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Effects of Native Rhizobia on Soybean [*Glycine max* (L.) Merrill] Production and Soil Properties in Daloa, Center-West of Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author KA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The field experiment study was conducted at the experimental station of Jean Lorougnon Guede University in Daloa to evaluate the potential effects of native rhizobia strains on soybean production and soils properties. The experiment was layout in a randomized complete block design with three replications. The treatments include seven native strains, one exotic strain (*Bradyrhizobium japonicum* IRAT FA3), one synthetic fertilizer NPK 12-22-22 formulation and one negative control (no fertilizer, no inoculation). Agronomic parameters (plant height, plant dry biomass, nodule number plant^{-1}, pod number plant^{-1}, pod weight plant^{-1}, seed yield ha^{-1}) and soil parameters (pH, total nitrogen, orgnanic carbon, organic matter and available phosphorus) were measured. Results showed that all tested native rhizobia enhanced nodules number, plant growth and soybean yield as compared to negative control. Among these native rhizobia, RSC119 and RSC504 induced more nodules than the introduced strain *B. japonicum* IRAT FA3. RSC119, RSC309 and RSC508 produced more pods and seed yield than the introduced strain *B. japonicum* IRAT FA3 and the synthetic fertilizer NPK 12-22-22. RSC508 induced the highest pods number plant^{-1} (102 pods) and seed yield (4.2 t.ha^{-1}). Moreover, these local bacteria had positive effects on soil properties following the soybean's harvest. Among these bacteria, RSC119 enhanced mostly total nitrogen, organic matter and available phosphorus, then RSC508 significantly reduced soil acidity. This study therefore suggests that local rhizobia are effectiveness and could be use as inoculum to improve soybean productivity and soil properties restoration.

Keywords: Native rhizobia; inoculation; soybean; soil properties; Côte d'Ivoire.

1. INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important annual grain legume in the world. Its seed are an excellent source of protein (40%) and edible oil (20%) [1]. It also appears as the best crop to improve livelihoods and income for rural smallholder farmers in various developing countries. Soybean also increases soil fertility and productivity for its ability to fix and reduce atmospheric nitrogen (N_2) into biological usable forms in symbiosis with rhizobia [2]. Soybean-rhizobia symbiosis could provide easy and inexpensive way to enhance soil fertility and improve crop production while reducing synthetic fertilizers need in agricultural practices [3]. Successful soybean-rhizobia symbiotic relationship is dependent for a large part on the effectiveness and competitiveness of bacterial strains used as inoculant [4]. Moreover, to ensure profitable production of soybeans outside its domestication zone, research recommends the exogenous supply of bacterial strains capable of fixing nitrogen such as inoculum [5]. In Côte d'Ivoire, an exotic strain of *Bradyrhizobium japonicum* (IRAT FA3) was used as inoculant for soybean production since two decades [6]. However, the response of soybean genotypes to this exotic bacterial strain was variable to agroecological conditions [7]. Thus, soybean vulgarization in some areas of the country with no history to soybean cultivation, requires effective strains. Recent studies reported that isolation and selection of native efficient rhizobia strains on different soybean cultivars [8,9]. Authors revealed that native rhizobia are able to improve soybean cultivars nodulation and growth better than introduced strain *B. japonicum* IRAT FA3 under controlled conditions. The capacity of these efficient local strains of rhizobia which are adapted to local agroecological conditions deserves to be evaluated in field conditions in order to promote them as elite strains for inoculum formulation. Thus, the objective of this study was to evaluate the symbiotic and agronomic potential of native rhizobia strains on soybean and their background effects on soil properties on farm.

2. MATERIALS AND METHODS

The field experiment was conducted at the experimental station of Jean Lorougnon Guede University in Daloa. It is located in the Centre-West of Côte d'Ivoire at 6°84' N latitude and 6°41′ W longitude. Average rainfall and temperature of Daloa area were respectively 1300 mm and 25.6°C per year [10]. The soils of the experimental site was desaturated reworked ferralitic type [11,12]. Previous cultivation of the experimental site was cassava (*Manihot esculenta* Crantz) and it had no history of soybean cultivation nor inoculation with Bacteria Fixing Nitrogen (BNF) since the three (3) last years.

