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Effects of commercial microbial inoculants and foliar fertilizers on soybean nodulation and yield in northern Guinea savannah of Nigeria

Clement Odon N'CHO[1]*, Ado Adamu YUSUF[2], Joséphine Tamia AMA–ABINA[1], Martin JEMO[3], Robert Clement ABAIDOO[3] and Issiaka SAVANE[1]

[1]Nangui Abrogoua University, Abidjan, Côte d'Ivoire.
[2]Ahmadu Bello University, Zaria, Nigeria.
[3]International Institute of Tropical Agriculture, Ibadan, Nigeria.

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ABSTRACT

The use of microbial inoculants and less expensive sources of plant nutrients such as foliar fertilizers are ways to practice low-input agriculture, especially in sub-Saharan Africa where smallholder farmers' soil nutrients are depleted. However, the application of microbial inoculants and foliar fertilizers are influenced by agroecological specific factors. Here we show the effects of microbial inoculants and foliar fertilizers application on soybean, under smallholder farmers' conditions in northern Guinea savannah. Three microbial inoculants; *Bradyrhizobium* spp. (RACA 6), arbuscular mycorrhizal fungi (Rhizatech) and *Trichoderma harzianum* (Eco-T) and two foliar fertilizers; Agroleaf high P and Agrolyser were evaluated. The trial was carried out during 2011 cropping season in randomized complete block design with 12 treatments and 4 replicates. The shoot dry weight was increased separately by the Reference treatment with N, P chemical fertilizers, Rhizatech and RACA 6 + triple superphosphate (TSP) over 21% compared to the control. Significant effect existed among treatments for soybean nodulation and shoot phosphorus concentration. Grain yield was relatively increased by the application of RACA 6 + TSP, RACA 6 + Eco-T + Agrolyser and RACA 6 + Rhizatech + Agrolyser compared to the control. The experiment suggested that soybean co-inoculation with rhizobial and fungal inoculants and application of foliar fertilizers in northern Guinea savannah could lead to improved grain yield.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is becoming one of the most cultivated grain legumes in sub-Saharan Africa. Among the grain legumes, it has the greatest potential of producing the cheapest source of food protein (Rao and Reddy, 2010) and other essential nutrients for farm households. Its capacity to fix atmospheric nitrogen (N_2) with rhizobia enables it to grow well on N-impoverished soil. Since biological nitrogen fixation (BNF) was discovered in the late 19th century, research works have reported on its potentiality as an alternative to inorganic N-fertilizer in agriculture. Indeed, despite the widespread encouragement to use inorganic fertilizers to replenish soil nutrients, most farmers do not have access to them while others cannot afford the cost of their procurement. Therefore, continuous cropping without replenishment of nutrients occurs during every cropping season. This results in negative balance of soil nutrients (especially

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Corresponding author. E-mail: ncho_oc@yahoo.fr. Tel: +22507525669.

nitrogen and phosphorus) in farmers' field in most of the tropical agroecological zones especially the northern Guinea savannah (Manyong et al., 2001). In addition, it is noteworthy that less than 50% of the N-fertilizer is taken up by plant (van Cleemput et al., 2008); the remaining contributes substantially to environmental pollution. In contrast, BNF provides an economically attractive and ecologically friendly renewable nitrogen (N) in agriculture through the reduction of external inputs and improving internal N resources. The average amount of soybean N derived from BNF is estimated at 75 kg N ha^{-1} (Keyser and Li, 1992). However, the symbiosis is specific and depends upon soybean genotype and rhizobia strains under various edaphic and climatic conditions (Abaidoo et al., 2007; Chalk et al., 2010). BNF could be limited by the acidity of tropical soils which is related to the complex of high aluminium and manganese toxicity and low calcium and available phosphorous (P).

