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Evaluation of Liming Materials and *Bradyrhizobium* Inoculation on the Productivity of Soya Bean in the Humid Tropical Ultisols of Southeastern Nigeria

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Abstract. Field experiments were conducted in 2009 and 2010 to evaluate the response of soya bean to pre-sowing seed inoculation with *Rhizobium japonicum* strains and liming the soil with different lime materials. The experiments were conducted on sandy loam soils at Calabar in the rainforest and Obubra in the southern guinea savanna zones of Cross River State, Nigeria. Treatments comprised factorial combinations of Rhizobium (with and without inoculation) and liming material (no lime, calcite, gypsum, and dolomite) laid in randomized complete block design replicated thrice. Liming the soil was effective in increasing root nodulation, plant vegetative growth and grain yield in Calabar, but not at Obubra. Pre-sowing seed treatment with *Rhizobium japonicum* was also more effective in Calabar than at Obubra. Root nodulation, plant height, pod-bearing branches, reproductive node number, dry matter yield, pods/plant and yield of grain were significantly influenced by the combined effect of inoculation and liming, but not the number of seeds and seed weight/pod. Sowing inoculated seeds in calcite- limed soils gave the best results but the difference between gypsum and dolomitic lime was not significant. The grain yield which averaged 2.34 t/ha in the Obubra grown crop was higher by 21.9 % compared to the Calabar grown crop. Differences in the response to seed inoculation and liming were attributed to weather and edaphic factors.

Key words: soya bean, seed inoculation, *Rhizobium japonicum,* liming material, acid soil, weather

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a rich source of plant protein (Bentley, 1975, Asiedu, 1989). Because it is one of the most efficient legumes for biological N fixation (Javaheri and Baudoin, 2003), it could be an important factor in the development of cropping systems keyed to limited inputs of fertilizer and other chemical agents. The cultivation of Soybean in Nigeria is concentrated mainly in the southern guinea ecological zone (Ashaye *et al.,* 1975), the main growing area being Benue state, followed by the Abuja area and Southern Zaria in Kaduna state. However, marginal but increasingly important areas of cultivation include the derived guinea savanna areas of Cross River State, and indeed the humid forest region, where Oko *et al.* (1991) reported successful late- season cropping.

For successful soybean cultivation, fields never planted to the crop or those with sandy soils need to be inoculated regularly with *Bradyrhizobium* (*Rhizobium japonicum*). But because landraces which may be compatible with the native strains of Rhizobium are seldom grown in this area, improved cultivars that are cultivated may require inoculation with the appropriate strain of Rhizobium. Moreover, if soils do not already contain a high population of *Rhizobium*, these bacteria can be added either as a liquid or granular peat inoculant, or as a peat-based powder. The different forms can be seed-applied or used in furrows. Furthermore, soils in this area have acid reaction (Akpan-Idiok and Esu, 2004) and would require liming for the attainment of sustainable high yields (Javaheri and Baudoin, 2001) for although Soybean cultivars differ in their tolerance to soil acidity and aluminum toxicity, they will never yield as much on acid soils as on soils without acidity constraints. There is an opportunity to increase the production of soya bean by better exploiting the colonization of the roots and rhizosphere through the application of bacterial fertilizer. This can minimize the use of nitrogenous fertilizer which is very expensive in Nigeria.

The aim of this field study was to investigate the effect of different liming materials and pre-sowing seed inoculation with *Rhizobium japonicum* on the productivity of Soya bean in a humid rainforest and southern savanna zones of Southeastern Nigeria.

MATERIAL AND METHODS

Field trials were conducted under rain-fed conditions during the late growing seasons of 2009 and 2010 at the University of Calabar Teaching and Research Farm and at the Cross River University of Technology Research Farm at Obubra. Calabar which is situated in the humid rainforest zone is co-ordinated by latitude 05^0 3' and 04^0 27'N and longitude $07^0$15' and 09^0 28' E, while Obubra situated in the southern guinea ecological zone is located at longitude 8^0 16'E and latitude 5^0 59'N with an altitude of 184 m above sea level. The land used for the study had been left fallow for about five years and was not known to have been cultivated to a legume crop for several years before then. A piece of farmland measuring 50m X 20 m was prepared manually with machete and spade and subsequently divided into three replications each containing 8 plots of 4.0 m X 4.0 m and with a boundary of 2.0 m separating them. A determinate, medium-maturing soya bean variety TGX 536-02D bred by the International Institute of Tropical Agriculture (IITA) was the test variety. This variety is the result of a back-cross between Bossier (ex USA) and the recurrent parent TGM 737P (ex Indonesia). It incorporates a good level of resistance to bacterial pustule and shattering and has a high capacity of promiscuous nodulation.

