N_2 Fixation by Soybean in the Nigerian Moist Savanna: Effects of Maturity Class and Phosphorus Fertilizer

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Summary

A study was carried out to determine the effect of P fertilizer and maturity class (days to maturity) on nodulation and N_2 fixation by soybean in the Nigerian moist savanna (NMS). One early (TGx1485-1D), one medium (TGx536-02D), and two late (TGx923-2E and TGx1670-1F) soybean varieties were sown at four sites in NMS with P application rates of 0, 30 and 60 kg.ha^{-1}. Nodulation increased with days to maturity and P rate. In late varieties, application of P substantially increased number of nodules.plant^{-1} by 84-134%, and nodule dry weight.plant^{-1} by 20-50%. Increasing P rate significantly increased specific nodulation (number of nodules per root dry weight) by 24-39% for all varieties but depressed nodule mass to whole plant mass ratio in medium and late varieties. Weight nodule^{-1} significantly reduced with increasing P rate and days to maturity class. Although nodule efficiency (g N fixed/nodule g weight) increased with P rate and days to maturity, only in medium (TGx536-02D) and late (TGx1670-1F) varieties was it significantly increased with P application. Over all varieties, P application significantly increased nodule efficiency by 6-10%. At harvest, N derived from the atmosphere was 61-83 kg.ha^{-1} in early and medium varieties, and 78-109 kg.ha^{-1} in late varieties. It increased significantly by 100-148% when P was applied. With an initial available soil P content < 7 mg.kg^{-1} at most sites, N_2 fixation increased in soybean with application of P and days to maturity.

Résumé

Fixation d'azote par le soja dans les savanes humides du Nigeria: effets de la période de maturité et de l'engrais phosphaté

Une étude a été conduite dans les savanes humides du Nigeria (SHN) pour déterminer l'effet de l'engrais phosphaté et de la classe de précocité (nombre de jours jusqu'à maturité) sur la nodulation et la fixation de N_2 par le soja. Quatre variétés de soja dont une à maturité précoce (TGx1485-1D), une à maturité moyenne (TGx536-02D), et deux à maturité tardive (TGx923-2E et TGx1670-1F) ont été semées dans quatre sites des SHN avec application de trois doses de P (0, 30 et 60 kg.ha^{-1}). Les résultats obtenus montrent que la nodulation a augmenté avec la durée du cycle de la plante et la dose de P appliquée. Pour les variétés tardives, l'application de P a contribué de 84-134% à l'augmentation du nombre de nodules et de 20-50% à celle du poids sec des nodules par plante. Une augmentation de la dose de P a accru de 24-39% la nodulation spécifique (nombre de nodules par poids sec des racines) pour toutes les variétés mais a déprimé le rapport de la masse des nodules sur la masse totale de la plante chez les variétés à maturités intermédiaire et tardive. Le poids des nodules a diminué significativement avec l'augmentation de la dose de P et de la durée du cycle de la culture. Bien que l'efficience des nodules (g N fixé/ g poids nodule) ait augmenté avec l'augmentation de la dose de P et du nombre de jours jusqu'à maturité, cette augmentation n'a été statistiquement significative que pour la variété à maturité moyenne (TGx536-02D) et la variété à maturité tardive (TGx1670-1F). L'application de P a augmenté significativement de 6-10% l'efficience des nodules. A la récolte, la fixation de N atmosphérique était de 61-83 kg.ha^{-1} pour les variétés précoce et moyenne alors qu'elle était de 78-109 kg.ha^{-1} pour les variétés tardives. Avec l'application de P, la fixation a augmenté de 100-148%. Avec une teneur initiale en P disponible dans le sol < à 7 mg.kg^{-1} dans la plupart des sites, la fixation de N_2 a augmenté dans le soja avec l'application de P et avec la durée du cycle de la plante.