Co-inoculation of soybean with rhizobia and soil fungi such as arbuscular mycorrhizal fungi (de Varennes and Goss, 2007) and *Trichoderma* sp (Shaban and El-Bramawy, 2011) sometime shows synergistic tripartite interaction and is thought as means for alleviating nutrient deficiencies effect on BNF through the enhancement of plant nutrients uptake (Bethlenfalvay, 1992; Chalk et al., 2006; Bisht et al., 2009, Verma et al., 2007). In addition, *Trichoderma* strains are known as important biological soil-borne pathogen and diseases control agents (Vinale et al., 2008) and constitute an attractive alternative to many pesticides for environmental safety reasons (Lüebeck and Jensen, 2002). Furthermore, to reduce fertilizer application to soil, new formulations of foliar fertilizer (micro- or macro-nutrients or both) are available worldwide and could be more effective than soil applied fertilizer in reducing effect of nutrient deficiencies on BNF (Zahran, 1999). Foliar fertilization provides more rapid utilization of nutrients and permits the correction of observed deficiency symptoms in less time than would be required by soil application (Fageria et al., 2009). Oko et al. (2003) reported that foliar application of urea increased soybean grain yield; between 6 and 68% higher than the control. Moreover, the effect of foliar fertilization on grain yield depends upon the application period of the different growth stages of soybean (Mallarino et al., 2001). It is also most successful for supplying micronutrients, and more effective and economical because some of the nutrients, such as iron, are easily immobilized in the soil (Fageria et al., 2009).

In Nigeria, soybean cultivation is increasing within the northern Guinea savannah agro-ecological zone (AEZ), where it is well adapted. The use of microbial inoculants and foliar fertilizers by smallholder farmers in soybean production are not popular in this AEZ. At the same time, soybean yield through microbial inoculation and foliar fertilization is an increasing interest brought about by the findings of companies providing their respective products.

This study was therefore conducted to assess the combination of microbial inoculants and foliar fertilizer effects on soybean nodulation, N content and grain yield under smallholder farmers' conditions.

MATERIALS AND METHODS

Sites description and experimental treatments

The experiment was carried out during 2011 cropping season in Kaya, Giwa Local Government Area of Kaduna State in the northern Guinea savannah of Nigeria. Four farmers were selected in Kaya and surrounding villages. The length of the cropping season is 151–180 days with a unimodal rainfall distribution of about 800–1200 mm (Weber et al., 1996). The major soils that cover more than 50% of the northern Guinea savannah (NGS) area of Nigeria include Luvisols, Vertisols and Lithosols (Jagtap, 1995).

One *Bradyrhizobium* strain (RACA 6) and two fungal inoculants, arbuscular mycorrhizal fungi (AMF) (Rhizatech) and *Trichoderma harzianum* (Eco-T) constituted the microbial inoculants used in the experiment (Table 1). Agroleaf high P and Agrolyser micronutrient fertilizer are some new formulations of foliar fertiliser produced by Scotts Company (Ohio, USA) and Cybernetics Ltd. (Nigeria), respectively. The final amounts of micronutrient and macronutrient received by each plot are indicated in Table 2.

The field experiment was carried out during rainy season in 2011. The experimental layout was randomized complete block design with four replications. Each replication was represented by one farmer's field. The experimental plot measured 3 m × 4.5 m with 6 rows per plot. Rows were 0.75 m apart. Each plot was separated from one another by one non-planted row as buffer. The promiscuous medium-maturing soybean variety TGx 1448-2E was used as test crop.

Before planting, 4 spoons of RACA 6 and 1 g of Eco-T (as recommended by the manufacturer) were used to coat 1 kg of the seeds respectively either in single or combined inoculation. Moreover, 50 g of Rhizatech was mixed with 500 g of soil and applied in the sowing furrow. The foliar fertilizers frequency of applications was modified to avoid leaf injury. However, the recommended rate (5 kg ha^{-1}) by the companies was considered. The foliar fertilizers were applied at three stages of the growth period: 3rd and 6th weeks after planting (WAP) and at the flowering stage (8 WAP). A litre of Agroleaf was sprayed for a 13.5 m^2 plot at 2.5 g L^{-1} for the first two initial growing periods and 5 g L^{-1} at flowering. Agrolyser was sprayed at rate of 2.05 g L^{-1} at 3 and 6 WAP and 4.1 g L^{-1} at flowering.

Mineral fertilizers at the rate of 30 kg P_2O_5 ha^{-1} and 120

Table 1. Origin and active agents of the microbial products.