Seeds were washed with distilled water and surface sterilized with 70 % ethanol solution before inoculation with an improved strain of *Rhizobium japonicum* (Bradyrhizobium) in the form of Legumefix^R. The inoculant was mixed with water to form slurry which was mixed evenly without soaking through and the seeds sown on seed beds immediately after. The proportion of materials used was 25kg seed: 250ml water: 110g inoculant (Nieuwenhuis and Nieuwelink, 2005). Four seeds were sown per hole at a depth of 2-4 cm and at a spacing of 50 cm X 10 cm. Seedlings were thinned to two per stand at two weeks after sowing (WAS).

Treatments comprised factorial combinations of Rhizobium (with and without inoculation) and liming material (calcite limestone, dolomitic limestone, gypsum each applied at the rate of 3487.04 kg ha^{-1}) laid out in a randomized complete block design. The control treatment was the combination of no inoculant X no limestone. The amount of lime applied was determined based on exchangeable acidity, mass per 0.15 m furrow slice and bulk density of the soil. Basal fertilizer N was not applied as this is not beneficial to the crop in this area (Oko et al., 2003). The lime, P and K at the rate of 60 kg ha^{-1} were worked into the soil 3 weeks before sowing, while weeds were controlled by hand pulling and hoeing at 4 and 7 WAS.

Agronomic data was based on ten randomly selected plants per plot. Nodulation parameters (number of nodules $plant^{-1}$, nodule volume, nodule dry weight) were taken at 7 WAS with an active nodule being one with an internal pink colour. The number of pod bearing branches per plant, retained pods per plant; seeds per pod and seed weight were the average from each of the ten randomly selected plants. Pods per branch were obtained by dividing the total number of viable pods from the branches by the branch number. Pod measurements were obtained following the procedure cited in Board and Tan (1995). Total pod number was the product of maximal pods per reproductive node and reproductive node number. A reproductive node was one with at least a harvestable pod. Maximal pods per reproductive node were obtained two weeks after full blossoming. Final pods per reproductive node were those retained to maturity. A harvestable pod was one with at least one filled seed. Weight of seeds was determined by the division of total weight per plant by the number of seeds per plant. The determination of grain yield was as described by Akhter and Sneller (1996). Data obtained were statistically analyzed using Genstat Discovery Edition 1 (2003) package for randomized complete block design and means comparison by Least Significant Difference (LSD) at 5% probability level as described by Gomez and Gomez (1984). The trials were analysed separately with data for the two years pooled together. No transformation of data was necessary.

Before sowing, composite soil samples from three locations in each site were taken with a soil auger to a depth of 20 cm and used for physico-chemical analysis. Soil pH was measured in 1:2.5 soils: water; total N was determined by the Kjeldhal method (Pearson, 1976) and the organic matter content of the soil was determined by the wet oxidation method of Walkley and Black (1934).

RESULTS AND DISCUSSION

The soil texture at both locations was sandy loam and acidic. Average pH of the soil was 4.4 in Calabar and 5.2 at Obubra. Compared to the Calabar soil, the soil at Obubra was less sandy, containing more organic matter and nitrogen. It was also less acidic. In general however, both soils were low in N but moderate in P and K (Chude et al., 2004). Calabar experienced higher average monthly rainfall than Obubra, the total amount from August to November being 1633.0 mm and 1246.35 mm for Calabar and Obubra, respectively. Rainfall in these areas is typically bimodal with peaks in July and September and its distribution during the period of study appeared to have been favourable for soya cultivation (Table 1). Because of leaching due to high rainfall, coupled with the sandy nature and low organic matter content, soils in Southeastern Nigeria have low cation exchange and buffering capacities and therefore lose fertility rapidly (Chude et al., 2004). Such soils as well as those fields never planted to soya bean may be expected to require inoculation with the appropriate Rhizobium strain for high yields.

