

# Response of Indigenous Rhizobia to the Inoculation of Soybean [*Glycine max* (L.) Merrill] Varieties Cultivated under Controlled Conditions in Côte d'Ivoire

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### Abstract

Soybean [Glycine max (L.) Merrill] is an important crop known to improve population nutritional status and increase soil fertility and its productivity through biological nitrogen fixation. In Côte d'Ivoire, the introduced Bradyrhizobium japonicum used as inoculum had slight compatibility to several soybean varieties compromising their vulgarization. Therefore, the present study was conducted to examine the infectiveness and the effectiveness of indigenous rhizobial isolates on three soybean varieties (Canarana, Doko and Piramana) cultivated in Côte d'Ivoire. The experiment was conducted with potted plant filled with sterilized sand and was statistically laid in Completely Randomized Design (CRD) with sixteen (16) natives rhizobia, one (01) reference strain and uninoculated control (with or without nitrogen) with three replications. The results showed that inoculation significantly improved nodule number, nodule dry weight, plant height and total dry matter of soybean over the negative control treatment. Among the inoculated treatments, five indigenous rhizobia RSC119, RSC324, RSC502, RSC504 and RSC508 significantly (P < 0.05) increased the nodulation and plant growth parameters than B. japonicum (IRAT FA3) with Doko and Piramana. RSC502 produced highest nodule number (64) on Piramama, nodule dry weight was most promoted with RSC504 (321 mg·plant<sup>-1</sup>) compared to IRAT FA3 strain (95 mg·plant<sup>-1</sup>) on Doko. The higher effectiveness was recorded with RSC119, RSC504 and RSC502 with 206.73%; 201.79% and 200.45% respectively compared to TN

(100%). The correlation analysis indicated significant association of nodule number and total biomass indicating the importance of symbiotic nitrogen fixation. Based on their infectiveness and their effectiveness, the isolates RSC119, RSC324, RSC502, RSC504 and RSC508 could be used as elite local rhizobia and tested in field conditions to establish their potential contribution on soybean productivity.

## **Keywords**

Selection, Indigenous Rhizobia, Inoculation, Soybean, Côte d'Ivoire

# **1. Introduction**

Soybean [*Glycine max* (L.) Merrill] is one of the most important grain legumes cultivated in the world for its richness in protein (40%) and oil (20%). It is the most available crop that provides a cheap and high quality source of protein comparable to meat, poultry and eggs. It is used for production of cooking oil, human food, stock feed and industrial products [1] [2]. This legume was introduced in Côte d'Ivoire for crops diversification and its contribution to improve population nutritional status. Soybean also increases soil fertility and productivity through biological nitrogen fixation (BNF) [3].

The BNF plays an essential role in crop establishment and fulfills most of plants need for nitrogen. The amount of nitrogen fixed by soybean in symbiosis with *Bradyrhizobium japonicum* is estimated yearly to 450 kg·ha<sup>-1</sup> [4]. It considerably reduces the need of synthetic nitrogen fertilizers supply of crops which continuous application could be harmful to soil fertility and accelerate environmental pollution [5] [6] [7]. Thereby, maintaining this natural significant nitrogen input should be better for economical and ecological sustainable soybean yields. However, the ability of this crop to fix the desired amount of nitrogen depends on many factors such as the effectiveness of the rhizobia strain and plant varieties [8] [9].

In Côte d'Ivoire, initiative researches have been undertook to vulgarize soybean crop in several areas of the country. Hence, researchers have evaluated in these studies the effective compatibility of soybean varieties to the introduced *B. japonicum* strain IRAT FA3 used as inoculum [10] [11] [12] [13]. Results of these studies have shown that *B. japonicum* IRAT FA3 was compatible with some soybean cultivars than others, compromising therefore their vulgarization in the country. However, recent investigations reported by N'Gbesso *et al.* [14] and Amani *et al.* [15], have revealed that indigenous rhizobia isolated in Cote d'Ivoire soil was able to improve other soybean cultivars growth and productivity. Thus, the present investigation was carried out to 1) evaluate the infectiveness and the effectiveness of indigenous rhizobia onthreesoybeans [*Glycine max* (L.) Merrill] varieties under controlled conditions and to 2) select all strains that hold potential to be included in the inoculums formulations for soybean production under Ivorian agro-climatic conditions.

#### 2. Materials and Methods

#### 2.1. Biological Materials

Soybean [*Glycine max* (L.) Merrill] cultivars Canarana, Doko and Piramama provided by the National Agricultural Research Centre (CNRA) in Bouake, Côte d'Ivoire, were used as plant material in this experiment.

Sixteen (16) indigenous authentic rhizobiaisolated from soybean nodules in Côte d'Ivoire and one (01) reference strain (*Bradyrhizobium japonicum* IRAT FA3) were used in this work. The origins and the sources of these bacteria are described in **Table 1**.