Soybean [*Glycine max* (L.) Merrill] cultivar Piramama obtained from the Crops Research Station of the National Center for Agronomic Research (CNRA) in Bouaké (Côte d'Ivoire), was used as plant material in this experiment. This soybean cultivar cycle is 98 days. Rhizobial strains used in this work consisted of seven (07) local rhizobia (RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508) isolated from soybean and one (01) exotic strain (*Bradyrhizobium japonicum* IRAT FA3) used as reference. Local and exotic rhizobia were provided respectively by the Laboratory of Agrovalorization of Jean Lorougnon Guede University in Daloa (Côte d'Ivoire) and the Central Laboratory of Soils, Water and Plants (LCSEP) of The CNRA in Bouake (Côte d'Ivoire). Each bacterial strain was used to produce inoculum at LCSEP. When the density of each pre-culture was estimated at 10^9 bacteria/mL, it is packaged in solid form in previously treated and sterilized peat.

Experiment was carried out according to a randomized complete block design with three replications during the short rainy season between july and september in 2019. The main plot was inoculation with 10 modalities of which seven (07) native strains (RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508), one (01) exotic strain (*Bradyrhizobium japonicum* IRAT FA3), one (01) synthetic fertilizer NPK 12-22-22 formulation used as positive control (TN) and one (01) without treatment used as negative control (T0). Each elementary plot measured 6 m^2 (3 m x 2 m) with an alley of 1 m between plots. Each elementary plot was composed of seven (7) sowing lines with 10 pockets per line. Consecutive blocks were separated of 2 m. In each plot, effective area was 3 m^2.

Before sowing, seeds were inoculated by coating. Three (3) seeds were sowing per pocket with a spacing of 50 cm between rows and 20 cm between pockets. Controls plots were sowed first in the experiment in order to avoid contamination with tested bacteria in the inoculum. The fertilized control received basal fertilizer at the rate of 150 $kg.ha^{-1}$. Thinning was done seven (7) days after sowing, to have two (2) plants per pocket.

Symbiotic and agronomic parameters were measured at different phenological stages. Plant nodulation and growth parameters measured were nodules number, plant height and dry biomass. These parameters were assessed at the beginning of the flowering stage from five plants randomly selected from each plot. Plant height was measured with a meter rule. Nodules were carefully detached from the root of plants previously dug up and counted. Then, fresh biomass (root and shoot) of these same plants were oven-dried at 70 °C for 72 h and their dry weight recorded. At maturity, the average number of pods per plant was counted on five randomly selected plants. Then, seeds yield was determined.

Before setting up the test, soil samples were taken from the site using an auger at a depth of 0 to 20 cm using the diagonal method. These soil samples were mixed to obtain a composite sample. Other soil samples were taken 30 days after soybean harvest on each plot to constitute composite sample for each treatment in order to evaluate the effect of inoculation on quality soils. Analysed parameters were granulometry by densimetric method using Robinson's pipette [13], pH_{H2O} with a pHmeter according to a soil/distilled water ratio of 1: 2.5 [14], organic carbon content by the Walkley and Black [15] method, then converted to organic matter (OM) using the factor 1.724, total nitrogen according to Kjeldahl method [16] and available phosphorus by modified Olsen method [17]. Data collected were subjected to analysis of variance (ANOVA) using STATISTICA 7.1 version when ANOVA assumptions are verified. Fisher's Least Significant Difference (LSD) was used to separate means at a significance level of 5%.

3. RESULTS AND DISCUSSION

3.1 Effect of Inoculation on Soybean Nodulation and Growth Parameters

Inoculation had a significant influence ($P \leq .05$) on the number of nodules, plant height, and dry biomass of soybean (Table 1). In terms of the different rhizobia tested, RSC119 and RSC504 induced higher number of nodules (42 and 50 nodules respectively). These were 20% to 43% higher that the nodules observed on the reference strain IRAT FA3 (35 nodules per plant). Results showed that native rhizobia strains tested were capable of inducing nodule formation on the soybean root system. Native rhizobia strains such as RSC119, RSC309 and RSC504 increase significantly nodules number compared to the exotic *Bradyrhizobium japonicum* IRAT FA3 strain used as soybean inoculum in Côte d'Ivoire. These results confirm observations made by Amani et al. [9] realized on pots under controlled conditions with the same strains. Moreover, works carried out in Bouaké, in the center of Côte d'Ivoire by N'Gbesso et al. [18] on the same soybean cultivar revealed that local bacterial strain 26D4 was able to increase nodule dry weight compared to introduced strain IRAT FA3. Other works carried out in the province of Kabul in Afghanistan showed that native strains of *Ensifer* (GS4 and GE6W) and *Bradyrhizobium* (GE3) induced formation of more nodules on soybean varieties Stime3300 and Enrei compared to the reference strain USDA110 [19]. On the other hand, nodules were observed on the roots of the negative control plants (3 nodules per plat) and positive control (5 nodules per plant). These nodules had a whitish colouration on inside. The presence of few nodules in non-inoculated plots reflects the lack of efficient indigenous rhizobia strains in the soils of the field trial. The formation of nodules on the uninoculated plants could also be due to indigenous rhizobia of the genus *Bradyrhizobium* which usually enter into symbiosis with cowpea (*Vigna unguiculata*) often grown in the trial area. However, the nodules formed were non-functional because the bacteria do not have nitrogen-fixing genes specific to soybean. Similar conclusions were drawn by previous studies, such as Nzabi et al. [20] and Argaw [21], who worked on various soybean genotypes in Ethiopia and Kenya respectively.