Product	Origin	Formulation	Type	Active microorganism
RACA 6	IITA, Ibadan (Nigeria)	Peat	Laboratory	*Bradyrhizobium* spp.
Rhizatech	Kenya	Granules	Commercial	Spores and mycelia fragments of AMF
Eco-T	South Africa	Powder	Commercial	*Trichoderma harzianum* strain Rfai KRL AG2

Table 2. Amount of nutrients received per plot from foliar fertilizers (mg).

Elements	N	P	K	Fe	Mg	Cu	Zn	B	Mn	Ca	Mo	Na	S
Agroleaf high P	1200	5200	500	10	6	6	6	2	A	A	A	A	A
Agrolyser	A	A	A	T	19	19	11	T	T	2014	T	104	272

A, Absent; T, present in trace quantity.

kg N ha^{-1} were applied with triple superphosphate (TSP, 46% P_2O_5) and urea (46% N), respectively. Phosphorus was applied at planting while N was applied in three equal splits: at planting, 3 WAP and at 80–100% flowering. The combined application of 120 kg N and 30 kg P_2O_5 ha^{-1} was called Reference treatment.

Measurements and data analysis

Before planting, 15 soil samples were taken randomly from each field (within 0–15 cm) and bulked to form a composite. The soils were air-dried and processed to determine the chemical and physical properties at the Analytical Service Laboratory of the International Institute of Tropical Agriculture (IITA).

Plant samples for biomass measurement were taken within a square demarcated within the net plot, that is, 0.5 m from each corner. The samples were taken at 80–100% flowering stages. Soybean plants were cut above ground, their root carefully excavated and the nodules were picked. Shoot and root were oven-dried for 72 h at 60°C and the dry weight was recorded. The nodules were counted, dried and weighed.

After the measurement of the shoot dry weight, the shoot was separated into leaves and stems and the two organs were ground separately. One volume of milled leaves and two volumes stems were mixed for the measurement of the shoot N and phosphorus (P) content (IITA, 1982).

Soybean grain yield per plant was estimated for individual plant within an area of 2.5 × 1.5 m^2. One hundred (100) seed weight was recorded after drying at 60°C for 72 h. The percentage moisture content of the 100 seeds weight was then used to estimate the dried weight of grain yield.

Analysis of variance (ANOVA) was performed using the PROC GLM in SAS version 9.2 (SAS, 2009). Post ANOVA multiple means separation was conducted with Duncan's Multiple Range Test at $\alpha=0.05$. The relationship between all the measured traits was verified using PROC CORR in SAS.

RESULTS

The results of soil analyses are shown in Table 3. The soils were acidic; the pH ranged from 4.8–5.4 and the available P ranged from 4.42–35.42 mg kg^{-1}. The characteristic organic carbon varied from 4.54–6.02 g kg^{-1} while their textural classes ranged between loamy to sandy loam.

The variation between farms on growth performance, nodulation, nutrient concentration and grain yield of soybean are shown in Table 4. Significant differences were observed among farms for all the parameters. The lowest shoot and root dry matter, nodulation and nutrient concentration were found in Farm 2 while Farm 4 showed consistency in soybean growth and grain yield increase.

The treatment effect on shoot dry weight did not show significant difference (Table 5). The shoot dry weight per plant ranged from 6.2–13.8 g $plant^{-1}$. The application of the commercial AMF inoculant Rhizatech, the Reference, and the interaction between rhizobial inoculants and TSP (RACA 6 + TSP) favoured soybean shoot dry weight production. The percentage increase was 41, 21 and 21% for the Reference (120 kg N + 30 kg P_2O_5 ha^{-1}), Rhizatech, and RACA 6 + TSP, respectively.

Soybean root dry weight per plant ranged from 1.1–2.2 g. However, it was not significantly influenced by the treatments. Rhizatech gave 66% increase over the control.

Significant ($P\leq0.05$) difference existed among the treatments for nodule dry weight. The nodules dry weight

Table 3. Soil physical and chemical properties of the farms at planting.