#### 2.2. Treatments and Experiment Design for the Pot Experiment

A pot experiment was conducted under the controlled conditions at Jean Lorougnon Guede University in Daloa (Cote d'Ivoire). The pot experiment was statistically laid in Completely Randomized Design (CRD) on sterile sand with nineteen (19) treatments including sixteen (16) indigenous rhizobial isolates, one (01) reference strain and two uninoculated treatments (one (01) uninoculated and unfertilized treatment as negative control (T0) and one (01) uninoculated and fertilized treatment (TN) as positive control) in three replications [16].

# 2.3. Evaluation of Indigenous Rhizobial Isolates Infectivity and Effectiveness

Seeds of each soybean cultivars were sterilized in Mercuric chloride (0.1%) for two min and then thoroughly rinsing with six changes of sterile distilled water. Thereafter, four seeds were sown on plastic pots containing sterile sand. Seven

Reference strain and local isolates	Host plants (Soybean cultivars)	Origins	Sources	
Reference strain:				
<i>Bradyrhizobium japonicum</i> IRAT FA3	-	Montpellier (France)	[14]	
Local rhizobia isolates:				
RSC309; RSC310; RSC312;	Piramama	Daloa (Cote d'Ivoire)	[15]	
RSC323; RSC324; RSC325	r 11 ailiailia	Daloa (Cole d Ivolle)	[15]	
RSC114; RSC115; RSC119	Doko			
RSC207	Canarana			
RSC412; RSC413	RSC412; RSC413 IT 235		This study	
RSC502; RSC504; RSC506; RSC508	Tracaja		otady	

**Table 1.** Origins and host plants of the indigenous rhizobial isolates and reference strain used in this study.

RSC: Rhizobia isolated from soybean nodules in cote d'Ivoire.

days after sowing, each plant of a pot except the controls was inoculated with 1 mL of broth culture of each isolate beforehand grown on YEM liquid medium to exponential phase. Plants were supplied with distilled water every two days and they were saturated once a week with a nitrogen-free nutrient solution. Furthermore, TN control received weekly 0.05% (w/v) KNO<sub>3</sub> as nitrogen source. For each treatment, plants were harvested 45 days after sowing (DAS) and nodule number, Plant height and matter weight were evaluated per treatment and per variety. Matter (root and shoot) of each treatment was dried three days at 70°C and was used to calculate the Relative effectiveness (RE) of isolates according to Maâtallah *et al.* [17] by the following equation:

 $RE = \frac{Inoculated plant dry matter}{N-Fertilized plant dry matter} \times 100$ 

Nitrogen fixing effectiveness classified as: Ineffective (RE < 35%); Lowly-effective (35% < RE < 50%); Effective 50% < RE < 80%; and highly effective (RE > 80%). Relationship between nodule number-plant height and nodule number-total matter were examined.

## 2.4. Statistical Analysis

The data of measured parameters recorded were pooled together and subjected to statistical analysis using the STATISTICA program (7.1). Plant growth and nodulation parameters were subjected by analysis of variance. The difference between the treatments means were evaluated at 5% level of significance using Fisher's LSD test.

#### 4. Results and Discussion

# 4.1. Impact of Indigenous hizobia on Nodule Number and Nodule Dry Weight on Soybean Varieties

This study was performed in sterile sand to evaluate the infectivity of indigenous rhizobia isolates on three soybean varieties (Canarana, Doko and Piramama). The results revealed that inoculation was affected significantly (P < 0.05) nodule number and nodule dry masson soybean varieties (Table 2).

All indigenous rhizobial isolates were able to form nodules on tested varieties. In the other hand, uninoculated control did not show any nodule, demonstrated that aseptic conditions were met in the experimental set up. Results revealed that all indigenous isolates were able to form nodules on three tested varieties of soybean. In the other hand, uninoculated control did not show any nodule, demonstrated that aseptic conditions were met in the experimental set up. The same report was made by Amani *et al.* [15] and Kumar and Reddy [18] during authentification of soybean and French bean rhizobial isolates respectively, in the same conditions. Therefore, analysis of variance showed that nodulation varied significantly (P < 0.05) between tested isolates and soybean varieties.

The nodule number per plant ranged from 0 for isolates RSC114 and RSC207 to 52 for isolate RSC504 with Doko, from 0 for isolate RSC114 to 28 for RSC119

