year. The direct availability of the fixed N to the host allows it to grow in environments that are low in N reserves. Grain legumes are therefore involved in rotation and intercropping systems in order to increase the yield of cereal crops.

Legumes have been shown to have stimulatory effects on the availability of soil and fertilizer N to associated grasses. In field experiments, a recovery of applied N was three times greater in the mixed swards than in the sole grass [14]. This increase in availability of N on introduction of the legumes may result from reduced

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immobilization of N, or enhanced re-mineralization of N which had been immobilized due to the addition of N rich legume residues to the soil. Thus, the introduction of legumes may improve the N economy of the savanna soil by increasing the release of native organic N from the soil, in addition to the benefits from N_2-fixation.

Bationo and Vlek [15] reported that legume-cereal rotation increases not only the yields of succeeding cereal crop but also its nitrogen use efficiency. The beneficial effect of legumes on succeeding crops is usually exclusively attributed to the increased soil N fertility as a result of N_2-fixation. The quantity of N fixed by properly nodulated legumes averages about 75% of the total N used for plant growth.

In Sudano-Sahelian zone of north-eastern Nigerian, grain legumes such as groundnut, cowpea and bambara groundnut are very important components of cropping systems due to their roles in provision of a large cash income to the farming community, provision of cheaper and large quantities of dietary protein and edible oil for the population, provision of fodder for livestock and improving soil fertility and productivity. However, deficiencies of soil nutrients such as N, P and B and Mo may limit the development of population of free-living rhizobia in the rhizosphere, reduce nodule formation, nitrogenase activity and growth of many legumes [16]. Even though dozens of the legume cultivars, both indigenous and exotic have been commonly cultivated in this agro-ecological region, information on their boron requirement for optimum N_2 fixation is lacking. Therefore, this study was carried outto determine the effect of boron on the nodulation and N_2-fixation by of cowpea, groundnut and bambara groundnut in the Sudano-Sahelian zone of Nigeria.

MATERIALS AND METHODS

A two-year field experiment was conducted during the 2007 and 2008 growing seasons at the Department of Soil Science Teaching and Research Farm, University of Maiduguri, Nigeria (11° 51^{1,} N,13° 15^{1} E). Maiduguri falls in Sudano-sahelian savanna agroecology with semi-arid climate. The experimental site received a total rainfall of 809mm during the first cropping season and 642mm in the second cropping season.

The treatments consisted of there grain legume varieties; cowpea (var, Bornoji - red); groundnut (var, kolji kanuri); bambara groundnut (var.Mallum Karekare); sorghum var. Paul Biya (as a reference crop) and three levels of B fertilizer (0, 0.5 and 1.0 KgB/ha). The treatments were arranged in randomized complete block design and replicated three times. A pre-plant soil sample of the site was taken and analyzed for physico-chemical properties according to Van Reeuwijt, [17]. The land was ploughed, harrowed and plots were laid out into plot sizes of 4 m long and 3 m wide. Three seeds were sown per hole according to BOSADP [18] recommended spacing. Seedlings were thinned down to two per hill one week after sowing. Boron was applied as boric acid by banding just after the emergence of the seedlings. Weeding was done with hoe. At 50 days after sowing, the crops were harvested from a 1 m^2 (quadrant) subplots. Post harvest soil samples were also taken from each plot and analyzed for total N as described by Van Reeuwijik [17]. Number of nodules was counted and the plant materials were oven dried at 65°C, weighed and analyzed for the N concentrations [19].

The amount of N fixed and percent N derived from biological fixation were estimated by the equations of Mary *et al.* [20].

Collected data were subjected to analysis of variance (ANOVA) and wherever differences between treatments were significant, these were compared using Duncan's multiple range test (DMRT) at 5% probability level [21]

RESULTS AND DISCUSSIONS

The soil of the experimental site is sandy loam in texture and neutral in reaction with low organic carbon (2% organic matter), total N (<1 g/kg soil) and available P (<10 mg/kg soil) according to FPDD [22] soil fertility ratings (Table 1).