Property	Unit	Farm 1	Farm 2	Farm 3	Farm 4	Mean
Sand	g kg^{-1}	664	664	484	544	589
Silt	g kg^{-1}	200	220	380	340	285
Clay	g kg^{-1}	136	116	136	116	126
pH (H_2O) 1:1		5.4	5.3	5.0	4.8	5.1
OC	g kg^{-1}	6.01	5.24	4.54	6.02	5.45
N	g kg^{-1}	0.57	0.5	0.43	0.59	0.52
Mehlich P	µg g^{-1}	7.45	4.42	35.42	14.37	15.42
Ca	cmol kg^{-1}	2.68	1.95	2.63	3.35	2.65
Mg	cmol kg^{-1}	0.88	0.6	0.44	0.92	0.71
K	cmol kg^{-1}	0.26	0.2	0.24	0.4	0.28
Na	cmol kg^{-1}	0.11	0.13	0.1	0.11	0.11
Exch. Acidity	cmol kg^{-1}	0.08	0.08	0.12	0.18	0.12
ECEC	cmol kg^{-1}	4.00	2.96	3.52	4.96	3.86
Zn	mg g^{-1}	3.84	2.02	6.16	1.69	3.43
Cu	mg g^{-1}	1.94	1.94	0.97	3.89	2.19
Mn	mg g^{-1}	22.93	21.85	36.57	39.38	30.18
Fe	mg g^{-1}	111	89	105	158	115.75

Table 4. Effect of farms' soils on the growth, nodulation and shoot N and P concentration of soybean.

Farm soil	SDWTP (g plant^{-1})	RDWTP (g plant^{-1})	NDWTP (mg plant^{-1})	NODNP	N_Conc (mg kg^{-1})	P_Conc (mg kg^{-1})	GDWTP (g plant^{-1})	100-seed (g)
1	6.8 b	1.23 c	58.6 b	17.2 ab	21.9 b	1.4 d	8.9 c	12.0 a
2	3.9 b	0.87 c	34.9 c	8.6 c	19.3 c	1.6 c	17.5 b	11.1 b
3	13.6 a	1.76 b	89.5 a	12.5 bc	18.2 c	2.7 a	25.2 a	11.8 a
4	13.2 a	2.30 a	73.8 ab	21.4 a	24.3 a	1.9 b	28.5 a	12,3 a

Figures followed by the same letters in the same column are not significantly different according to DMRT at P=0.05.
SDWTP, Shoot dry weight per plant; RDWTP, root dry weight per plant; NDWTP, nodule dry weight per plant; NODNP, nodules number per plant; N_Conc, shoot N concentration; P_Conc, shoot P concentration; GDWTP; grain yield per plant; 100–seed, 100 seeds weight.

per plant ranged from 42.3–137.1 mg. The lowest nodule dry weight was obtained from the plants inoculated with *T. harzianum* based product, Eco-T; about 31% lower than the control. RACA 6 and Rhizatech increased nodule dry weight with 74.8 and 75.13 mg plant^{-1}, respectively. The interaction between RACA 6 and TSP produced the highest nodule dry weight of (137.1 mg plant^{-1}) 125% over the control (Table 6).

Significant ($P \leq 0.05$) differences existed among treatments for soybean nodulation. Rhizatech induced more (about 9%) nodules number from the indigenous rhizobia over the un-inoculated control. Inoculation with RACA 6 led to the production of more nodules, about 18% compared to the indigenous rhizobia in the control. The highest nodule production per plant (about 109% over the control) was obtained from the application of RACA 6 and TSP. Furthermore, negative effect was observed on soybean nodulation from the plant co-inoculated with RACA 6 and Rhizatech (-24%). However, the application of Agrolyser on plant co-inoculated with the same microbial inoculants (RACA 6 + Rhizatech + Agrolyser) showed a positive effect on nodulation (Table 6). Meanwhile, the Reference plot showed the lowest nodules production (-30%).

