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Nodulation, Nitrogen Fixation and Productivity of Cowpea (*Vigna unguiculata*) Varieties as Influenced by Rhizobial Inoculation and Phosphorus Application on Farmers' Fields in Minna, Southern Guinea Savanna of Nigeria.

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Abstract
Cowpea is a very important crop in Nigeria but its yield on farmers' fields have remained very low due to poor soil fertility among other reasons. This study therefore aimed at exploiting varietal differences, rhizobial inoculation and phosphorus application as means of improving nitrogen (N) fixation and productivity of cowpea. The treatments consisted of three rhizobial inoculation levels (uninoculated, inoculated with either USDA 3384 or USDA 3451 rhizobial strain), three phosphorus rates (0, 20 and 40 kg P ha^{-1}) and three cowpea varieties (IT93K-452-1, IT99K-573-1-1, TVX3236) sown on three farmers' fields in Minna, southern Guinea Savanna of Nigeria. Each farmer's field served as a replicate. Data were collected on nodule number, nodule dry weight, total N in plant tissue, N-fixed, %N derived from the atmosphere (NDFA), shoot biomass yield and grain yield. The results showed that the highest and lowest values of all the parameters measured were obtained at 40 kg P ha^{-1} and 0 kg P ha^{-1} respectively. However, there was no significant difference (P>0.05) between the values obtained at 20 and 40 kg P ha^{-1} for most of the parameters measured. Though inoculated plants produced more nodules at lower phosphorus rate, they were not effective enough to fix higher nitrogen than the uninoculated plants. There was no significant difference between the inoculated and uninoculated plants in respect of nodule dry weight, total N in plant tissue and grain yield. Variety IT93K-452-1 recorded the highest tissue N-content and N-fixed; however, the highest grain yield was obtained in IT99K-573-1-1. It could be concluded from the results that the exotic rhizobial strains used in this study were not better than the indigenous rhizobial strains in fixing higher nitrogen and improving the productivity of the crop. Furthermore, application of 20 kg P ha^{-1}was sufficient for optimum performance of cowpea in the study area and variety IT99K-573-1-1 can be recommended for optimum grain yield.
Key Words: Rhizobial inoculation, Biological nitrogen fixation, Biomass yield, Grain yield, Cowpea varieties

Introduction

A major problem facing farmers in Nigeria is low soil fertility particularly low soil nitrogen (N) and phosphorus (P) which poses a serious threat to food security. The productivity of cowpea, an important food and feed crop that supplies 40% of the dietary protein requirement of the population in Nigeria is also limited by this factor (Kamai *et al*., 2014). This problem could be tackled using mineral fertilizers which however are not environmentally friendly and are often inaccessible to most resource-poor farmers. Exploiting biological nitrogen fixation (BNF) in cowpea appears to be a very viable option to improving crop productivity under the existing challenges of the farmers. BNF has been reported to contribute at least 70 million tonnes of nitrogen per year to the soil globally (Mabrouk and Belhadj, 2011) thereby reducing the need for mineral N fertilizer.

However, high phosphorus supply is needed for nodulation and biological nitrogen fixation in legumes (Elkoca *et al.*, 2007). Weisany *et al.* (2013) revealed that when legumes receive an inadequate supply of phosphorus they may suffer from nitrogen deficiency. Cowpea varieties differ in their ability to fix and utilize nitrogen under different soil P condition (Ankomah *et al.*, 1995). Ability of some rhizobial strains to nodulate their host under low soil P has also been documented (Leung and Bottomley, 1987). This could be a positive virtue especially in the tropics with inherent low soil P. Prior to this field trial, a glasshouse experiment was carried out on the need to inoculate cowpea in Nigeria savannas. The results (unpublished) confirmed that the growth of cowpea is limited by Nitrogen on Nigeria savanna soils hence, there is a nitrogen gap that needs to be bridged. This study therefore aimed at exploiting varietal differences, rhizobial inoculation and phosphorus application as means of improving nitrogen fixation and productivity of cowpea.
The hypotheses were:

i. P application has no effect on Nodulation, N-fixation, N content and yield of cowpea,
ii. rhizobial inoculation has no significant effect on Nodulation, N-fixation, N content and yield of cowpea,
iii. there is no significant difference in the varietal response of cowpea to rhizobial inoculation and P application.