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Introduction

Among all inputs applied in order to maximize agricultural output, nitrogenous fertilizers rank first (6). They make up 50% of all nutrient inputs (10). The efficiency of these fertilizers, however, is one of the lowest among plant nutrients. Nitrogenous fertilizers are subject to losses due to denitrification leading to release of nitrogen N_2 and NO_2 gases, volatilization of ammonia, run-off and leaching which can contaminate surface and underground water.

Under tropical conditions, the efficiency of applied nutrient has been estimated to be less than 50% N, 10-30% for P and about 40% for K (4). For instance, Subbiah *et al*. (33) reported that when 120 kg. $N.ha^{-1}$ (contained in N fertilizer compounds) was applied to maize in a single dressing, only 21% could be recovered from soils. High rates of N fertilization especially when using ammonium sulphate have also been shown to acidify soils (2, 20, 28, 32).

In addition to environmental problems, farmers in the Nigerian moist savanna (NMS) have to contend with the rising costs of N fertilizer, the availability of which is often erratic. Therefore, technologies that will reduce N fertilizer input by resource-poor farmers in the NMS are urgently needed. Nitrogen input through biological N_2 fixation (BNF) by grain legumes can help to maintain soil N reserves as well as substitute for N fertilizer requirement for large crop yields (26). Although soybean is a relatively new crop to the moist savanna ecological zone of Nigeria, its cultivation either in rotation or intercrop with cereals in NMS zone is increasing. After soybean grain harvest, N contents of root and harvest residues are made available to the soil through decomposition.

The amount of N derived from the atmosphere increases with days to maturity (crop duration) in grain legumes (15, 26). This is because increased crop duration in the field means a longer period of nodule activity. Moist savanna soils, however, have characteristically low available P making application of P through fertilizers necessary (9). Apart from P deficiency limiting plant growth, it can also limit symbiotic N_2 fixation as the latter has been noted to have a higher P requirement for optimal functioning than either plant growth or nitrate assimilation. If this P constraint is overcome, grain legumes in the cereal-based cropping system of NMS should be able to fix a greater amount of N_2. Consequently, greater N input through N_2 fixation will be possible in this type of cropping system. This study was, therefore, carried out to determine the effect of P application in low P soils and of days to maturity in soybean on N_2 fixation and N nutrition benefits.

Material and methods

This study was carried out at Mokwa (9°18'N, 5°04'E), Gidan Waya (9°28'N, 8°22'E), Kasuwan Magani (10°24'N, 7°42'E), and Fashola (7°56'N, 3°45'E) in the moist savanna ecological zone of Nigeria. Moist savannas make up about 71% of the 730,000 km^2 occupied by savannas in Nigeria (19, 21). With a precipitation/evaporation ratio ranging between 0.40 - 0.10 (16), this ecological zone is well-suited to annual crops of medium duration such as groundnut, maize and soybean (19).

The study was laid out as a split-plot in randomized complete block with three replications. Main plot treatments were four soybean varieties, grouped into three maturity classes discriminated by days to maturity. They included one early (TGx1485-1D), one medium (TGx536-02D), and two late (TGx923-2E and TGx1670-1F) varieties. They were obtained from Crop Improvement Division (CID) of the International Institute of Tropical Agriculture (IITA), Ibadan in Nigeria. The characteristics (according to CID) of the soybean varieties used are presented in table 1.

Table 1
Key characteristics of soybean varieties used in the study

Varieties	Days to maturity	Maturity class
TGx1485-1D	95	Early
TGx536-02D	100	Medium
TGx923-2E	115-120	Late
TGx1670-1F	115-120	Late

Source: Crop Improvement Division (CID) of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Most varieties of soybean that IITA has released in Nigeria (including the ones used in this study) are able to nodulate freely in farmers' fields (29). Sub-plot treatments were three P rates of 0, 30 and 60 kg. $P.ha^{-1}$ applied as triple superphosphate (TSP). Each sub-plot measured 5 x 4.5 m^2. Before sowing, TSP was applied along the sowing rows and mixed with the soil by hand. Soybean seeds were drilled in rows 5 m long and 75 cm apart. Three weeks after sowing, soybean plants were thinned to obtain a within row spacing of 8 cm. Rice was grown (at the three P rates) as a control crop to estimate the amount of N derived from the atmosphere (Ndfa) using the N-difference method. In this method, Ndfa is determined by subtracting the amount of N accumulated at harvest (with or without P fertilizer application) in the non-fixing crop (rice in this case) from that of the fixing crop (soybean) (27). The proportion of N derived from the atmosphere was calculated as the amount of N derived from the atmosphere in soybean as a percentage of total N in soybean. Total N comprised the N contained in standing biomass at harvest and fallen litter.

