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Response of Soybean (*Glycine max* (L.) Merrill) to Bradyrhizobia Inoculation and Phosphorus Application

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ABSTRACT

For many tropical countries, particularly in Africa, biological nitrogen (N) fixation continues to be an alternative or supplement to the use of chemical N fertilizers for sustainable agriculture. Two field experiments were established to determine the effectiveness of introduced bradyrhizobia on nodulation, growth and yield of a promiscuous and a non-promiscuous soybean variety under varying levels of phosphate fertilizer (0, 30 and 60 kg ha^{-1}). Bradyrhizobia inoculation had significant ($p<0.01$) effects on nodulation, dry matter, total N and seed yield of both varieties, being more pronounced in the case of the promiscuous variety than the non-promiscuous variety. Under natural conditions, increased application of phosphorus had quite prominent effects on nodulation and other growth and yield parameters of 'Bengbie' (promiscuous variety) but it was almost the reverse in the case of the other variety, 'Bragg' (non-promiscuous), where only seed yield was increased. P application at low dose (30 kg ha^{-1}) coupled with inoculation with bradyrhizobia significantly favoured all the parameters studied, in the two varieties. Application of high P (60 kg ha^{-1}) under inoculated conditions proved beneficial only in the case of 'Bragg' and not 'Bengbie'. Inoculation of promiscuous soybean with effective bradyrhizobia may prove to be a better strategy for increasing soybean yields than addition of P fertilizer in West African soils.

Key Words: Bradyrhizobia; Phosphate fertilizer; Promiscuous nodulation; Soybean; West Africa

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) production requires good supply of nitrogen (N) for high seed yield. The crop, like many other annual legumes has the ability to meet most of its N requirement through inoculation with bradyrhizobia. Following infection of soybean by bradyrhizobia to form nodules, N from the air is converted into a form readily available to the plant. The International Institute of Tropical Agriculture (IITA) developed promiscuous soybean varieties, which are capable of establishing symbiotic relationship with indigenous bradyrhizobia, as a practical alternative to inoculation by African farmers (Dashiell *et al*., 1983). Recent studies with these promiscuous soybean varieties, however, have shown considerable variability in the effectiveness and population of communities of indigenous bradyrhizobia in a given location (Sanginga *et al.*, 1999; Fening & Danso, 2002). Sanginga *et al.* (1995) also found a direct relationship between bradyrhizobia cell counts and promiscuous soybean response. Thus, promiscuous soybean may also need inoculation with exotic bradyrhizobia depending on effectiveness and population of indigenous bradyrhizobia in the locality (Okereke *et al.*, 2000), as well as the degree of promiscuity of variety (Sanginga *et al.*, 1999).

Several studies have also reported that soils deficient in phosphorus (P) limit the extent of nodulation, N_2 fixation and seed yield of legume crops (Pereira & Bliss, 1989; Israel, 1993; Ankomah *et al*., 1995). Phosphorus, apart from its effect on the nodulation process and plant growth, has been found to exert some direct effects on soil rhizobia (Singleton *et al.*, 1992).

Since P deficiency is common in most West African soils (Adetunji, 1995), present studies were initiated to determine the effectiveness of introduced bradyrhizobia on nodulation, growth and seed yield of promiscuous and non-promiscuous soybean varieties under varying levels of soil P.

MATERIALS AND METHODS

Two experiments were conducted at the University of Ghana Farm, Legon, in the major growing seasons (April–July) during 1999 and 2000. Land preparation was attained by double ploughing, followed by harrowing. Phosphorus (P) as single super phosphate was applied in the rows, two days before planting. Planting was done after two successive rains in mid-April in both years. Both fields had previously been cropped to maize (*Zea mays*). The soil at the experimental locations belonged to the Adenta series, a savanna Acrisol (FAO, 1990). Soil samples collected from 0-20 cm depth had pH (H_2O) 5.4, 0.08% total N, 0.6% organic matter and 6.9 ppm available P.

Two varieties of soybean namely 'Bengbie', a promiscuous variety (100-seed weight of 10 g and maturing in 120 days) and 'Bragg', a non-promiscuous variety (100-

seed weight of 16 g and maturing in 100 days) were used. Seeds were first coated with gum-Arabic as a sticker and then a peat-based inoculant containing *Bradyrhizobium japonicum* strain TAL 102 was applied. Four-row plots were used with 60 cm between rows and 5cm between plants in a row. Each row was 6 m long. There were 12 treatment combinations consisting of a factorial arrangement of two varieties, three P rates and inoculation versus no inoculation. Thus each variety had the following treatments: 1) Control (uninoculated, no P fertilizer); 2) uninoculated + 30 kg P ha^{-1}; 3) uninoculated + 60 kg P ha^{-1}; 4) inoculated (no P fertilizer); 5) inoculated + 30 kg P ha^{-1}; 6) inoculated + 60 kg P ha^{-1}.