Materials and Methods

The study was conducted on three farmers' fields (09^o 27.832' N 006^o 25.375' E, 09^o 31.203' N 006^o 27.678' E, 09^o 28.026' N 006^o 25.325' E) during the 2015 cropping season in Minna, Niger state, southern Guinea Savanna agro-ecological zone of Nigeria. It was a factorial combination of three levels of rhizobial inoculation (uninoculated and inoculated either with USDA 3384 or USDA 3451), three cowpea varieties (IT93K-452-1, IT99K-573-1-1, TVX-3236) and three P-rates (0, 20 and 40 kg P ha^{-1}) arranged in a Randomized Complete Block Design. Each farmer's field served as a replicate. On each farm, soil samples were collected from 12 points along diagonal transects. They were bulked and thoroughly mixed to obtain composite samples. The composite sample of each field was analysed for its physical and chemical properties following the procedures of Okalebo *et al.* (1993). The plot size was 4 m by 3.75 m and there were five ridges per plot, the three inner ridges served as the net plot from which data were collected. Rhizobial inoculation was done by applying the inoculants to the seeds at the rate of 5 g per kg seed using the slurry method which was done by first coating the seeds with a sticker (solution of 85 ml water and 15 g sugar) before applying the inoculant to the coated seeds (IITA and N2Africa**,** 2014).Three seeds were sown per hole on ridges at a spacing of 75 cm x 20 cm inter and intra row spacing respectively and the resultant seedlings were later thinned to two per stand at ten days after planting. The different rates of P fertilizer were applied at planting to the plots that received P treatment using single super-phosphate as the source. All the plots received a basal application of 40 kg K_2O ha^{-1} at planting using muriate of potash as the source.

Data collection

At 50% flowering, the shoot of plants within 1 m distance along the ridge in each plot were cut at the base, the roots carefully dug out with a spade and gently washed to recover the nodules. The nodules were then counted and oven dried at 70^o C and weighed on a Mettler balance. The shoot samples were also oven dried and the dry weight determined. The dried samples were then used for the determination of total N in shoot for N fixation estimation using the macro Kjeldahl's oxidation method involving digestion and distillation. N-difference method as descried by Yakubu *et al*. (2010) was used for estimation of N-fixed and maize was used as the reference crop. % N derived from atmosphere (NDFA) was calculated using the formula:

$$\text{Total N in plants} = \frac{\text{Dry matter weight x \%N in plants}}{100}$$

N fixed (NDFA) = Total N in legume-Total N in reference crop

$$\%\text{NDFA} = \frac{\text{Total N in legume} - \text{Total N in reference crop}}{\text{Total N in legume}} \times 100$$

(Yakubu *et al*., 2010)

Collected data were subjected to analysis of variance using statistical analysis system (SAS) and means separated using LSD (P=0.05).

Results

The result of the soil analysis of the fields is presented on Table 1. The soils are sandy loam, slightly acidic, generally very low in organic carbon, nitrogen and potassium, moderate in phosphorus, low in calcium, magnesium and sodium according to the ratings of Federal Fertilizer Department (2012).