Although differences may occur in the capacities of the fixing and non-fixing plants to use soil N, it has been reported that with low soil N and control plant accumulating much less N than the legume, error due to plant type will be minimized (27).

The amount of rainfall during the growing season ranged from 623 to 1,089 mm with experimental sites. Doorenbos and Pruit (11) have reported an optimum water requirement in the range of 450-825 mm for soybean. Twenty soil core samples collected from 0-20 cm depth were bulked for each site, air-dried and crushed. Samples were sieved through 2 mm and 0.05 mm meshes for determination of particle size, total nitrogen (N), % organic carbon, soil organic matter, and available P. Soil samples were subjected to Kjeldahl digestion at 360 °C for 2 hours with lithium sulphate, 30% hydrogen peroxide and concentrated H_2SO_4 in the presence of selenium as catalyst in a block digestor. Total N was determined from the digest by steam distillation with excess NaOH. NH_4^+ collected in the distillate was neutralised by titrating with HCl. Available P was also determined from the extract using Bray II method. Percentage organic carbon was determined by oxidizing soil sample with $K_2Cr_2O_7$ solution and H_2SO_4 at 150 °C for 30 minutes. This solution was titrated with ferrous ammonium sulphate solution after cooling. The various procedures followed for soil analysis were as outlined by Okalebo *et al.* (25). Organic matter content was obtained by multiplying % organic C with 1.729. Soil particle size was determined by the pipette method (13). Soil pH was measured by a pH meter using a soil to water ratio of 1:1.

Soil sample analysis showed that total soil N was low at all sites and averaged 0.8 $g.kg^{-1}$ (Table 2).

Low total soil N level has been reported to be favourable to nodulation and atmospheric N fixation (5). Consequently, the nodulating soybean varieties used in the experiment were expected to be able to derive more N from the atmosphere. Table 2 shows that initial available soil P averaged 8.3 $mg.kg^{-1}$ for all experimental sites. According to soil fertility status elaborated for Nigerian soils (12), soil P status was high (16.2 $mg.kg^{-1}$) at Mokwa, but low (<7 $mg.kg^{-1}$) at other sites.

At podding stage which was about 54 days after planting (DAP) in the early and medium length varieties and 75 DAP in the late varieties, five soybean plants randomly selected within the five central rows of each plot were sampled for nodulation. Soil around the roots of individual plant to be sampled was loosened using a fork to a depth of about 20 cm. Each plant was then carefully pulled up. All nodules were separated from roots, counted, oven-dried at 65 °C for 48 hours and weighed. Specific nodulation at podding was calculated as number of nodules per of root dry weight while nodule efficiency was calculated as Ndfa per nodule g weight (g N fixed/g nodule) (methods explained above).

At final harvest in soybean (about 100 DAP in early and medium, and 124 DAP in late varieties) respectively, standing plants within three central rows of each plot were cut at the base just above the ground. Because all leaves had fallen from soybean plants at time of harvest, leaf litter including petiole within harvest area was collected using a 0.5 x 0.5 m^2 quadrat. Rice plants were also cut above the soil surface within a harvest area of 1 x 1.5 m^2. Harvest biomass of soybean and rice, and soybean litter samples were oven-dried at 65 °C for 48 hours and milled to pass through a 1 mm sieve. Nitrogen contents of the various plant samples were determined by digestion with conc. H_2SO_4 and subsequent steam distillation with excess NaOH before the distillate was neutralized by titrating with HCl (25).