	5						
	Nodule Number			Nodule Dry Weight			
Treatments	(plant <sup>-1</sup> )			(mg·plant <sup>-1</sup> )			
	Doko	Canarana	Piramama	Doko	Canarana	Piramama	
RSC114	-	-	11 ± 2.08hi	-	-	80 ± 8.56f	
RSC115	39 ± 2.00c	10 ± 1.52ge	58 ± 4.51b	266.67 ± 8.77b	46.67 ± 5.77d	125 ± 8.23de	
RSC119	45 ± 3.00b	28 ± 4.00a	42 ± 2.38d	263.33 ± 6.70bc	80 ± 10.00a	253.33 ± 12.28a	
RSC207	-	10 ± 1.52ge	13 ± 3.05h	-	15 ± 5.00e	113.33 ± 8.79e	
RSC309	$40 \pm 3.05c$	16 ± 2.00d	42 ± 2.86d	250 ± 10.00c	53.33 ± 5.77d	133.33 ± 5.77cde	
RSC310	10 ± 1.52f	13 ± 2.00ef	38 ± 3.25de	166.67 ± 4.86e	45 ± 5.00de	130 ± 10.00cde	
RSC312	18 ± 2.65e	8 ± 1.73e	30 ± 2.08f	183.33 ± 5.77d	$15 \pm 2.50e$	116.67 ± 9.27e	
RSC323	$1 \pm 0.58h$	10 ± 1.53ge	36 ± 1.53e	10 ± 2.86j	15 ± 3.00e	120 ± 9.87e	
RSC324	35 ± 3.05d	18 ± 2.00d	50 ± 2.08c	110 ± 10.00f	66.67 ± 6.25b	213.33 ± 10.33b	
RSC325	7 ± 1.53fg	12 ± 2.00fg	39 ± 3.57de	73.33 ± 3.05h	36.67 ± 7.63e	146.67 ± 12.55cd	
RSC412	8 ± 1.53f	18 ± 2.00d	15 ± 0.58h	77.67 ± 2.52h	21.67 ± 2.89f	78 ± 5.77f	
RSC413	4 ± 1.53g	13 ± 2.00ef	9 ± 1.15i	46.67 ± 2.89i	21.67 ± 2.77f	80 ± 10.00f	
RSC502	46 ± 1.56b	15 ± 2.00de	64 ± 3.51a	181.33 ± 10.26d	56.67 ± 5.77cd	246.67 ± 8.31a	
RSC504	52 ± 2.00a	21 ± 3.00c	36 ± 1.53e	321 ± 13.26a	69 ± 8.89c	153.33 ± 5.77c	
RSC506	34 ± 1.73d	12 ± 2.00fg	26 ± 2.89g	190 ± 10.00d	36.67 ± 7.63e	146.67 ± 12.55cd	
RSC508	47 ± 2.52b	24 ± 4.00b	41 ± 3.05d	273.33 ± 5.77b	73.33 ± 7.73bc	193.33 ± 11.54b	
IRAT FA3	34 ± 1.73d	27 ± 5.00a	35 ± 1.53ef	95 ± 3.84g	89.67 ± 10.25a	133.33 ± 10.27cde	
TN	-	-	-	-	-	-	
T0	-	-	-	-	-	-	
LSD 5%	3.67	2.33	5	14.66	10	26.67	

**Table 2.** Impact of indigenous rhizobia, introduced strain and nitrogen fertilizer on nodule number and nodule dry weight on three soybean cultivars grown on sterile sand at flowering stage.

TN: uninoculated and fertilized control; T0: uninoculated and unfertilized control. Means in the same column followed by the same letter are not significantly different at the 5% probability level by LSD's test.

with Canarana and from 9 for isolate RSC413 to 64 for isolate RSC502. Thus, across all tested treatments, isolate RSC502 produced the higher nodule number

(64) than other tested isolates and the reference strain on Doko.

Considering the varieties, Piramama and Doko were more receptive to inoculation with local rhizobial isolates than Canarana. Indeed, RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508 induced the formation of high nodules relative to the introduced strain IRAT FA3 with varieties except Canarana. Tested isolates RSC508, RSC502 and RSC119 induced the largest number of nodulesper plant respectively 52; 47; 46 and 45 against 34 nodules for the strain IRAT FA3 on Doko. On Piramama, tested rhizobia strains RSC502, RSC115 and RSC324 were revealed more infective with 64; 58 and 50 nodules respectively against 35 to the strain IRAT FA3 (Figure 1). Hungria et al. [19] also reported that inoculation of soybean significantly increased the nodule number over the control. Attempts realized in Mozambique were also found that some indigenous rhizobia were effective than five reference strains used in the country to improve soybean production [20]. Unlike Doko and Piramama varieties, local rhizobia were unable to increase the number of nodules on Canarana compared IRAT FA3 excepted RSC119, which induced nodules production like the latter. Based on nodulation indigenous rhizobia RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508 were more infective than the introduced strain IRAT FA3. When the analysis of variance was performed separately for each cultivar, it appeared that Piramama and Doko had best response to inoculation



**Figure 1.** Nodules induced on roots of the soybean cultivars by local rhizobia. (a) Canarana cultivar without inoculation; (b) Canarana cultivar inoculated with isolate RSC119; (c) Doko cultivar inoculated with isolate RSC508; (d) Piramama cultivar inoculated with isolate RSC502.

comparing to Canarana. There were more nodules on these varieties than Canarana. The greater number of nodules due to inoculation suggested that there is better combining and symbiotic relationship between indigenous isolates and soybean cultivars Doko and Piramama. However, soybean cultivar Canarana with a fail response to inoculation may possess a particular Rj gene that play a role in controlling the plant's compatibility with specific rhizobial strains. In addition, indigenous tested rhizobia may show a preference for particular genotypes among the compatible genotypes [21] [22].