All rhizobia tested improved soybean height compared with negative control (T0). Native rhizobia RSC119 and RSC504 induced the highest plant heights at flowering with 44.4 cm and 44.5 cm respectively. Their effect on plant growth was similar to that of chemical fertilizer. Like these bacteria, RSC309, RSC324 and RSC508 had positive impact on soybean growth. Moreover, tested rhizobia strains except RSC115 increased plant dry biomass compared with negative control (T0). Local rhizobia RSC119 and RSC504 induced 4.8 g and 4.4 g of plant biomass respectively. In addition, the dry biomass produced by these bacteria was statistically similar to biomass induced by synthetic fertilizer (4.8 g). Regarding plant growth, parameters such as height and dry biomass were significantly influenced by native rhizobia tested. Indeed, all of these rhizobia improved the height and dry biomass of soybeans compared to the negative control. These rhizobia generally induced the same effects as the synthetic fertilizer NPK 12-22-22 for these different parameters. The improvement in height and biomass observed in this work would be due to the addition of nitrogen during the symbiotic fixation of atmospheric nitrogen. These results confirm those of Alam et al. [22] in Bangladesh. These authors showed that the height and dry biomass of soybean genotypes Shohag, BARI Soybean6, MTD10 and BGM02026 were improved by the local strain of *Rhizobium* sp. BARIGm901 compared to the uninoculated control. Regar et al. [23] similarly observed such enhancements in the Rajasthan region of India, leveraging a local rhizobial strain. Other work showed that inoculation practice improved plant height and biomass as well as synthetic nitrogen fertilization [24].

Table 1. Effect of local rhizobia, introduced strain and synthetic fertilizer NPK 12 22 22 on soybean nodules number, height and dry biomass of soybean at flowering stage

Treatments	Nodules number	Plant height (cm)	Plant dry biomass (g)
RSC115	33±3e	32,50±1,5e	2,7±0,5f
RSC119	42±4b	44,40±2,33ab	4,8±0,4a
RSC309	37±2cd	41,30±3,2cd	3,9±0,3bc
RSC324	33±2de	40,20±1,6cd	3,1±0,3de
RSC502	27±4f	39,40±1,1d	3,1±0,5de
RSC504	50±3a	44,50±2,3ab	4,4±0,6ab
RSC508	38±3c	41±3,2cd	3,4±0,4cd
IRAT FA3	35±3cde	42,60±1,2bc	3,5±0,3cd
TN	5±4g	46,20±2a	4,8±0,2a
T0	3±3g	31,12±1,8e	2,4±0,3f
Mean	30±15	40,3±5,1	3,6±0,9
CV (%)	49,4	12,7	24
LSD (5%)	4,4	3,1	0,6

In each column, means followed by the same letter are not significantly different at 5% threshold according to Fisher's LSD test. T0: Untreated control; TN: Synthetic fertilizer NPK 12-22-22

3.2 Effect of Inoculation on Soybean Pods and Grain Yield

Local tested rhizobia improved significantly (P= .008) both number and weight of pods per plant compared to negative control (Table 2). The number and weight of pods varied respectively from 44 to 102 pods per plant and from 30.9 to 56.1 g for all treatments. Native rhizobia RSC508 induced both the most pods (102 pods) per plant and the highest pod weight (56.1 g). RSC119 and RSC309 also boosted pod production per plant compared with synthetic fertilizer and reference strain IRAT FA3. These rhizobia induced the production of 99 and 96 pods per plant respectively, compared to pods induced by synthetic fertilizer (80 pods per plant) and reference strain IRAT FA3 strain (85 pods per plant). Soybeans inoculated with local rhizobia showed an increase in both number and weight of pods compared with the negative control. Furthermore, plants inoculated in this study with local rhizobia RSC119, RSC309 and RSC508 produced more pods than the introduced strain *B. japonicum* IRAT FA3 and the synthetic fertilizer NPK 12-22-22. According to Abdel-Fattah et al. [25], the inability of synthetic fertilizer to increase the number and weight of pods compared with inoculation is due to their low availability in the soil solution. However, soybean plants inoculated with rhizobial strains may produce pod numbers that are statistically similar to those fertilized with synthetic fertilizers [26]. These results corroborate those obtained by Argaw [21] who compared the local Ethiopian *Bradyrhizobium japonicum* TAL-379 strain with an exotic *Bradyrhizobium* sp. strain UK isolate on soybean nodulation and growth.