A split-plot design was used with the four Variety-Inoculation status combinations as main plots and P rates as subplot factor. There were four replications of each three-way treatment combination. Both experiments were carried out at under rain-fed conditions. Manual weeding was done at two weeks after emergence and three weeks later.

At 48 days after planting (DAP), 10 randomly selected plants were harvested from the two middle rows for nodule count. Plants were dug out with a ball of earth and the soil carefully removed. Roots were carefully washed; nodules were removed, counted and then oven-dried for 72 h at 70°C and weighed. Plant shoots were cut into small pieces and also dried at 70°C to constant weight. The dried plant samples were milled and samples used for plant nitrogen and phosphorus determinations. N concentration was determined by the Kjeldahl procedure and P concentration by nitro-perchloric digestion and molybdenum-ascorbic acid dosage method. Seed yield was taken from an area of 4.8 m^2 from the centers of the two middle rows of each plot. Pods were removed and threshed by hand. Seed yield was determined at 10% moisture content.

Analysis of variance (ANOVA) was carried out on data combined from the two studies using Genstat statistical software version 6.1 (GenStat, 2000). Significant differences were assessed at 5% level. Mean separation was carried out by least significant difference (Lsd) procedure. Square root transformation of data on nodule counts was carried out prior to ANOVA.

RESULTS

There were significant ($p<0.05$) interactions between soybean variety and inoculation status for all traits scored, indicating that differences between the two varieties were dependent upon whether the plants were inoculated or not. Nodule number and dry weight for 'Bengbie', the promiscuous variety, increased five-fold and four-fold respectively with inoculation (Table I). For 'Bragg', the non-promiscuous variety, the corresponding increases were 25-fold and 11-fold, respectively. Without inoculation, 'Bengbie' produced about three times as many nodules as 'Bragg', but when inoculated, 'Bengbie' produced only 69% of the nodule production of 'Bragg'. The number of nodules and nodule dry weight appeared to have reached their maximum at 30 kg P ha^{-1} in 'Bengbie', but both traits increased as rate of applied P was increased for inoculated 'Bragg'

Accumulated phosphorus (P) of inoculated plants was significantly ($p<0.01$) higher than uninoculated plants at all P levels (Table II). Phosphorus uptake per plant was on the average significantly ($p<0.01$) greater in 'Benbgie' than in 'Bragg' at all P levels without inoculation. However, the difference in P uptake between the two varieties was not significant under bradyrhizobia inoculation. Phosphorus additions significantly ($p<0.01$) increased P uptake in 'Bengbie' irrespective of inoculation status, but in 'Bragg' such increase was so only under inoculation.

Response of total nitrogen (N) to inoculation was similar to that of P, with the two soybean varieties being significantly different only when the plants were not inoculated (Table II). Total N in "Bengbie' was significantly ($p<0.01$) higher than in 'Bragg' except at the highest level of applied P.

Growth and yield response of the two soybean varieties to inoculation was significant ($p<0.01$), but were independent of applied P level (Table III). Shoot dry weight of plants at 48 DAP was significantly ($P<0.01$) greater in 'Bengbie' than in 'Bragg' without inoculation, but the two varieties produced similar shoot dry matter yields with inoculation. Phosphorus addition did not significantly affect shoot dry matter production of the two varieties, irrespective of inoculation status.

Inoculation led to a significant ($p<0.01$) increase in seed yield at all P levels in both varieties (Table III). The difference in seed yield between the two varieties under uninoculated conditions was significant ($P<0.001$) only when there was no P addition. For inoculated plants, however, differences in seed yield between 'Bengbie' and 'Bragg' were significant ($p<0.01$) at all P levels.

DISCUSSION

There were high increases in nodulation after inoculation for both the promiscuous ('Bengbie') and non-promiscuous ('Bragg') varieties. Nodulation of 'Bengbie' by indigenous bradyrhizobia was rather low and could not be supportive of high yield. Similar findings have been reported for promiscuous soybean grown in the moist savanna of West Africa (Okereke *et al.,* 2000). Kumaga and Etu-Bonde (2000), from pot studies, demonstrated that nodulation and N_2 fixation of promiscuous soybean may be increased by inoculation with effective bradyzhizobia. The current results on nodulation agree with the findings of Fening and Danso (2002) that bradyrhizobia numbers and effectiveness vary considerably among locations. Sanginga *et al.* (1999) found that need for inoculation for some elite promiscuous soybean breeding lines depended on effectiveness of indigenous bradyrhizobia in a given locality. Besides, the fields used in the present study had

Table I. Effects of bradyrhizobia inoculation and phosphorus application on nodulation of two soybean varieties at 48 DAP