Grain yield per plant varied from 16.9 to 25.8 g (Table 6). The statistical analyses did not show significant difference among the treatments. The combined application of RACA 6 and TSP produced 52% of grain yield over the control plot and more than the single application of either. It was followed by the interactions treatments; RACA 6 + Eco-T + Agrolyser and RACA 6 + Rhizatech + Agrolyser that increased the grain yield by 45 and 37%, respectively. The application of TSP alone increased grain yield (about 21%) more than the

Table 5. Soybean shoot and root dry weight (g plant^{-1}) as influenced by the treatments at eight weeks after planting.

Treatment	Shoot dry weight		Root dry weight	
	Means	Relative response (%)	Means	Relative response (%)
Control	9.8	-	1.3	-
Eco-T	9.0	-8	1.6	19
Rhizatech	11.8	21	2.2	66
TSP (30 kg P_2O_5 ha^{-1})	9.5	-3	1.5	11
Agroleaf high P	7.4	-24	1.3	-3
RACA 6	7.4	-24	1.4	6
RACA 6 + Agroleaf	9.7	0	1.3	-2
RACA 6 + Rhizatech	6.2	-37	1.1	-15
RACA 6 + TSP (30 kg P_2O_5 ha^{-1})	11.8	21	2.0	50
RACA 6 + Eco-T + Agrolyser	7.2	-26	1.4	6
RACA 6 + Rhizatech + Agrolyser	9.1	-7	1.3	-2
Reference	13.8	41	2.2	63

Table 6. Soybean nodulation, shoot N concentration at eight weeks after planting and grain yield.

Treatment	Nodules number plant^{-1}		Nodules dry weight (mg plant^{-1})		Grain yield (g plant^{-1})		Shoot N concentration (mg plant^{-1})	
	Mean	Relative response (%)	Mean	Relative response (%)	Mean	Relative response (%)	Mean	Relative response (%)
Control	14.7 b	-	60.9 b	-	16.9	-	20.7	-
Eco-T	12.2 b	-17	42.3 b	-31	19.1	13	20.9	1
Rhizatech	16.0 b	9	75.1 b	23	22.1	31	22.4	8
TSP (30 kg P_2O_5 ha^{-1})	11.5 b	-22	51.8 b	-15	20.5	21	20.2	-3
Agroleaf high P	11.7 b	-20	54.0 b	-11	18.1	6.8	21.2	2
RACA 6	17.4 b	18	74.8 b	23	16.9	0	20.4	-2
RACA 6+Agroleaf	13.1 b	-11	52.9 b	-13	19.0	12	21.0	1
RACA 6+Rhizatech	11.2 b	-24	48.9 b	-21	17.0	1	21.2	2
RACA 6+TSP (30 kg P_2O_5 ha^{-1})	30.7 a	109	137.1 a	125	25.8	52	21.4	3
RACA 6+Eco-T+Agrolyser	13.5 b	-8	63.3 b	4	24.6	45	20.1	-3
RACA 6+Rhizatech+Agrolyser	16.8 b	14	64.6 b	6	23.1	37	20.0	-4
Reference	10.3 b	-30	45.1 b	-26	17.0	0	21.4	3

Figures followed by the same letters are not significantly different according to DMRT at α=0.05.