Such as nodule number, nodule weight of soybean varieties was significantly promoted by inoculation with local rhizobia (Table 2). However, it varied according to the treatment and the variety. In fact, with Doko variety, ten (10) local isolates increased the dry weight of the nodules compared to IRAT FA3. RSC504 induced the highest weight of nodules (32 mg·plant<sup>-1</sup>) than IRAT FA3 (95 mg·plant<sup>-1</sup>). Regarding Piramama variety, isolates RSC119, RSC324, RSC502 and RSC508 induced higher nodular dry weight compared to IRAT FA3 that was statistically similar to the weight produced by the isolates RSC115, RSC207, RSC309, RSC310, RSC312, RSC323, RSC325, RSC504 and RSC506. RSC119 promoted the highest nodule dry weight (253.33 mg·plant<sup>-1</sup>) than IRAT FA3 strain (113.33 mg·plant<sup>-1</sup>) and other isolates. Contrary to Doko and Piramama, all local tested isolates did notexhibited nodules dry weight higher than IRAT FA3 on Canarana variety except RSC 119 which favored identical nodule dry weight as this strain. Researchers such as Htwe et al. [23] were found the same results with other soybean cultivars. These authors exhibited that the local strains Bradyrhizobium spp. SHY6-1 and B. elkanii SAY3-4 isolated in Nyanmar have given the highest nodule dry weights than the exotic B. japonicum USDA110.

# 4.2. Indigenous Rhizobial Isolates Contribution on Plant Height and Matter Production

The results revealed that inoculation was affected significantly (P < 0.05) plant height and plant dry matter of soybean varieties (Table 3).

Inoculation of soybean varieties with native rhizobia and reference strain increased plant height and influenced total dry matter compared to the uninoculated negative control (**Table 3**). The table shows that local isolates RSC502 and RSC508 respectively provided the most plant height with Doko and Piramama followed to the positive control and other treatments. In example, the height of Doko plant inoculated with RSC502 was 84.47 cm against 64 cm for positive control (TN). The higher height with Piramama was recorded with the isolates RSC508 (55 cm) which was statistically similar to the isolates RSC119, RSC324, RSC325, RSC502 and RSC506. These isolates promoted plant height than the uninoculated and nitrogen fertilizer control (TN). None local rhizobia did not improve Canarana cultivar growth more than the positive control. Therefore, local isolate RSC324 and the reference strain IRAT FA3 were statistically identical