The effects of boron on the nodulation and N_2-fixation of legumes crops in the first and second growing seasons are shown in Tables 2 and 3, respectively. There was significant ($P<0.05$) differences between the rates of boron in their effects on all the parameters measured, except soil available N. Application of 0.5 kg B/ha consistently gave the higher values than those of 1 kg B/ha fertilization, due to toxicity effect in both growing seasons indicating that the sufficiency range of the applied B nutrient might have been exceeded. The crops differed significantly ($P<0.05$) in their responses to the B fertilizer. Averaged over the two years (Table 4) the results of experiment showed that application of 0.5 kgB/ha improved the nodulation, N_2-fixation and N content of the legume crops significantly ($P<0.05$), but 1kgB/ha had detrimental effects on all the parameters tested in bambara groundnut and groundnut.

Table 1: Texture and some selected chemical characteristics of soil of the experimental site

Parameter	Values
Sand (g/kg)	690
Silt (g/kg)	155
Clay (g/kg)	155
Texture	Sandy loam
pH	6.70
Organic carbon (g/kg)	4.30
Total nitrogen (g/kg)	0.50
Available phosphorus (mg/kg)	5.35
*Available B	Traces

* Safyanu (2004).

Table 2: Effects of boron rates on nodulation, N content and nitrogen fixation by cowpea, groundnut and bambara groundnut, in the first cropping season.

	Rate (kg B/ha)			
Crops	0	0.5	1.0	Mean
Nodules/plant				
Cowpea	7.33	28.33	39.33	25.00^b
Groundnut	41.76	61.00	33.67	46.44^a
Bambara groundnut	20.67	38.67	6.00	21.78^b
Mean	23.22^b	42.67^a	27.33^b	
SE±	1.55 (rate)			
SE±	1.55 (crop)			
SE±	2.69 (interaction)			
Total N in plant (kg/ha)				
Cowpea	14.31	33.17	42.70	30.06^a
Groundnut	15.81	27.50	24.60	22.64^b
Bambara groundnut	14.32	30.68	9.92	18.31^c
Mean	14.81^c	30.45^a	25.74^b	
SE±	0.69 (rate)			
SE±	0.80 (crop)			
SE±	1.38 (interaction)			
N-fixed (kg/ha)				
Cowpea	10.08	27.94	33.83	23.95^a
Groundnut	11.58	25.61	15.73	17.64^b
Bambara groundnut	10.09	25.45	1.15	12.23^c
Mean	10.58^c	26.33^a	16.90^b	
SE±	0.86 (rate)			
SE±	0.86 (crop)			
SE±	1.48 (interaction)			
% NDFA				
Cowpea	69.94	83.98	79.21	77.71^a
Groundnut	72.82	80.82	63.86	72.50^b
Bambara groundnut	68.95	82.91	11.28	54.38^c
Mean	70.57^b	82.57^a	51.45^c	
SE±	1.49 (rate)			
SE±	1.49 (crop)			
SE±	2.58 (interaction)			
% Soil N				
Cowpea	0.047	0.065	0.051	0.054^a
Groundnut	0.056	0.075	0.047	0.059^a
Bambara groundnut	0.037	0.047	0.033	0.039^b
Mean	0.047^b	0.062^a	0.044^b	
SE±	0.0024 (rate)			
SE±	0.0027 (crop)			
SE±	0.005 (interaction)			

Means in columns and rows followed by similar letter(s) are not significantly different at the 5% probability level of the Duncan's Multiple Range Test (DMRT).

Table 3: Effects of boron rates on nodulation, N content and nitrogen fixation by cowpea, groundnut and bambara groundnut, in the second cropping season.