Soybean seeds yield per hectare was significantly influenced by inoculation. Seeds yield per hectare varied from 0.7 t/ha to 4.3 t/ha. Seed yields were improved more by native rhizobia RSC119, RSC309 and RSC508. These rhizobia produced higher yields than synthetic fertilizer. Among these, RSC508 induced the highest yield of 4.3 t/ha compared with 3.5 t/ha for the positive control and 0.7 t/ha for the negative control. Patra et al. [27] achieved similar results with soybean cultivar PK-416 in India. According to Shahid et al. [28], the variation in seed yield between inoculated cultivars is largely due to the combined effect of bacterial isolates on vegetative and production parameters, which are themselves influenced by environmental conditions. Other authors mention that the increase in seed yield of different soybean varieties by rhizobial bacteria is linked to the low nitrogen content of tropical soils. This low nitrogen content in soils is at the origin of the initiation of symbiosis between the soybean and its symbiont [29,30]. However, according to N'Cho et al. [31] and Dabesa and Tana [32] inoculation of soybean seeds with the *Bradyrhizobium* strain alone did not increase soybean yields compared with synthetic fertilizer. They suggested that the bacterial inoculum should be combined with a phosphorus-based fertilizer to increase soybean yield.

Table 2. Effect of native rhizobia, exotic strain IRAT FA3 and synthetic fertilizer NPK 12-22-22 on the number and weight of pods and seed yield of soybean

Treatments	Pods		Seeds yield (t/ha)
	Number	Weight (g)	
RSC115	64±7c	37,1±2,6f	3,2e
RSC119	99±4a	52,9±1,5ab	3,7c
RSC309	96±8a	50,9±6,2bc	3,9b
RSC324	86±8b	47,4±4,4cd	3,5d
RSC502	68±3c	32,6±1,9gh	2,6f
RSC504	69±4c	35±1,3fg	3,2e
RSC508	102±9a	56,1±4,1a	4,3a
IRAT FA3	85±4b	48,8±0,8cd	3,6cd
TN	80±3b	45,8±1,9e	3,5d
T0	44±4d	30,9±1,81i	0,7g
Mean	79±18	43,7±9,1	3±0,5
CV (%)	23	20,9	36,3
LSD (5%)	10,2	4,2	0,2

In each column, means followed by the same letter are not significantly different at 5% threshold according to Fisher's LSD test. T0: Untreated control; TN: Synthetic fertilizer NPK 12-22-22

3.3 Background Effects of Local Rhizobia on Soil Properties

The fallowing of soils, which was the most important method for reconstituting soil fertility, almost no longer exists. To overcome this situation and increase agricultural production, farmers have turned to use of synthetic fertilizers with harmful consequences for human health and the environment. Actually, innovative agricultural practices capable of restoring soil fertility must be proposed in order to reduce synthetic fertilizers using in production systems. It is in this context that the effect of native rhizobia inoculated with soybean on soil quality has been assessed. Analyses carried out on soil samples taken 30 days after soybean harvest showed that inoculation had a significant influence (P =0.006) on the physico-chemical properties of the soil (Table 3).

All tested rhizobia tended to decrease the pH (H_2O) of the soil by 0.3 to 0.5 unit, except RSC508 strain which increased the pH by 0.2 unit compared to the initial soil state. The pH level induced by these bacteria was between 5.5 and 6.4. Results related to pH showed that some local rhizobia tended to make the environment more acidic. This soil acidity is always satisfactory for good biological activity. These results are not surprising because during rhizobia-legume symbiosis process, the different partners produce organic acids which tend to acidify the environment. In contrast, the local rhizobium RSC508 increased soil pH. This increase in soil pH is linked to several factors including the ability of the bacteria to induce the production of a large quantity of biomass.