Treatments —	Plant Height (cm)			Plant Dry Matter (g·Plant <sup>-1</sup> )		
	Doko	Canarana	Piramama	Doko	Canarana	Piramama
RSC114	53 ± 3.61hi	29.97 ± 0.90fg	46.10 ± 1.01de	1.60 ± 0.35j	1.41 ± 0.02fg	2.57 ± 0.40cd
RSC115	73.13 ± 1.98bc	32.33 ± 2.52cdef	48.43 ± 1.25cd	4 ± 0.26b	$1.17 \pm 0.04$ gh	2.6 ± 0.10cd
RSC119	76 ± 2.00b	33.93 ± 2.30cd	51.93 ± 0.38ab	4.5 ± 0.5a	2.18 ± 0.13bc	4.17 ± 0.17a
RSC207	$55.10\pm4.71\mathrm{h}$	$29.80\pm0.72 \mathrm{fg}$	$44.10 \pm 1.45 ef$	$2.02\pm0.27\mathrm{i}$	1.77 ± 0.06de	$1.83 \pm 0.12e$
RSC309	67.40 ± 2.76cde	32.6 ± 0.53cdef	45.67 ± 0.47def	2.98 ± 0.06ef	1.98 ± 0.03cd	2.63 ± 0.21cd
RSC310	57.03 ± 0.95gh	31.67 ± 2.08cdefg	42.67 ± 1.52f	2.56 ± 0.16gh	1.66 ± 0.25gh	1.43 ± 0.12fg
RSC312	$56.27 \pm 1.55h$	30.27 ± 0.25efg	48.17 ± 2.75cd	2.69 ± 0.11fg	1.13 ± 0.06gh	$2.4 \pm 0.26$ d
RSC323	$48.23 \pm 1.32 \mathrm{i}$	31.20 ± 1.93defg	42.33 ± 2.31f	$1.62 \pm 0.06j$	$1.31 \pm 0.02$ gh	$2.4 \pm 0.26$ d
RSC324	65.83 ± 0.96def	$38.4\pm0.66a$	52.87 ± 2.05ab	3.52 ± 0.33cd	2.5 ± 0.43a	2.77 ± 0.40bcd
RSC325	63.40 ± 2.95efg	$30 \pm 0.56$ fg	53.50 ± 0.75ab	$2.23 \pm 0.25$ hi	$1.62 \pm 0.08ef$	2.38 ± 0.33d
RSC412	56.93 ± 4.15gh	30.26 ± 0.25efg	44.10 ± 1.45ef	2.54 ± 0.17gh	$1.14 \pm 0.03$ gh	1.47 ± 0.19efg
RSC413	59.17 ± 1.96fgh	29.06 ± 1.21g	43.37 ± 2.51ef	2.13 ± 0.21i	$1.28 \pm 0.03$ gh	1.8 ± 0.20ef
RSC502	$84.47 \pm 6.70a$	33.17 ± 1.61cde	54.23 ± 1.90ab	$4.47\pm0.30a$	1.71 ± 0.06def	2.63 ± 0.23cd
RSC504	76.17 ± 3.62b	$34.57 \pm 2.50b$	$47.50\pm0.50\mathrm{d}$	4.61 ± 0.14a	2.3 ± 0.10ab	2.5 ± 0.10cd
RSC506	68 ± 4.00cde	30.06 ± 1.01fg	53.67 ± 1.67ab	3.62 ± 0.15cd	1.85 ± 0.13de	2.87 ± 0.23bc
RSC508	70.67 ± 2.57bcd	32.33 ± 2.52cdef	55 ± 1.00a	$3.85 \pm 0.04 bc$	2.23 ± 0.06abc	$4.16\pm0.40a$
IRAT FA3	64 ± 4.36def	38.83 ± 3.56a	48 ± 4.33cd	3.33 ± 0.23de	2.46 ± 0.19ab	$3.13 \pm 0.15b$
TN	73.67 ± 3.78bc	37.53 ± 2.20ab	$51.20 \pm 3.14$ bc	2.23 ± 0.06hi	$2.5 \pm 0.43$ a	$3.07 \pm 0.15b$
T0	54.77 ± 3.68hi	$29.03\pm0.84g$	$38.67 \pm 2.08$ g	$1.40 \pm 0.20 \mathrm{j}$	$1.02\pm0.14h$	$1.12 \pm 0.10$ g
LSD 5%	6.87	3.23	3.4	0.41	0.31	0.44

**Table 3.** Impact of indigenous hizobia, introduced strain and nitrogen fertilizeron plant height and plant matter production on three soybean cultivars at flowering stage.

TN: uninoculated and fertilized control; T0: uninoculated and unfertilized control. Means in the same column followed by the same letter are not significantly different at the 5% probability level by LSD's test.

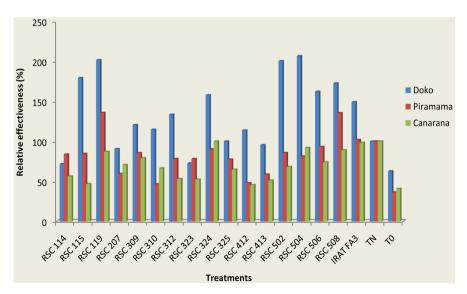
> with the positive control. Thus, soybean varieties inoculated with local rhizobia and the reference strain produced greater biomass compared to the negative control. Total biomass produced on Doko with isolates RSC504 (4.61 g·plant<sup>-1</sup>), RSC119 (4.5 g·plant<sup>-1</sup>) and RSC502 (4.47 g·plant<sup>-1</sup>) were greater than positive control (2.23 g·plant<sup>-1</sup>) and the reference strain IRAT FA3 (3.33 g·plant<sup>-1</sup>). Greater biomass increasing on Piramama were obtained with isolates RSC119 (4.17 g·plant<sup>-1</sup>) and RSC508 (4.16 g·plant<sup>-1</sup>) compared to TN (3.07 g·plant<sup>-1</sup>) and the reference strain IRAT FA3 (3.13 g·plant<sup>-1</sup>). Considering Canarana variety, local tested rhizobia did not produced greater biomass compared with positive control. Nevertheless, isolates RSC324 and RSC504 had stimulated similar effect with TN and IRAT FA3 (**Table 3**).

> The Results revealed that plant height and total biomass were significantly affected by inoculation on tested soybean varieties compared to uninoculated and unfertilized control. These results were similar to the findings of Tahir *et al.* [24] who reported that inoculation increase soybean growth in Pakistan. According to Sobral *et al.* [25], soybean growth promotion may be due to the capacity of