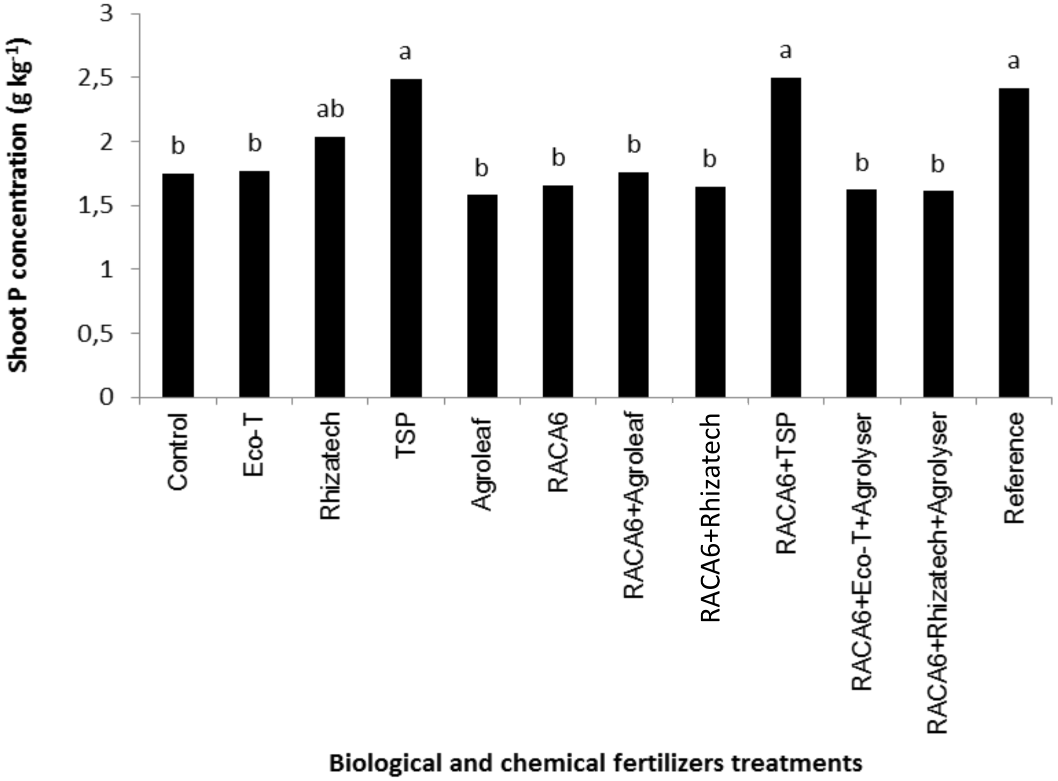

Figure 1. Effect of biological and chemical fertilizers on soybean shoot P concentration at 8 weeks after planting (Bars with the same letters are not significantly different according to DMRT at α=0.05).

Table 7. Relationship between shoot N and P concentration and other parameters.

Parameter	Shoot N concentration	Shoot P concentration
Shoot dry weight	0.20268^{ns}	0.61331^{***}
Root dry weight	0.44521^{*}	0.46871^{*}
Nodules dry weight	0.14770^{ns}	0.47799^{*}
Nodules number	0.52528^{*}	0.01636^{ns}
100-seeds weight	0.34098^{*}	0.26588^{ns}
Grain yield	0.09136^{*}	0.42201^{*}

ns, not significant; *, significant.

Reference treatment where N and P were applied. However, none of the treatment increased the 100 seeds dry weight.

The concentration of N in the shoot ranged from 20.00–22.41 g kg^{-1} (Table 6). No significant difference was observed among the treatment for shoot N concentration. Plants inoculated with Rhizatech had the greatest shoot N concentration. It was followed by the Reference (21.4 g kg^{-1}) and the combined application of RACA 6 and TSP (21.4 g kg^{-1}). Due to the different treatments, soybean shoot P concentration varied from 1.6 to 2.5 g kg^{-1} as indicated in Figure 1. The plants treated with RACA 6 and TSP had the highest shoot P concentration, followed by the application of TSP (2.5 g kg^{-1}), Reference (2.4 g kg^{-1}) and Rhizatech (2.0 g kg^{-1}).

The relationship between the different parameters and the nutrient concentration are presented in Table 7. Positive correlation existed between the concentration of nutrients in the shoot (N and P) and the measured parameters. High significance and slight correlation existed between shoot N concentration and nodules number (r=0.53: P=0.0001). The shoot P concentration was also significantly correlated with the shoot dry weight (r=0.61; P<0.0001) in Table 7.