The soya bean crop grown at Obubra produced a higher number of active root nodules per plant as well as nodule volume and dry weight compared with that grown in Calabar (Table 2). However, whereas soil liming was ineffective at Obubra where the soil was less acidic, it significantly influenced root nodulation in the more acid soils of Calabar ($p>0.05$). Under low soil pH conditions toxic concentrations of aluminum and manganese occurs and the availability of such nutrients as Ca, Mg, P and Mo becomes sub-optimal. In addition, because low soil pH curtails soil microbial activities, it adversely affects organic matter mineralization and the subsequent availability of N, P and S. Inoculating soya bean seeds with *Rhizobium japonicum* was beneficial to root nodulation at both Obubra and in particular, Calabar. In both locations, inoculation was most effective when combined with liming the soil with calcite, but the effect of gypsum and dolomite did not seem to differ significantly.

The mean individual and combined effects of soil liming and bacterial inoculation of soybean seed on some growth attributes is presented in Table 3. Across all treatments, plant height averaged 54.6 and 61.1 cm at Calabar and Obubra, respectively. Although, neither the incorporation of lime nor the bacterial inoculation of seeds significantly affected the height of soya bean plants grown at Obubra, treatments that included rhizobial inoculation and soil liming performed significantly better than others in both locations. The vegetative growth of the limed crop was better than the unlimed one at Calabar. Differences in branches per plant, total and reproductive nodes and dry matter yields were statistically significant between the inoculated and limed plants and the uninoculated and limed ones. In both locations, the best combination was calcite with inoculation, but there was no marked difference between gypsum and dolomite when used as liming materials with inoculated soya bean seeds.

The number of seeds per pod in both locations of study was similar and unaffected by soil liming, seed inoculation or the effect of their interaction (Table 4). Inoculated and limed crops produced significantly more pods but the seeds they contained were not heavier than those that were either uninoculated or unlimed. The yield of grain which averaged 2.34 t/ha in the Obubra grown crop was higher than that grown in Calabar by 21.9 %.

The results of seed inoculation obtained largely support those of similar studies with legumes. Rani and Kodandaramaiah (1997) obtained up to 27 % higher soya bean grain yield with *Bradyrhizobium* inoculation compared to the uninoculated control. In Chickpea, Bhuiyan et al. (2008) found that inoculated plants gave significantly higher nodule number, nodule weight, Stover yield and seed yield compared to the uninoculated crop, while Gupta and Namedo (1996) obtained yield increases up to 27.9 % with seed inoculation of the same crop. Ara et al., (2009) reported that inoculation with either *Bradyrhizobium* or *Azotobacter* or their combination significantly increased nodulation, root and shoot weight, number of seeds per pod and seed yield in Mungbean.

However, contrary to the view that root nodulation and grain yield of legumes are always positively affected when acid soils are limed, the present study found that at Obubra, liming did not seem to have significantly influenced the growth and yield of the crop. Disparities in response to liming may be due to differences in such edaphic factors as degree of sandiness, organic matter content and severity of acidity, as well as to such weather factors as the intensity and distribution of rainfall. In Calabar where soil liming was effective in increasing nodulation and grain yield, calcite performed better than either gypsum or dolomite, both of which did not differ significantly from each other.

The combined use of soil liming and seed inoculation resulted in significantly better nodulation, vegetative growth and consequently higher grain yield. The increase in yield in limed and inoculated treatments might be attributed to increased nodulation and nitrogen fixation, resulting in higher dry matter accumulation and translocation of more photosynthate to the sink. Bekere et al. (2013) reported similar results in Ethiopia.

A major finding of this study is that the effectiveness of liming and bacterial inoculation of soya bean seed depends not only on the presence of the appropriate strain of Rhizobium, but also of edaphic and meteorological conditions of the location.