Inoculation status	Variety	Nodules plant^{-1}				Nodule dry weight (mg plant^{-1})			
		$^{a}P_0$	P_{30}	P_{60}	Mean	P_0	P_{30}	P_{60}	Mean
Uninoculated	Bengbie	5	8	4	5.7	45	75	34	52.7
	Bragg	2	2	1	1.7	35	27	17	26.3
	Mean	3.5	5.0	2.5	-	40.0	53.0	25.5	-
Inoculated	Bengbie	27	35	28	30.0	184	273	212	223.0
	Bragg	37	40	53	43.3	211	320	383	304.5
	Mean	32.0	37.5	40.5	-	197.5	296.5	297.5	-

DAP: days after planting; ^{a}Phosphorus level (kg P ha^{-1}); Lsd $_{p=0.01}$ (Nodules plant^{-1}): Inoculation status =5.0; Inoculation x Variety = 7.1; Lsd $_{p=0.01}$ (Nodule dry weight): Inoculation status = 65; Inoculation X Variety = 92

Table II. Effects of bradyrhizobia inoculation and phosphorus application on plant total nitrogen (N) and phosphorus (P) at 48 DAP

Inoculation status	Variety	Total N (mg plant^{-1})				Total P (mg plant^{-1})			
		$^{a}P_0$	P_{30}	P_{60}	Mean	P_0	P_{30}	P_{60}	Mean
Uninoculated	Bengbie	131	160	189	160	27	34	42	34
	Bragg	76	76	51	68	19	15	13	16
	Mean	104	118	120	-	23	25	28	-
Inoculated	Bengbie	224	258	228	237	39	51	53	48
	Bragg	182	231	290	234	37	39	55	44
	Mean	203	245	259	-	38	45	54	-

DAP: days after planting; ^{a}Phosphorus level (kg P ha^{-1}); Lsd $_{p=0.01}$ (Total N): Variety =27; Inoculation status = 27; Inoculation x Variety =38; Lsd $_{p=0.05}$: (Total P) P level = 8; Variety =7; Inoculation status = 6; Inoculation x Variety = 9

Table III. Effects of bradyrhizobia inoculation and phosphorus application on shoot dry weight at 48 DAP and seed yield at maturity

Inoculation status	Variety	Shoot dry weight (mg plant^{-1})				Seed yield (kg ha^{-1})			
		$^{a}P_0$	P_{30}	P_{60}	Mean	P_0	P_{30}	P_{60}	Mean
Uninoculated	Bengbie	3.48	4.35	4.95	4.26	241	281	303	275
	Bragg	2.06	1.97	1.97	2.00	285	319	271	292
	Mean	2.77	3.16	3.24	-	263	300	287	-
Inoculated	Bengbie	6.02	6.26	6.27	6.18	600	845	587	677
	Bragg	5.05	6.10	7.16	6.10	1100	1327	1379	1269
	Mean	5.54	6.18	6.93	-	850	1086	983	-

DAP: days after planting; ^{a}Phosphorus level (kg P ha^{-1}); Lsd $_{p=0.01}$ (Shoot dry weight): Inoculation status = 0.6; Variety = 0.6; Inoculation x Variety = 0.9; Lsd $_{p=0.05}$ (Seed yield): Variety =142; Inoculation status=152; Inoculation x Variety = 201

previously been cropped to maize, which might have reduced the indigenous bradyrhizobia population. While previous inoculation increased yield of both promiscuous and non-promiscuous soybean, numbers of indigenous bradyrhizobia were very low in soils previously cropped to maize (Sanginga *et al.,* 1995). Increased nodulation observed in the current study could be due to high competitive ability of inoculant bradyrhizobia used (Okereke *et al.,* 2000).

Although P additions resulted in high P uptake, except for uninoculated 'Bragg', this did not result in significant increases in shoot growth and seed yield. This is in contrast to the findings of Israel (1993), who observed increased whole plant growth of soybean in response to increased soil P supply. Working with rhizobia inoculation and P supply in leucaena (*Leucaena leucocephala*) and gliricidia (*Gliricidia sepium*), Sanginga (1992) reported that at low levels of soil P, the P requirement for symbiotic N_2 fixation appeared to be greater than for plant growth, depending upon plant genotype. In this study, increased nodulation corresponded with increased P uptake. Hence uninoculated 'Bragg' did not respond to varying P levels most likely due to very low nodulation activity.

Without inoculation, variety 'Bragg' had lower shoot dry weight and total N than variety 'Bengbie', but had a higher seed yield. This may be due to the dry spells towards the later stage of the experiment in both years (Sanginga, 1992). Variety 'Bragg' matured earlier and hence had most of its pods well filled compared to 'Bengbie'. The increase in seed yield under inoculation was highly significant, indicating the potential for increasing seed yield of both varieties through inoculation with effective and competitive foreign bradyrhizobia.

CONCLUSIONS

It is concluded that inoculation of promiscuous soybean with effective bradyzhizobia may be a more important strategy for increasing soybean yields than addition of phosphate fertilizers in the savanna Acrisol.

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