Analysis of variance and means separation were conducted using the General Linear Model of Statistical Analysis System Institute Inc. (31).

Results

Nodulation in soybean

The number of nodules per soybean plant determined by counting at 54 DAP was significantly ($p< 0.01$) affected by variety, P application and their interaction. Number of nodules per soybean plant increased in all varieties as P level increased. Table 3 shows that with increasing P application there were more nodules. $plant^{-1}$ in late soybean varieties than in early and medium varieties. Compared with no P treatment, 30 kg $P.ha^{-1}$ significantly increased number of nodules. $plant^{-1}$ by 53-68% in early and medium soyabean varieties, and by 82-88% in late maturing varieties. Relative to 30 kg $P.ha^{-1}$, the application of 60 kg $P.ha^{-1}$

Table 2
Soil chemical and physical properties from samples taken from 0-20 cm depth at the experimental sites

	Sites			
Properties	Mokwa	Fashola	Gidan Waya	Kasuwan Magani
pH in water (1:1)	6.1	6.1	4.9	5.6
Org. matter ($g.kg^{-1}$)	0.88	1.02	1.50	1.30
Total N ($g.kg^{-1}$)	0.60	0.69	1.13	0.78
Bray-II P ($mg.kg^{-1}$)	16.2	5.2	6.2	5.7
Sand ($g.kg^{-1}$)	770	860	650	590
Silt ($g.kg^{-1}$)	190	110	210	260
Clay ($g.kg^{-1}$)	40	30	140	150
Textural class	Loamy sand	Loamy sand	Sandy loam	Sandy loam

did not significantly increase number of nodules.plant^{-1} in the early and medium varieties. The additional 24-28% increase in number of nodules.plant^{-1} observed in late varieties was, however, significant. Number of nodules.plant^{-1} in late varieties was at least double that in the early and medium varieties. Over all varieties, application of P at the rate of 30 kg P.ha^{-1} significantly increased number of nodules.plant^{-1} by 75% while there was a further 21% increase with 60 kg P.ha^{-1} treatment.

The effects of variety, P application and their interaction were significant ($p < 0.01$) on nodule dry weight per soybean plant. Application of 30 kg P.ha^{-1} increased nodule dry weight.plant^{-1} by 12-13% in early and medium varieties, and 20-34% in late varieties (Table 3). Compared to the effect of 30 kg P.ha^{-1}, a significant increase in nodule dry weight.plant^{-1} was observed only in TGx923-2E (a late maturing variety) when 60 kg P.ha^{-1} was applied. At all P rates, nodule dry weight.plant^{-1} was largest in TGx923-2E.

Consequently, the significantly largest nodule dry weight.plant^{-1} was observed in this variety.

Table 3 also shows that over all varieties, the application of 30 and 60 kg P.ha^{-1} significantly increased nodule dry weight.plant^{-1} of soybean by 19 and 27%, respectively.

The ratio of nodule mass to whole plant mass was significantly ($p < 0.01$) affected by variety, P application and their interaction (Table 3). P application reduced this ratio in all varieties except in TGx1485-1D (the early variety). Increasing P application reduced nodule mass to whole plant mass ratio by 25% in TGx536-02D, 25% in TGx923-2E, and 36% in TGx1670-1F. When P was applied at 30 or 60 kg P.ha^{-1}, nodule mass to whole plant mass ratio was significantly reduced by at least 24%.

Weight.nodule^{-1} in soybean was significantly affected by variety ($p < 0.01$). Late soybean varieties had significantly lower weight.nodule^{-1} compared to early and medium varieties (Table 3). Weight.nodule^{-1} was highest at 27.1 mg.nodule^{-1} in the medium variety (TGx536-02D). It decreased significantly ($p < 0.01$) with P application. For all varieties, weight.nodule^{-1} was depressed by 34 and 44% when 30 and 60 kg

Table 3
Effect of soybean variety by P rate interaction on nodulation and nodule efficiency