some Bradyrhizobium isolates to produce Indole Acid Acetic (IAA), solubilize phosphate and fix nitrogen. However, plant height and matter yield promoted by inoculation varied according to soybean cultivars. In the present study, the local rhizobia RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508 induced the higher plant height and improved total biomass than another. In addition, in comparison with the positive control (TN) representing the 100% level of plant total dry matter (biomass), most indigenous rhizobia tested showed higher biomass benefit. However, their relative effectiveness (RE) varied among soybean cultivars (Figure 2). Local isolates may be classed on three groups: highly effective (RE > 80%), effective (80% > RE > 50%) and slowly effective (RE < 50%). More than 88% of the isolates were found to be highly effective and 11% were effective on Doko. The higher effectiveness was recorded with RSC119, RSC504 and RSC502 with 206.73%, 201.79% and 200.45% respectively compared to TN (100%). Considering Piramama variety, 58% of local rhizobia were highly effective, 29% were effective and 11% were slowly effective. In opposite to Doko and Piramama, only 11% of local isolates were highly effective, 58% were effective and 11% were slowly effective on Canarana cultivar. Beyond soybean cultivars, isolates RSC115, RSC119, RSC324, RSC502, RSC504 and RSC508 were found to provide the best plant matter benefit on sterile sand. These results corroborated the finding of Guei et al. [26] who reported that nine local strains had good relative effectiveness than reference strain on Bambara groundnut cultivated in the Center West of Côte d'Ivoire.

# 4.3. Relationship between Nodule Production-Plant Height and Nodule Production-Total Dry Matter on Tested Soybean Cultivars

Correlation between nodule number-plant height and nodule number-total biomass was recorded in Figure 3. The relationship between nodule number



**Figure 2.** Relative effectiveness (RE) of indigenous rhizobia on soybean varieties on sand pot. RE = (Inoculated plant dry matter/N-fertilized plant dry matter)  $\times$  100.

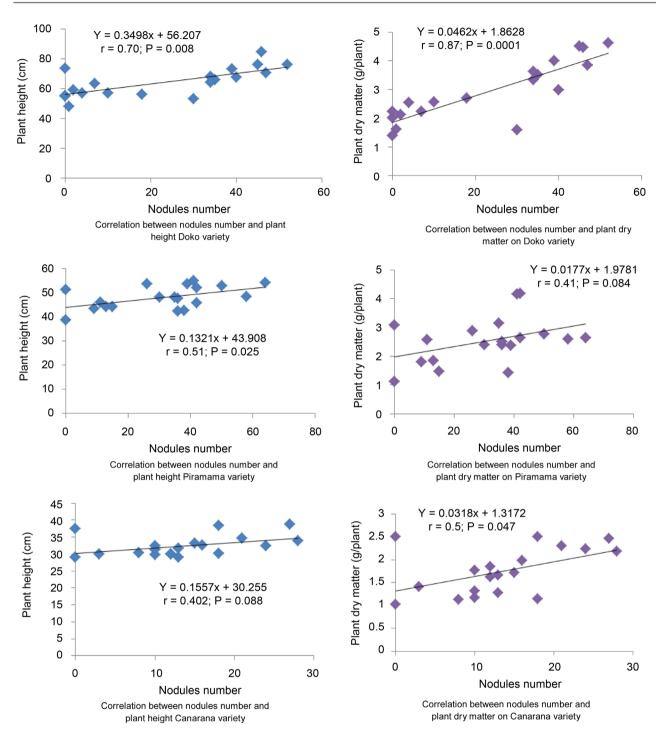


Figure 3. Correlation between nodulation and plant growth parameters of soybean cultivars grown in sterile sand.

with plant height was positively and significantly (P < 0.01) correlated on Doko and Piramama (r = 0.70 and r = 0.51 respectively) whereas, these parameters were negatively correlated on Canarana. A positive correlation was also recorded between nodule number and plant biomass on Doko (r = 0.87 and P < 0.001) and Canarana (r = 0.5 and P < 0.05) but negatively correlated on Piramama (r = 0.41 and P = 0.08). From the correlation analysis, it is observed that, both plant height and plant biomass had significant positive correlation nodule number. These results showed that nodule number is an important factor which affects soybean growth. That's have been previously reported by over authors [24] [27] [28]. Nevertheless, these results were contrary to those of Maâtllah *et al.* [17] who revealed that local rhizobia isolated in Morocco had increased matter advantage less than fertilizer application.

### 5. Conclusion and Perspectives

The results of present investigation demonstrated that the Ivorian soil contains native rhizobia capable of effectively inducing nodulation and improving the productivity of soybean varieties grown in Côte d'Ivoire. The three soybean varieties did not nodulate freely even without inoculation. Doko and Piramama nodulated better with the native rhizobia than Canarana variety. Indigenous rhizobia RSC115, RSC119, RSC324, RSC502, RSC504 and RSC508 increased nodule number (42 to 64) and nodule dry weight (213.33 to 321 mg·plant<sup>-1</sup>) than the reference strain IRAT FA3 (34 nodule per plant and 95 to 133.33 mg·plant<sup>-1</sup>) on Doko and Piramama. The higher effectiveness was recorded with isolates RSC119, RSC504 and RSC502 with 206.73%, 201.79% and 200.45% respectively compared to TN (100%) in the sterile sand. Based on the good symbiotic and agronomic performances of native rhizobia, six local isolates RSC115, RSC119, RSC324, RSC508 have been selected to examine their potential performances under reel field conditions in order to select the best indigenous isolates for local inoculum production.