The result of the effect of phosphorus application and rhizobial inoculation on nodulation, nitrogen fixation, tissue N-content, biomass and grain yield of selected cowpea varieties are presented on Table 2. The highest and lowest values of nodule number and weight, tissue N content, N-fixed, shoot biomass and grain yield were obtained at 40 kg P ha^{-1} and 0 kg P ha^{-1} respectively. There was however no significant difference between the values obtained at 20 and 40 kg P ha^{-1} for all the parameters except tissue N content. Application of 20 kg P ha^{-1} and 40 kg P ha^{-1}increased the grain yield by 48.7 % and 51.8 % respectively over that of control. Though plants that received 40 kg P ha^{-1} derived the highest % nitrogen from the atmosphere (NDFA), the value was not significantly different from those derived at other P levels.

Significantly higher nodule number was obtained in plants inoculated with USDA 3384 (*ca* 20) than the uninoculated plants (*ca* 15). However, the uninoculated plants had significantly higher biomass yield (20.78 g/plant) ($P<0.05$) than those inoculated with USDA 3451 (17.34 g/plant) but there was no significant difference between the biomass yield of plants inoculated with USDA 3384 rhizobium strain (19.01 g/plant) and the uninoculated plants. There were no significant difference between the inoculated and uninoculated plants in respect of nodule dry weight, tissue N-content, % NDFA, N-fixed and grain yield.

Variety IT99K-573-1-1 produced the heaviest nodules (0.121 g/plant), which was however at par with the value obtained in IT93K-452-1 (0.111 g/plant). The lightest nodules were obtained in TVX 3236 variety (0.086 g/plant). IT93K-452-1 plants produced significantly higher number of nodules (*ca* 22) compared to the other two varieties; the number (*ca* 12) recorded in IT99K-573-1-1 was significantly lower than those of the other varieties. Variety IT93K-452-1 fixed significantly higher nitrogen (0.51 g/plant) than IT99K-573-1-1 and TVX 3236 which fixed similar amount of N (0.38 and 0.32 g/plant respectively). The highest tissue N-content and biomass yield were recorded in variety IT93K-452-1 but the values were not significantly different from those obtained in IT99K-573-1-1. There was no significant difference between the varieties in respect of %NDFA ($p>0.05$).

The interaction effects between phosphorus and inoculation, phosphorus and variety, phosphorus and inoculation and variety were significant for nodule dry weight. Phosphorus and inoculation, phosphorus and variety interaction effects were significant in respect of nodule number per plant. Fig. 1 shows the interaction effect of phosphorus and inoculation on nodule dry weight. When the uninoculated plants were fertilized with 40 kg P ha-^{1} the highest nodule dry weight (0.208 g) was obtained but the value was statistically similar to the one recorded in plants inoculated with USDA 3384 fertilized with 20 kg ha^{-1} (0.200 g). Plants inoculated with USDA 3451 had the highest nodule dry weight at 40 kg P ha-^{1} (0.169g) which was similar to the 0.152 g obtained at 20 kg P ha^{-1}. The least values were obtained in unfertilized plants with or without inoculation.

Table 1. Soil physical and chemical properties of the experimental sites

Soil parameters	Farm 1	Farm 2	Farm 3
Particle size distribution (g kg $^{-1}$)			
Sand	743	704	758
Silt	105	115	88
Clay	152	181	154
Texture	Sandy loam	Sandy loam	Sandy loam
Chemical properties			
pH (H_2O)	5.99	6.52	6.50
Organic carbon (g kg $^{-1}$)	1.35	0.60	1.27
Total Nitrogen (g kg $^{-1}$)	0.15	0.18	0.10
Available phosphorus (mg kg $^{-1}$)	20.07	18.90	18.50
Exchangeable bases (cmol kg^{-1})			
Ca	0.65	0.85	0.70
Mg	0.95	0.90	1.80
Na	0.25	0.26	0.24
K	0.17	0.19	0.16
ECEC	2.02	2.20	2.90

Table 2. Effect of rhizobial inoculation and phosphorus application on nodulation, nitrogen fixation, tissue N-content and yield of some cowpea varieties