	Variety				
P rate (kg.ha^{-1})	TGx1485-1D (Early)	TGx536-02D (Medium)	TGx923-2E (Late)	TGx1670-1F (Late)	Mean
	----------number of nodules per plant----------				
0	28	30	51	50	40
30	47	46	93	94	70
60	49	54	119	117	85
Mean	41	44	87	87	
	----------nodule dry weight per plant (g.plant^{-1}) ----------				
0	0.70	0.69	0.92	0.79	0.79
30	0.79	0.77	1.23	0.95	0.94
60	0.82	0.83	1.38	0.97	1.00
Mean	0.77	0.76	1.18	0.90	
	------------nodule mass: whole plant mass------------				
0	0.112	0.124	0.089	0.074	0.100
30	0.097	0.093	0.067	0.047	0.076
60	0.115	0.080	0.055	0.038	0.072
Mean	0.108	0.099	0.070	0.053	
	----------------weight per nodule (g.nodule^{-1})----------------				
0	29.8	37.1	21.2	19.0	26.8
30	18.1	25.6	14.1	12.7	17.6
60	18.8	18.8	12.3	10.7	15.1
Mean	22.2	27.1	15.8	14.1	
	------nodule efficiency (g N_2 fixed /g nodule weight)------				
0	0.27	0.23	0.37	0.42	0.32
30	0.29	0.27	0.38	0.58	0.38
60	0.25	0.33	0.42	0.66	0.42
Mean	0.27	0.28	0.39	0.55	

Standard error for number of nodule per plant: P rate= 2.6; Variety= 3.1; Variety*P rate= 5.3.
Standard error for nodule dry weight per plant: P rate= 0.014; Variety= 0.016; Variety*P rate= 0.027.
Standard error for nodule mass:whole plant mass: P rate= 0.0035; Variety= 0.0041; Variety*P rate= 0.0067.
Standard error for weight per nodule: P rate= 1.73; Variety= 1.99; Variety*P rate= 3.45.
Standard error for nodule efficiency: P rate= 0.014; Variety= 0.017; Variety*P rate= 0.027.

P.ha^{-1} were applied, respectively. Although results show that increasing P rate depresses weight.nodule^{-1} in the varieties studied, ANOVA showed no significant variety by P rate interaction effect on this parameter. Specific nodulation (number of nodules per dry weight of root) in soybean was significantly ($p < 0.05$) increased by 24% when 30 kg P.ha^{-1} was applied (Figure 1). A further 15% increase with another 30 kg P.ha^{-1} increment was, however, not significant. Variety and variety by P rate interaction had no significant effect on specific nodulation in soybean.

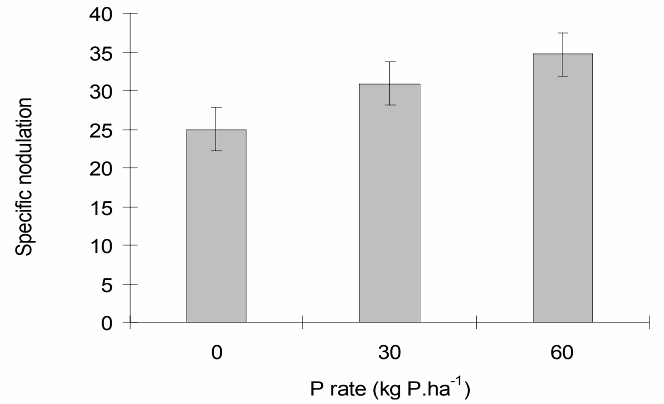

Figure 1: Effect of P fertilizer on specific nodulation in soybean.