# **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

#### References

- Fekadu, G., Hussein, M. and Getinet, A. (2009) Genotype x Environment Interactions and Stability of Soybean for Grain Yield and Nutrition Quality. *African Crop Science Journal*, 17, 87-99. <u>https://www.researchgate.net/publication/27792086</u>
- [2] Mahamood, J., Abayomi, Y.A. and Aduloju, M.O. (2009) Comparative Growth and Grain Yield Responses of Soybean Genotypes to Phosphorous Fertilizer Application. *African Journal of Biotechnology*, 8, 1030-1036.
- [3] Chen, L.S., Figueredo, A., Villani, H., Michajluk, J. and Hungria, M. (2002) Diversity and Symbiotic Effectiveness of Rhizobia Isolated from Field Grown Soybean Nodules in Paraguay. *Biology and Fertility of Soils*, 35, 448-457. https://doi.org/10.1007/s00374-002-0493-1
- [4] Zablotowicz, R.M. and Reddy, K.N. (2004) Impact of Glyphosate on the *Bradyrhi-zobium japonicum* Symbiosis with Glyphosate-Resistant Transgenic Soybean: A Mini-Review. *Journal of Environment Quality*, **33**, 825-831. https://doi.org/10.2134/jeq2004.0825
- [5] Yusuf, A.A., Iwuafor, E.N.O., Abaidoo, R.C., Olufajo, O.O. and Sanginga, N. (2009) Effect of Crop Rotation and Nitrogen Fertilization on Yield and Nitrogen Efficiency

in Maize in the Northern Guinea Savanna of Nigeria. *African Journal of Agricultural Research*, **4**, 913-921.

- [6] Hossain, A.K.M.M., Mian, M.H., Hakim, M.A., Islam, M.M. and Ferdous, J. (2012) Isolation and Selection of *Bradyrhizobium* from the Root Nodules of Indigo Plants (*Indigofera tinctoria* L.). *African Journal of Biotechnology*, **11**, 12183-12191. https://doi.org/10.5897/AJB12.1288
- [7] De Bruijn, F.J. (2016) "Biological Nitrogen Fixation" Book Summary. Advances in Microbiology, 6, 407-411. <u>https://doi.org/10.4236/aim.2016.66040</u>
- [8] Bhargava, Y., Murthy, J.S.R., Kumar, T.V.R. and Rao, M.N. (2016) Phenotypic, Stress Tolerance and Plant Growth Promoting Characteristics of Rhizobial Isolates from Selected Wild Legumes of Semiarid Region, Tirupati, India. *Advances in Microbiology*, 6, 1-12. <u>https://doi.org/10.4236/aim.2016.61001</u>
- [9] Sharma, S.R., Rao, N.K., Gokhale, T.S. and Ismail, S. (2012) Isolation and Characterization of Salt-Tolerant Rhizobia Native to the Desert Soils of United Arab Emirates. *Emirates Journal of Food and Agriculture*, 25, 102-108. https://doi.org/10.9755/ejfa.v25i2.7590
- [10] N'Gbesso, M.F.D.P., N'Guetta, A.S.P., Kouamé, N.C. and Foua Bi, K. (2010) Evaluation de l'efficience de l'inoculation des semences chez 11 génotypes de soja (*Glycine max* L. Merrill) en zone de savane de Côte d'Ivoire. *Sciences & Nature*, 7, 59-67. <u>https://doi.org/10.4314/scinat.v7i1.59931</u>
- [11] Ama-Abina, T.J., Beugre, G.F., N'Gbesso, M.F.D.P., Brou, N.D. and Yoro, G.R. (2012) Effets d'un herbicide et de l'inoculation sur les facteurs de rendement du soja cultivé sur un sol gravillonnaire de plateau. *International Journal of Biological and Chemical Sciences*, 6, 1970-1978. https://doi.org/10.4314/ijbcs.v6i5.7
- Beugre, F.G., N'Gbesso, P.D.F.M., Ama-Abina, J.T. and Yoro, R.G. (2013) Influence d'un herbicide et de l'inoculation des semences sur la croissance du soja [*Glycine max* L. Merrill] cultivé sur un sol gravillonnaire de plateau. *Agronomie Africaine*, 25, 221-229. <u>https://www.ajol.info/index.php/aga/article/view/100645</u>
- [13] N'Zi, J.-C., Koua, A.P., Kouassi, K.D., Kahia, J., Kouassi, J.-L., N'Guetta, A.S-P. and Kouamé, C. (2015) Effect of Inoculating Seeds with *Bradyrhizobium japonicum* on the Agronomic Performance of Five Varieties of Soybean (*Glycine max*) in Côte d'Ivoire. *African Journal of Agricultural Research*, **10**, 3671-3677. https://doi.org/10.5897/AJAR2015.10202
- [14] N'Gbesso, M.F.D.P., Fondio, L., Coulibaly, N.D. and Kouame, N.C. (2017) Efficacité symbiotique de cinq souches locales de rhizobiums sur les paramètres de croissance du soja. *International Journal of Biological and Chemical Sciences*,11, 2327-2340. https://doi.org/10.4314/ijbcs.v11i5.30
- [15] Amani, K., Konate, I., N'Gbesso, M.F.D.P., Attien, Y.P., Fondio, L., Filali-Maltouf, A. and Tidou, A.S. (2019) Phenotypic and Symbiotic Diversity of Rhizobia Isolated from Root Nodules of Soybean [*Glycine max* (L.) Merrill] in Côte d'Ivoire. *International Journal of Current Microbiology and Applied Science*, 8, 766-774. <u>https://doi.org/10.20546/ijcmas.2019.803.094</u>
- [16] Somasegaran, P. and Hoben, H.J. (1994) Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer-Verlag, Inc., New York, 450. <u>https://doi.org/10.1007/978-1-4613-8375-8</u>
- [17] Maâtallah, J., Berraho, E.B., Munoz, S., Sanjuan, J. and Lluch, C. (2002) Phenotypic Characterization of Rhizobia Isolated from Chickpea (*Cicer arietinum* L.) Growing in Moroccan Soils. *Agronomie*, 22, 321-329. <u>https://doi.org/10.1051/agro:2002013</u>
- [18] Kumar, N.R. and Reddy, R.S. (2018) Screening of Bradyrhizobial Isolates for Plant