Treatments	Nodule dry weight (g/plant)	Nodule number per plant	Total N in tissue (g/plant)	%NDFA	N-fixed (g/plant)	Biomass yield (g/plant)	Grain yield (t/ha)
Phosphorus (P)							
Control	0.011	4.49	0.39	43.23	0.18	10.44	0.81
20 kg P ha^{-1}	0.148	23.21	0.88	48.72	0.46	22.52	1.58
40 kg P ha^{-1}	0.160	24.39	1.07	50.69	0.56	24.17	1.68
LSD(P=0.05)	0.023	4.52	0.14	NS	0.13	3.40	0.16
Inoculation (I)							
Uninoculated	0.101	15.06	0.81	46.86	0.43	20.78	1.36
USDA 3451	0.113	17.11	0.77	46.74	0.39	17.34	1.43
USDA 3384	0.105	19.92	0.76	49.03	0.38	19.01	1.28
LSD(P=0.05)	0.023	4.52	0.14	NS	0.13	3.40	0.16
Variety (V)							
IT93K-452-1	0.111	22.27	0.88	52.71	0.51	21.11	1.26
IT99K-573-1-1	0.121	11.77	0.75	45.44	0.38	18.24	1.61
TVX 3236	0.086	18.06	0.69	44.49	0.32	17.67	1.19
LSD(P=0.05)	0.023	4.52	0.14	NS	0.13	3.40	0.16
Interaction							
P x I	*	*	NS	NS	NS	NS	NS
I x V	NS	NS	NS	NS	NS	NS	NS
P x V	*	*	NS	NS	NS	NS	NS
P x I x V	*	NS	NS	NS	NS	NS	NS

LSD- least significant difference *-significant (P≤0.05), NS-Not significant

Fig. 2 shows the interaction effect of phosphorus and variety on nodule dry weight. IT93K-452-1 plants had the highest nodule dry weight (0.211 g) when fertilized with 40 kg P ha^{-1}. This was however not significantly different from the value obtained in IT99K-573-1-1 plants that received 20 P kg ha^{-1} (0.172) and closely followed by the value recorded in TVX 3236 plants that received 20 kg P ha^{-1} (0.156). The least nodule dry weight values (0.006-0.017 g/plant) were recorded at 0 kg P ha^{-1} in all the varieties. The phosphorus x inoculation x variety interaction effect on nodule dry weight also revealed that the uninoculated IT93K-452-1 plants that received 40 kg P ha^{-1} had the highest nodule dry weight (0.378 g/plants) which was followed by (but statistically higher than) the values obtained in IT99K-573-1-1 (0.23 g/plant) and TVX 3236 (0.22 g/plant) plants inoculated with USDA 3384 at 20 kg P ha^{-1} (data not shown).

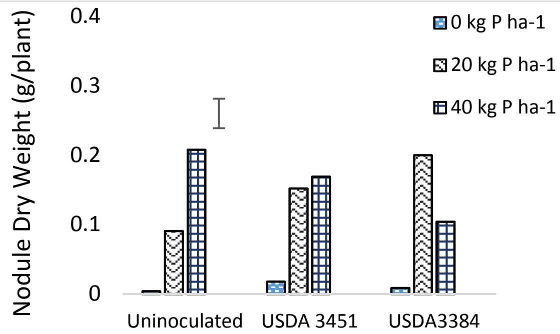

Fig. 1. Interaction effect of phosphorus and rhizobial inoculation on nodule dry weight

I-LSD (P=0.05)

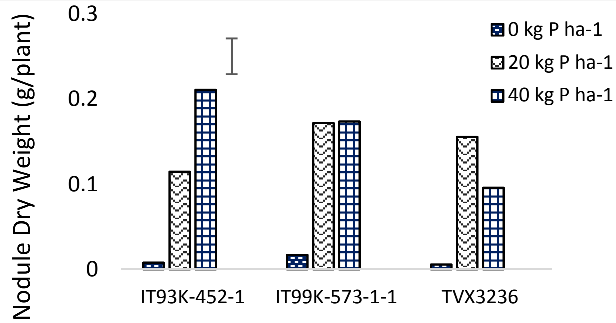

Fig. 2. Interaction effect of phosphorus and variety on nodule dry weight

I-LSD (P=0.05)