Amount of N_2 derived from the atmosphere

The efficiency with which atmospheric N was fixed in the nodules, determined as the amount of N fixed divided by weight of nodules (g N fixed/g nodules), was significantly (p< 0.01) affected by variety, P application and their interaction. The significant variety by P rate interaction effect on nodule efficiency resulted from the responses of medium (TGx536-02D) and late (TGx1670-1F) varieties and no response in the early variety (Table 3). Starting from 0.23 g.g^{-1} without P applied, nodule efficiency was significantly increased by 43% in TGx536-02D when 60 kg P.ha^{-1} was applied. In TGx1670-1F on the other hand, the 38% and 57% increases in nodule efficiency due to 30 and 60 kg P.ha^{-1}, respectively, were significant. At each P rate, nodule efficiency was significantly higher in the late maturing varieties. Relative to when no P was applied, efficiency of N_2 fixation in nodules was significantly increased by 18% with 30 kg P.ha^{-1} and 31% with 60 kg P.ha^{-1}. Table 3 also shows that averaged over all P rates, root nodules in late varieties were significantly more efficient in fixing N_2 compared to early and medium varieties. Nodule efficiency was significantly higher in the late variety, TGx1670-1F (0.55 g.g^{-1}). This was at least double nodule efficiency in early and medium varieties.

The amount of nitrogen derived from the atmosphere (Ndfa) in soybean was significantly affected by variety (p< 0.01). Results presented in figure 2 show that longer cycle in soybean varieties significantly increased the amount of Ndfa. Among varieties tested, TGx1485-1D (the early maturing variety) had the significantly lowest

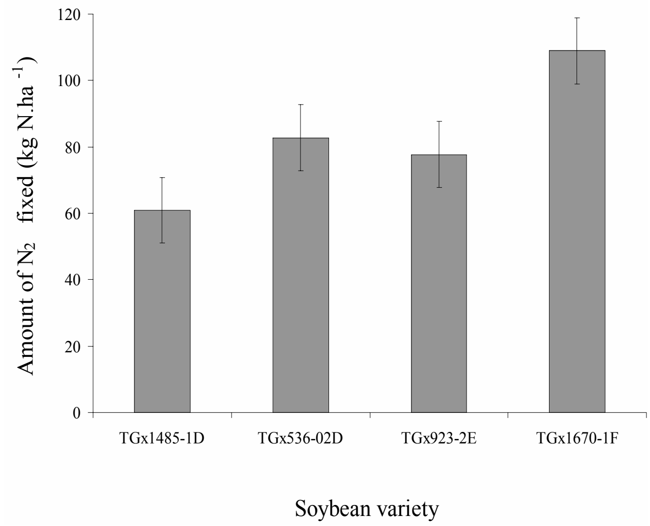

Figure 2: Effect of variety on the amount of N_2 fixed at final harvest in soybean.

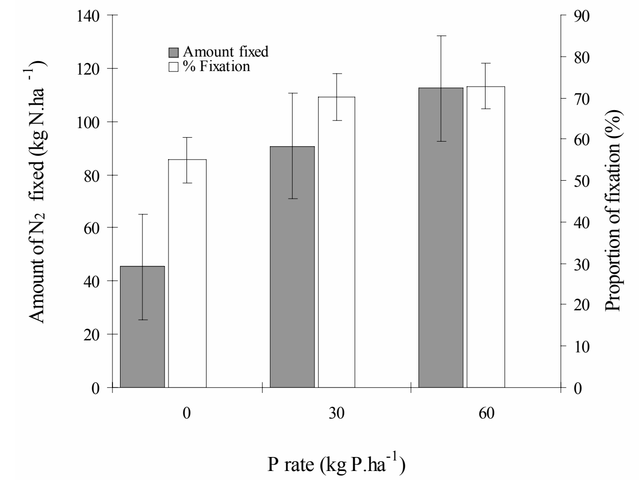

Figure 3: Effect of P fertilizer on the amount and proportion of N_2 fixed at final harvest in soybean.

amount of Ndfa (60.9 kg N.ha^{-1}), while the significantly largest amount of Ndfa (108.9 kg N.ha^{-1}) was in one of the late varieties (TGx1670-1F). Also, application of P at the rates of 30 and 60 kg.ha^{-1}, significantly (p< 0.01) increased Ndfa in soybean by 100 and 148%, respectively (Figure 3). No significant effect of variety was observed on the proportion of N_2 derived from the atmosphere (pfix). However, relative to when no P was applied, 30 and 60 kg P. ha^{-1} treatments significantly (p< 0.01) increased pfix in soybean by 15 and 17% respectively (Figure 3). The effect of variety by P rate interaction on Ndfa or pfix was not significant in this study.