	Rate (kg B/ha)			
Crops	0	0.5	1.0	Mean
Nodules/plant				
Cowpea	11.33	31.00	27.00	23.11^b
Groundnut	48.00	70.00	40.67	52.89^a
Bambara groundnut	29.67	21.00	5.00	18.56^c
Mean	29.67^b	40.67^a	24.22^c	
SE±	1.12 (rate)			
SE±	1.12 (crop)			
SE±	1.93 (interaction)			
Total N in plant (kg/ha)				
Cowpea	31.80	44.82	50.04	42.22^b
Groundnut	39.71	80.91	47.59	56.07^a
Bambara groundnut	47.83	35.53	17.67	33.68^c
Mean	39.78^b	53.75^a	38.43^b	
SE±	1.59 (rate)			
SE±	1.84 (crop)			
SE±	3.19 (interaction)			
N-fixed (kg/ha)				
Cowpea	23.88	36.40	35.74	32.01^b
Groundnut	31.80	72.50	33.53	45.94^a
Bambara groundnut	39.91	27.12	3.36	23.46^c
Mean	31.86^b	45.34^a	22.21^c	
SE±	1.94 (rate)			
SE±	1.94 (crop)			
SE±	3.36 (interaction)			
% NDFA				
Cowpea	74.89	81.04	71.29	75.74^b
Groundnut	79.83	89.56	70.42	79.94^a
Bambara groundnut	83.46	75.72	19.31	59.50^c
Mean	79.39^a	82.11^a	53.67^b	
SE±	1.39 (rate)			
SE±	1.39 (crop)			
SE±	2.40 (interaction)			
% Soil N				
Cowpea	0.056	0.079	0.092	0.076^a
Groundnut	0.065	0.074	0.069	0.070^a
Bambara groundnut	0.068	0.048	0.044	0.053^b
Mean	0.063^a	0.067^a	0.068^a	
SE±	0.004 (rate)			
SE±	0.004 (crop)			
SE±	0.007 (interaction)			

Means in columns and rows followed by similar letter(s) are not significantly different at the 5% probability level of the Duncan's Multiple Range Test (DMRT)

Table 4: Effects of boron rates on nodulation and nitrogen content and fixation by cowpea, groundnut and bambara groundnut, combined for two seasons.

	Rate (kg B/ha)			
Crops	0	0.5	1.0	Mean
Nodules/plant				
Cowpea	9.33	29.67	33.17	24.06^{b}
Groundnut	44.83	65.50	38.67	49.67^{a}
Bambara groundnut	25.17	29.83	5.50	20.17^{b}
Mean	26.44^{b}	41.67^{a}	25.78^{b}	
SE±	2.17 (rate)			
SE±	2.17 (crop)			
SE±	3.77 (interaction)			
Total N in plant (kg/ha)				
Cowpea	23.05	39.00	46.37	36.14^{b}
Groundnut	27.76	54.21	36.10	39.36^{a}
Bambara groundnut	31.08	33.10	13.79	25.99^{c}
Mean	27.30^{c}	42.10^{a}	32.09^{b}	
SE±	0.86 (rate)			
SE±	0.99 (crop)			
SE±	1.22 (interaction)			
N-fixed (kg/ha)				
Cowpea	16.98	32.17	34.79	27.98^{b}
Groundnut	21.69	49.05	24.63	31.79^{a}
Bambara groundnut	25.00	26.28	2.26	17.85^{c}
Mean	21.22^{b}	35.84^{b}	20.56^{b}	
SE±	1.04 (rate)			
SE±	1.04 (crop)			
SE±	1.79 (interaction)			
% NDFA				
Cowpea	72.42	82.51	75.25	76.73^{a}
Groundnut	76.33	85.19	67.14	76.22^{a}
Bambara groundnut	76.21	79.31	15.30	56.94^{b}
Mean	74.98^{b}	82.34^{a}	52.56^{c}	
SE±	1.02 (rate)			
SE±	1.02 (crop)			
SE±	1.76 (interaction)			
% Soil N				
Cowpea	0.051	0.072	0.072	0.065^{a}
Groundnut	0.061	0.074	0.058	0.064^{a}
Bambara groundnut	0.053	0.047	0.038	0.044^{b}
Mean	0.055^{a}	0.065^{a}	0.056^{a}	
SE±	0.002 (rate)			
SE±	0.003(crop)			
SE±	0.006(interaction)			

Means in columns and rows followed by similar letter(s) are not significantly different at the 5% probability level of the Duncan's Multiple Range Test (DMRT)

This study therefore, showed groundnut and bambara groundnut are low B-requiring crops and prone to B toxicity. This finding is in agreement with those obtained by Havlin *et al.* [23] that groundnut is among the crops sensitive to B toxicity. Boron is essential for normal nodule development and functioning and translocation of sugars, N and P from the roots into the nodules.