The interaction effect of phosphorus and inoculation on nodule number is presented on Fig. 3. The result revealed that plants inoculated with USDA 3384 produced the highest number of nodules at 20 kg P ha^{-1} (33.51). This was followed by the values obtained in uninoculated plants that received 40 kg P ha^{-1} (28.82). There was no significant difference between the number of nodules produced by USDA 3451 inoculated plants that received 20 and 40 kg P ha^{-1} (24.89 and 22.06 respectively). For the uninoculated plants, the highest number of nodules were recorded in plants that received 40 kg P ha^{-1} (28.82) and this was statistically higher than the value obtained in plants that received 20 kg P ha^{-1} (11.23). Nodule production increased as phosphorus rates increased in IT93K-452-1 variety. Application of 20 kg P $ha^{-1}$and 40 kg P ha^{-1} increased the number of nodules produced by 86.6% and 88.6 % respectively over the control plants. Though the values obtained in in the two rates were at par. The same trend was observed in IT99K-5731-1. However, increasing phosphorus rate up to 40 kg P ha^{-1} slightly reduced the nodule number produced in TVX 3236. The least number of nodules (3.86-5.73) were recorded in plants that did not receive phosphorus fertilizer (Fig. 4).

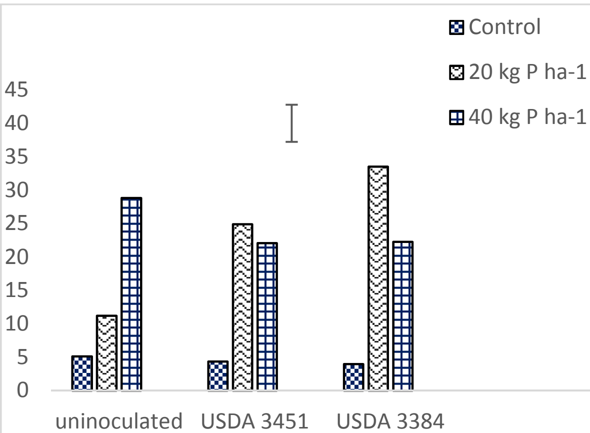

Fig. 3. Interation effect of phosphorus and rhizobial inoculation on nodule number

I-LSD (P=0.05)

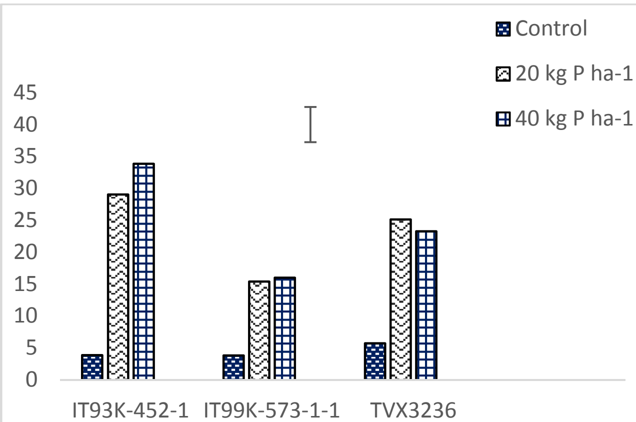

Fig. 4. Interaction effect of phosphorus and variety on nodule number

I-LSD (P=0.05)