Discussion

Results from this study showed that longer duration to maturity in varieties and P application significantly increased the number of nodules.plant^{-1} in soybean but depressed weight.nodule^{-1}. Visual observations also showed that with increasing number of nodules. plant^{-1}, nodules became smaller in size and weighed less, presumably because of competition for photosynthate. Nodules have also been reported by other authors (15) to be smaller such that nodule mass.plant^{-1} is reduced at intermediate nitrate concentrations. Reduction in mean nodule size observed in this study was associated with significantly higher nodule efficiency (expressed as g N fixed/g nodules) with significant reductions in weight.nodule^{-1}, suggesting better rhizobial activity.

In agreement with earlier reports (17, 18), increasing days to maturity and P rate increased nodule dry weight but reduced nodule mass to whole plant mass ratio. There was greater proportional increase in total plant dry weight arising from increased vegetative growth. Enhanced growth with increased nodulation suggests that nodules with rhizobia in them had a positive effect on soybean N nutrition. The non-significant effect of maturity class on specific nodulation seems to suggest that irrespective of variety, there is a maximum nodulation capacity per root dry weight of soybean plants. Of course, the number of nodules present is dependent on the amount of assimilate available to them. Carrol and Mathews (7) have inferred that early infection by rhizobia in legume roots results in a signal that is translocated to the root and thus suppresses further development of nodules. In this phenomenon of autoregulation, a legume root system will only develop a certain number of active nodules, beyond which no further new infection occur (15). However, application of P increased number of nodules more than soybean root dry weight, showing the importance of P in increasing specific nodulation. Application of P when available soil P is low has similarly been shown to increase number and dry weight of nodules (23, 30).

The effect of days to maturity in soybean was evident on the amount of N_2 fixed as late varieties (TGx1670-1F and TGx923-2E) had more Ndfa than early (TGx1485-1D) and medium (TGx536-02D) varieties. N_2 fixation has equally been reported to increase with increasing crop duration (1, 14). This is expected because longer growth duration allows for a longer period of N_2 fixation in the nodules. While soybean could fix 15-162 kg N.ha^{-1} (22), an estimate of 26-188 kg N.ha^{-1} in the tropics has been made (15). In the moist savanna environments of Nigeria, Ndfa was in the range of 61 to 109 kg N.ha^{-1} in the soybean varieties studied. On the average, soybean Ndfa in this study was 66% of the total aboveground N against the 70-90% from previous estimates (15).

Available soil P at the study sites averaged less than 10.5 mg.kg^{-1} which Aune and Lal (3) noted as being the critical value for grain legumes. As a result, the application of P positively increased all nodulation parameters, and ultimately increased the amount of Ndfa in soybean. Cassman *et al*. (8) have similarly reported the enhancement of nodulation and N_2 fixation in soybean when P is applied. Higher Ndfa resulting from longer days to maturity and higher P rate is attributable to higher yield potential and higher levels of nodule efficiency.

Since late maturing soybean varieties were able to fix more N_2 than early and medium varieties, greater N contribution to any cropping system is expected through their roots, litter and harvest residues. A positive N balance by soybean crop in NMS has thus been reported elsewhere due to the effect of increased crop duration and P application (24). Late maturing soybean varieties are, therefore, able to give higher N benefit compared to early and medium varieties for the improvement of the cropping systems of the moist savanna. This is apart from the possible higher yield resulting from longer duration. The positive effect of P application on most parameters underline the importance of P fertilization under low available soil P conditions. Results, however, show that for most parameters the effects of 30 and 60 kg P.ha^{-1} treatments were not significantly different. Post experiment soil analyses (data not shown) indicated that P rate of 30 kg.ha^{-1} gave rise to available soil P build-up above the reported critical level (3). This rate of P application appears sufficient under the low soil available P conditions of the Nigerian moist savanna.

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