Application of 0.5 kgB/ha enhanced nodulation in all the crops tested. The nodule number increased by 20, 21.and 5 per plant over the control in cowpea, groundnut and bambara groundnut, respectively. This agrees with Brady and Weil [1] who reported that B is essential for cell division in the process of nodule formation. Muofhe and Dakora [24]observed a marked increased in number of nodules in bambara groundnut plants as a result of boron application, However, in this study application of 1 kgB/ha suppressed nodulation in bambara groundnut and groundnut. This finding is in line with the report of Agbenin *et al.* [25] that application B at the rate of 1.5 - 3kg/ha depressed nodulation in cowpea in Guinea Savanna zone of Nigeria. This may be due to toxicity of B on peribacteriod and infection thread. According to Zehirov and Georgiev [26] boron deficiency can cause reduction in nodule number and increase in size of nodules. They also observed that B starvation inhibits the development of cell wall and changes cell permeability.

N content of the crops increased by 69, 95 and 6% over the control in cowpea, groundnut and bambara groundnut, respectively indicating that B may influence N nutrition. This result is in agreement with the reports of [27, 28] who observed a significant ($P<0.05$) increase in N content in beans as a result of B fertilization. In contrary, Agbenin *et al.* [25] reported that application of 1.5-3kgB/ha had no effect on N concentration of index leaves of cowpea in Guinea Savanna zone of Nigeria. In this study, the highest amount of N (39.36 kg/ha) was found in groundnut. Therefore, it could be inferred that boron exerted greatest influence on N nutrition in groundnut.

The study also showed that application of 0.5 kg B/ha increased the amount of N fixed by 69, 126 and 5% over the control in cowpea, groundnut and bambara groundnut, respectively, suggesting the role of B on nitrogenase activity. This concurs with the reports of Tipath *et al.* [29] and Zehirov and Georgiev [30] that B application improved nitrogenase activity in nodules of legumes. Since groundnut fixed the highest amount of N (31.79 kg/ha), it means the influence of B on nitrogenase activity is greatest in groundnut. The percentage of nitrogen derived from fixation also increased as a result of application of 0.5 kgB/ha.The percentage increases over the control were 10.09, 8.86 and 3.10 in cowpea, groundnut and bambara groundnut respectively. A similar observation was made by Rondon *et al.* [27] that a remarkable increase in the percentage nitrogen derived from fixation due to B fertilization, indicating the specific role of Boron in nodule functions. Bolanos *et al.* [31] reported that B deficiency leads to formation of abnormal nodules (containing no bacteriods in the infected area) and the phenolic content is reduced [24], which may lead to a reduction of nodulation performance and a low N_2-fixation rate.

The study also revealed that available N in the soil under B-treated crops increased greatly compared to that of control. The available N in the soil under cowpea (0.065%) was higher than that of groundnut (0.064%), but the difference was not significant. This indicated that B fertilization enhanced N_2 fixation and N secretion by legume crops, which in turn, improved the N status of the soil as observed by Rondon *et al.* [27]. This result therefore, demonstrates the potential of boron application to improve N input by grain legumes into agro-ecosystems.

CONCLUSION

In this study, application of 0.5kgB/ha significantly increased N_2-fixation in cowpea, groundnut and bambara groundnut. However, application of 1kgB/ha had detrimental effects on the crops. Groundnut fixed the highest amount of N (31.79 kg/ ha) and is followed by cowpea (27.98kgN/ha). Therefore, cultivation of groundnut variety (Kulji Kanuri) and application of 0.5 kgB/ha could improve the N status of the soil of Sudano-Sahelian region of Nigeria.

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