Total Nitrogen (Nt), Organic Carbon (OC) and Organic Matter (OM) were significantly increased by native rhizobia RSC119 compared with the other treatments and the initial state of the soil. This strain induced 3.12% of OM against 1.27% for the initial soil. In addition, it induced the highest level of Available Phosphorus (Av.P) compared to all the treatments, with 64 mg.kg^{-1}. Moreover, RSC508 and RSC502 tended to maintain soil phosphorus levels. The C/N ratio was generally between 11 and 12 for all treatments except with RSC324, RSC502 and T0. Results obtained show that native rhizobia strains used as inoculum during soybeans crop establishment, have significant beneficial effects on the physico-chemical parameters of the soil 30 days after harvest. However, while soil organic matter (OM) and total nitrogen (Nt) levels increased, they did not reach the minimum threshold (2%) required for total nitrogen. These observations are in line with those made by N'Goran et al. [33] with an association of yam and soybean. The increase in these elements in the soil is due, among other things, to the early fall of soybean leaves before harvest. N'Diaye et al. [34] attributed the increase in soil organic carbon and Nt levels to a supply of organic matter coming from all parts of the plant. For these authors, soil carbon and nitrogen are closely linked to soil organic matter (OM) which gives the latter physico-chemical properties that promote the sustainable functioning of ecosystems. They pointed out that the different parts of a plant with different biochemical qualities have different levels of degradation, giving the soil a continuous supply of organic matter. This contribution of legumes to OM is responsible for the increase in corn productivity,

Table 3. Background-effects of native rhizobia inoculated to soybean on soil quality

Treatments	pH_{H2O}	OC (%)	N (%)	C/Nt	OM (%)	Av.P (mg.kg^{-1})
Initial soil	6,4a	0,7ef	0,06b	12c	1,27cd	66a
RSC115	5,9b	0,62f	0,06b	11d	1,07d	56bcd
RSC119	6b	1,81a	0,15a	12c	3,12a	64a
RSC309	6,1b	0,92cde	0,08b	11d	1,58bc	55bcd
RSC324	6,1b	0,76def	0,06b	14a	1,31cd	54cd
RSC502	6b	0,74ef	0,06b	13b	1,27cd	60abc
RSC504	6,1b	0,96bcd	0,08b	12c	1,64bc	50de
RSC508	6,6a	1,15b	0,10b	12c	1,98b	61ab
IRAT FA3	6,4a	0,98bc	0,08b	12c	1,68bc	56bcd
TN	6,4a	0,76def	0,07b	11d	1,31cd	46e
T0	6,1b	0,88cde	0,07b	13b	1,51cd	52de
Mean	6,2	0,9	0,08	12	1,6	56
CV (%)	8,1	33,3	20	16,7	31,2	10,7
LSD (5%)	0,3	0,2	0,05	1,0	0,4	6

In each column, means followed by the same letter are not significantly different at 5% threshold according to Fisher's LSD test. T0: Untreated control; TN: Synthetic fertilizer NPK 12-22-22

as demonstrated by Kouassi et al. [35]. Natural fallows generally play this same role at ground level, but it will still be necessary to observe for a longer period or even several years [36]. The C/N values obtained are generally between 9 and 12, reflecting effective mineralization of organic matter and intense microbial activity at the soil level. These results show the capacity of native rhizobia to induce significant production of soybean matter and an intensification of microbial activity. According to Konaté et al. [37], the acceleration of microbial biomass activity stimulates the decomposition and mineralization of pre-existing organic matter in the soil.

Assimilable phosphorus and total phosphorus levels decreased with all treatments. This drop in assimilable P could be linked to the sandy texture of the site soils. The sandy texture exposes the soil to leaching and the loss of mineral elements in general. This assertion partly justifies the results obtained by Kouadio et al. [38], who maintain that the leaching phenomenon to which sandy soils are subjected during the rainy seasons exposes them to nutrient depletion.

4. CONCLUSION

The present study evaluated the performance of native rhizobia on soybean productivity and soil fertility in Côte d'Ivoire. Results indicated that native rhizobia strains are efficient and competitive to enhance soybean nodulation, growth and yield. Native rhizobia strains RSC119 and RSC504 induced more nodules formation than the induced strain *Bradyrhizobium japonicum* IRAT FA3. Also, native rhizobia strains RSC119, RSC309, RSC504 and RSC508 induced more pods number and seed yield than synthetic fertilizer NPK 12-22-22 formulation. The highest number of pods per plant and seed yield were obtained with RSC508. Moreover; local rhizobia improved soil properties such as soil organic matter and total nitrogen on short periods after soybean harvest. Among these bacteria, the strain RSC508 increased soil pH. Results showed that soybean inoculation with infective and competitive local rhizobia strains significantly increased soybean productivity and helps to restore soil fertility over short periods.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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