Table 1: Some physico-chemical soil properties and rainfall received at the study sites

Mechanical properties	Calabar		Obubra	
	2009	2010	2009	2010
Sand (%)	69.1	72.4	63.8	65.7
Clay (%)	8.5	8.1	12.4	10.9
Silt (%)	22,4	19.5	23.8	23.4
Textural class	Sandy loam	‘’	Sandy loam	sandy loam
Chemical properties				
Organic matter (%)	2.16	2.58	2.79	2.53
Nitrogen (%)	0.15	0.17	0.17	0.20
Phosphorus (ppm)	14.37	14.54	15.68	16.92
Potassium (cmol kg^{-1} soil)	0.21	0.21	0.34	0.30
pH (H_2O)	4.6	4.0	5.1	5.3
Rainfall (mm)				
August	305.8	296.3	250.6	261.9
September	591.9	662.9	521.7	501.3
October	378.8	476.1	330.4	264.9
November	271.4	282.7	205.5	156.4
Total rainfall	1547.9	1718.0	1308.2	1184.5
Mean rainfall	387.0	429.5	327.1	296.1

Table 2: Effect of liming material and pre-sowing seed Rhizobium inoculation on soya bean root nodulation at Calabar and Obubra

Treatment	Nodule (no. plant) Calabar	Obubra	Nodule volume (ml plant-1) Calabar	Obubra	Nodule dry wt. (g plant-1) Calabar	Obubra
Lime source						
No lime	8.6	12.3	1.29	1.70	0.12	0.21
Calcite	15.9	16.7	1.78	1.95	0.35	0.48
Gypsum	11.4	16.5	1.59	1.94	0.26	0.42
Dolomite	13.0	15.2	1.74	1.87	0.33	0.39
Mean	12.23	15.18	1.60	1.87	0.27	0.38
Rhizobium inoculant						
No inoculation	9.3	15.8	0.53	1.79	0.24	0.35
Inoculation	36.8	40.2	2.09	3.71	0.93	1.65
Mean	23.05	28.0	1.31	2.75	0.59	1.0
Interaction effect						
No lime X no inoculant	8.4	15.2	1.48	1.87	0.21	0.39
No lime X inoculant	19.6	31.4	1.12	1.79	0.50	0.80
Calcite X no inoculant	14.9	18.8	1.68	1.84	0.30	0.38
Calcite X inoculant	42.6	49.6	2.42	3.96	1.08	1.76
Gypsum X no inoculant	14.0	20.4	1.80	1.16	0.36	0.52
Gypsum X inoculant	39.8	47.3	2.27	3.26	1.01	1.45
Dolomite X no inoculant	16.3	21.8	1.93	2.10	0.41	0.45
Dolomite X inoculant	41.5	58.2	2.36	2.42	1.05	1.47
Mean	24.64	30.4	1.88	2.30	0.62	0.90
LSD (0.05)for:						
Lime source	2.08	ns	0.11	ns	0.13	0.16
Rhizobium inoculant	6.94	10.3	1.02	0.53	0.19	0.33
Interaction	11.37	6.54	0.17	1.05	0.20	0.18

Table 3: Effect of liming material and pre-sowing seed Rhizobium inoculation on vegetative growth of soya bean grown in Calabar and Obubra

Treatment	Plant height (cm)		Branches/plant (no)		Nodes/plant (no)		Rep nodes/plant (no)		Shoot dry wt. (g)	
	Calabar	Obubra	Calabar	Obubra	Calabar	Obubra	Calabar	Obubra	Calabar	Obubra
Lime source										
No lime	50.2	56.9	5. 9	7.2	11.87	13.18	4.14	6.34	32.2	44.4
Calcite	58.3	55.2	8.3	10.8	19.44	22.41	10.66	12.11	48.3	52.7
Gypsum	56.7	59.3	7.1	10.5	18.76	18.22	8.35	10.23	36.9	50.3
Dolomite	56.1	59.8	6.5	8.8	17.17	20.19	7.18	11.94	40.0	50.8
Mean	57.0	57.8	6.7	9.3	16.81	18.50	7.58	10.16	39.35	49.55
Rhizobium inoculation										
No inoculation	43.3	59.8	3.6	5.5	12.13	13.46	4.95	5.55	28.7	41.1
Inoculation	50.7	68.0	5.2	12.7	20.03	26.0	19.73	21.38	36.4	51.6
Mean	47.0	63.9	4.4	9.1	16.08	19.73	12.34	13.47	32.55	46.35
Interaction effect										
No lime × no inoculation	54.9	58.4	5.1	6.8	10.86	14.25	4.29	6.19	29.3	40.9
No lime × inoculation	57.3	59.6	6.3	7.7	12.38	16.33	6.17	11.43	33.1	36.7
Calcite × no inoculation	59.7	59.9	7.6	8.4	19.76	20.86	8.36	11.69	39.6	43.2
Calcite × inoculation	69.0	62.6	13.8	15.2	20.16	26.51	18.60	20.41	50.1	55.8
gypsum × no inoculation	55.4	57.2	5.7	7.0	18.44	19.04	10.41	10.65	31.8	43.0