Discussion

The higher values obtained for most of the parameters at 20 and 40 kg P ha-^{1} application compared to the unfertilized confirms the fact that adequate P nutrition is needed for legumes (Weisany *et al*., 2013). High phosphorus supply has been reported to be required for nodulation (Elkoca *et al*., 2007) as nodules themselves are strong sink for P. Weisany *et al.* (2013) reported that the phosphorus content per unit dry weight is considerably higher in the nodules than in the roots and biomass, particularly at low external phosphorus supply. Phosphorus is known to promote early root formation and the formation of lateral, fibrous and healthy roots, which play an important role in N_2 fixation, water and nutrient uptake (Niu *et al*., 2012). This explains the reason for the higher N content in the plant tissue at higher P application rate. Increase in grain yield by 49-52% as a result of phosphorus application further confirms the importance of phosphorus in legume productivity. Several workers have reported that P increased the grain yield of legumes. Amjad *et al.* (2004) reported that pod yield of peas increased with increasing phosphorus rates and the authors obtained the highest yield at 69 kg P_2O_5 ha^{-1}. Ndakidemi *et al.* (2006) reported that application of P to inoculated plants increased the grain yield of soybean and that application of 26 kg P ha^{-1} increased the profit margins by 84% - 102% in two districts of Tanzania. Magani and Kuchinda (2009) recorded an increase in the productivity of cowpea as a result of phosphorus application and therefore recommended the application of 37.5 kg P ha^{-1} in the northern guinea savanna of Nigeria. This is more than the optimum rate obtained in this study. The optimum phosphorus rate obtained in this study (20 kg P ha^{-1}) concur with the recommendation of Federal Fertilizer Department (2012) for cowpea in soils with medium P content. The grain yield obtained in this study (0.81 – 1.68 t/ha) is much higher than the average of 450 kg ha^{-1} obtained on farmers' fields (Omotosho, 2014). This could be as a result of better management practices and the cropping system used in this experiment. Most farmers in Nigeria don't plant cowpea as a major and sole crop. It's majorly planted as a minor crop in relay cropping system or as an intercrop. The cropping system adopted by the farmer and the management practices are contributory factors to the low yield obtained on farmer's field.

The higher nodulation recorded in inoculated plants at 20 kg P ha^{-1} suggests that the elite strains are more efficient P user compared to the indigenous strains that need higher P supply to nodulate. Leung and Bottomley (1987) reported that some rhizobial strains have relatively higher capacity to nodulate their host under low soil P. IT93K-452-1 variety nodulated better but appears to need higher quantity of P than the other two varieties for nodulation. Cowpea varieties exhibit variation in their ability to thrive under low soil P condition. Genotypic difference in the effect of P on nodulation was reported by Ankomah *et al.* (1995). The higher N-fixed in variety IT93K-452-1 than the other varieties confirms the fact that crop varieties vary in their genetic ability to fix nitrogen (Omondi *et al.,* 2014).

Cowpea varieties fixed about 50% of their total N requirement in this study. This is still far from the potential of 90% fixed N achievable in cowpea (Eaglesham *et al*., 1977). The exotic inoculant strains used appeared to be less effective than the indigenous rhizobial population which are likely to be more robust to the environmental conditions in the area. Successful competition for nodule sites by indigenous rhizobia is one of the reasons for the failure to achieve a response to inoculation with elite rhizobia strains (Theis *et al*., 1991). Very few positive responses to rhizobial inoculation have actually been recorded in cowpea probably because it is a very promiscuous legume (nodulate with a wide range of rhizobia present in most tropical soils).

Conclusion

Application of P significantly affected N-fixation, tissue N-content and biomass yield of cowpea and the varieties responded differently to rhizobial inoculation and P application. We therefore reject the 1st and 3rd null hypothesis. Furthermore, the result of the study showed that the inoculant strains used were less effective than the indigenous strains; we therefore accept the 2nd null hypothesis. This suggests the need to test more inoculants and to develop more effective inoculants from indigenous cowpea rhizobia which are more likely to fix higher N to boost crop productivity. While 20 kg P ha^{-1} appeared to be adequate for optimum performance of cowpea in the study area, IT93K-452-1 variety fixed higher amount of N and IT99K-573-1-1 had the highest grain yield.

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