DISCUSSION

The experiment showed that fertilization of soybean with inorganic N in NGS resulted in low grain yield compared with soybean inoculated with rhizobial inoculant. This observation is in line with the findings of Albareda et al. (2009) who had earlier observed that rhizobial inoculant increased soybean seed yield than inorganic N fertilizer. The N applied to the soil decreased the BNF activity (Senaratne et al., 1987) and thus becomes the major source of N to satisfy the plant development because of the low soil N level of the zone (Okogun et al., 2005). The sandy-textured soil with low CEC (varying from 2.96–4.96 cmol kg^{-1}) in the location may have favoured leaching of N applied. As soybean is a high N consuming crop, the plant could not meet its N requirement through the application of fertilizer N only. Okogun et al. (2004, 2005) found that inoculation of TGx1448-2E did not significantly increase the shoot yield. Our trial confirmed these results but showed that the increment might be very well above the 11 and 3% observed in their two years (2004 and 2005) reports. The difficulty for a successful inoculation technology implementation in soybean production across farms might be due to the low soil fertility and its variation among farmers' field that exist in the zone. In fact, full BNF potential is not expected from a legume plant whose growth is restricted by unfavourable climate and edaphic factors as underlain by Zahran (1999) and Hardarson and Atkins (2003). Therefore, the low growth performance and nodulation of soybean in farm 2, and in some extent in farm 1, might be due to the soil P content that was low (Cassman et al., 1981). According to the study of Gibson et al. (1982), P has beneficial effect on the nodule number and dry weight. Consequently, nutrients whose deficiencies constraint the growth and the nodulation of soybean must be corrected for a successful BNF, taking into account within the AEZ soil fertility variation.

The supply of nutrient through foliar fertilization to diminish the negative effect of nutrient deficiencies, especially P (Cassman et al., 1981), on BNF did not show in this experiment. Applied foliar P directly mobilizes into the plant's leaves where it serves as energy carrier from the leaves to other parts of the plant but might not stimulate the nodulation because the P sprayed cannot reach the rhizobia population in the rhizosphere. In contrary, the bacteroids and the plant cell of the nodules could be benefit of high energy transfer and fixed more N to increase grain yield (Sa and Israel, 1991).

The expected outcome of the tripartite interaction rhizobium – AMF – soybean is higher nodulation, N_2-fixation and enhanced growth parameters due to P mobilization (Bethlenfalvay, 1992). Our results showed that soybean line TGx1448-2E required AMF to grow better than P applied at rate of 30 kg P_2O_5 ha^{-1}. But, the co-inoculation of soybean with Rhizatech and RACA 6 decreased the shoot and root dry weight. In fact, dual inoculation does not always have synergistic effect on plant growth and nodule yield (Nwoko and Sanginga, 1999). The poor growth performance from RACA 6 and Rhizatech co-inoculation might be due to the low P level; most of the farmers' fields have their P below the critical level of 15 mg P kg^{-1} suggested by Zingore and Giller (2012). This might constitute a limiting factor for AMF to increase the P uptake and satisfy both BNF and plant growth. On the other hand, it could be explained by the architecture of the soybean root that cannot explore enough volume of soil to extract enough amount of P. In fact, Wang et al. (2011) found that deep root genotype of soybean benefited more from co-inoculation than the shallow root genotype. In addition, low P might reduce photosynthetic activity due to the strong competition imposed by the symbioses (Marschner and Dell, 1994), and as well, the root growth might be influenced negatively (Piccini et al., 1988) and thereby becomes incapable to take up sufficient amount of nutrient. This is shown in the shoot P and N concentration that was higher in the rhizatech treatment than the one of the interaction between RACA 6 and Rhizatech.

The application of the foliar micronutrient Agrolyser on the double inoculation fungi-*Rhizobium* increased grain yield. This confirmed what was pointed out by Sillanpaeae (1982) on micronutrients stresses on crop yield in Nigeria. As well, Ross et al. (2006) and Bellaloui et al. (2010) indicated the importance of some micronutrients such as boron on soybean nitrogen fixation and seed yield. The double inoculation with the application of foliar micronutrient provided somehow a balanced nutrient supply to the plant which is also important for legume (Gibson et al., 1982).

Conclusion

The results showed that application of N at the rate of 120 kg ha^{-1} did not increase soybean grain yield and showed that rhizobia inoculation with adequate available P increased soybean development and grain yield in NGS. The results also suggested that application of micronutrient foliar fertilizer, Agrolyser in combination with microbial inoculants increased grain yield of soybean. However, the use of microbial inoculants in improving soybean yield demands more understanding of the biochemical compound secreted by each microbe when co-inoculated with others and also the required micronutrients by the interaction.

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