gypsum × inoculation	61.3	60.8	10.1	12.6	21.39	24.73	18.0	18.77	40.0	49.5
Dolomite × no inoculation	58.1	59.0	6.0	7.2	17.06	19.86	9.47	9.46	34.5	35.4
Dolomite × inoculation	62.7	61.2	12.4	12.9	20.11	23.52	14.36	19.22	45.7	49.8
Mean	59.8	59.8	8.4	9.73	17.52	20.64	11.21	13.48	38.01	44.29
LSD (0.05) for:										
Lime source means	3.06		2.01	ns	4.14		3.12		5.17	3.09
Inoculation means	2.18		4.11	ns	3.64		4.48		Ns	6.56
Interaction means	2.73		6.23	4.06	6.16		7.81		8.33 12.14	

Table 4: Yield components and soya bean grain yield as influenced by pre-sowing seed Rhizobium inoculation and soil liming at Calabar and Obubra

Treatment	**Pods/plant (no.) Calabar**	**Obubra**	**Seeds/pod (no) Calabar**	**Obubra**	**100 seed wt. (g) Calabar**	**Obubra**	**Grain yield (t/ha) Calabar**	**Obubra**
Lime source								
No lime	22.8	25.7	2.43	2.55	24.7	23.5	0.93	1.59
Calcite	28.9	28.6	2.53	2.55	26.3	25.3	2.19	2.67
Gypsum	26.4	28.8	2.67	2.60	25.9	27.4	2.06	2.43
Dolomite	25.6	28.0	2.55	2.57	23.6	23.7	2.10	2.55
Mean	25.9	27.8	2.55	2.57	25.1	24.6	1.82	2.31
Rhizobium inoculation								
No inoculation	20.6	23.9	2.35	2.47	23.9	25.0	0.86 1.48	
Inoculation	31.2	30.6	2.66	2.50	26.0	27.4	2.44	

							2.57	
Mean	25.9	27.3	2.51	2.48	25.0	26.2	1.65	
							2.03	
Interaction effect								
No lime × no inoculation	20.4	26.0	2.53	2.67	22.7	24.8	0.78	
							1.51	
No lime × inoculation	25.5	26.9	2.61	2.63	24.1	23.6	2.06	
							2.75	
Calcite × no inoculation	27.8	29.8	2.67	2.60	24.8	24.4	2.22	2
							78	
Calcite × inoculation	34.1	35.6	2.60	2.57	26.0	26.3	3.16	
							3.23	
Gypsum × no inoculation	23.9	29.5	2.66	2.54	25.3	25.9	2.0	
							2.43	
Gypsum × inoculation	26.4	30.7	2.56	2.66	26.2	27.0	2.99	
							2.96	
Dolomite × no inoculant	24.0	26.8	2.48		23.8	24.6	2.09	
			2.57				2.50	
Dolomite × inoculant	27.4	30.4	2.58		24.7	26.7	3.02	
			2.54				3.17	
Mean	26.2	29.5	2.59		24.7	25.4	2.29	
			2.60				2.67	
LSD (0.05) for:								
Lime source means	3.07	ns	Ns		Ns	Ns	1.20	
			Ns				1.13	
Inoculation means	6.19	5.06	Ns		NS	Ns	1.76	
			Ns				0.59	
Interaction means	12.44	5.12	Ns		NS	Ns	2.18	
			Ns				0.67	

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