Growth Promoting Properties *in Vitro* Conditions. *International Journal of Current Microbiology and Applied Science*, **7**, 2232-2237. https://doi.org/10.20546/ijcmas.2018.710.256

- [19] Hungria, M., Chueire, L.M.O., Megías, M., Lamrabet, Y., Probanza, A., Guttierrez-Mañero, F.J. and Campo, R.J. (2006) Genetic Diversity of Indigenous Tropical Fast-Growing Rhizobia Isolated from Soybean Nodules. *Plant & Soil*, 288, 343-356. <u>https://doi.org/10.1007/s11104-006-9125-0</u>
- [20] Chibeba, A.M., Kyei-Boahen, S., Guimarães, M.F., Nogueira, M.A. and Hungria, M. (2017) Isolation, Characterization and Selection of Indigenous *Bradyrhizobium* Strains with Outstanding Symbiotic Performance to Increase Soybean Yields in Mozambique. *Agriculture, Ecosystems and Environment*, **246**, 291-305. https://doi.org/10.1016/j.agee.2017.06.017
- [21] Saeki, Y., Akagi, I., Takaki, H. and Nagatomo, Y. (2000) Diversity of Indigenous Bradyrhizobium Strains Isolated from Three Different Rj-Soybean Cultivars in Terms of Randomly Amplified Polymorphic DNA and Intrinsic Antibiotic Resistance. Soil Science & Plant Nutrition, 46, 917-926. https://doi.org/10.1080/00380768.2000.10409157
- [22] Saeki, Y., Kaneko, A., Hara, T., Suzuki, K., Yamakawa, T., Nguyen, M.T., Nagatomo, Y. and Akao, S. (2005) Phylogenetic Analysis of Soybean-Nodulating Rhizobia Isolated from Alkaline Soils in Vietnam. *Soil Science & Plant Nutrition*, **51**, 1043-1052. <u>https://doi.org/10.1111/j.1747-0765.2005.tb00143.x</u>
- [23] Htwe, A.Z., Yamakawa, T., Moe, K. and Dien, D.C. (2015) Symbiotic Effectiveness of Different Indigenous *Bradyrhizobium* Strains on Selected *Ri*-Genes Harboring Myanmar Soybean Cultivars. *African Journal of Microbiology Research*, 9, 2345-2353. <u>https://doi.org/10.5897/AJMR2015.7751</u>
- [24] Tahir, M.M., Abbasi, M.K., Rahim, N., Khaliq, A. and Kazmi, M.H. (2009) Effect of *Rhizobium* Inoculation and NP Fertilization on Growth, Yield and Nodulation of Soybean (*Glycine max* L.) in the Sub-Humid Hilly Region of Rawalakot Azad Jammu and Kashmir, Pakistan. *African Journal of Biotechnology*, 8, 6191-6200. <u>https://doi.org/10.5897/AJB09.1039</u>
- [25] Sobral, J.K., Welington, L., Araújo, W.L., Mendes, R., Geraldi, I.O., Kleiner, A.A.P. and Azevedo, J.L. (2004) Isolation and Characterization of Soybean Associated Bacteria and Their Potential for Plant Growth Promotion. *Environmental Microbiology*, 6, 1244-1251. <u>https://doi.org/10.1111/j.1462-2920.2004.00658.x</u>
- [26] Guei, N.K.R., Konate, I., Bakayoko, S., Ouattara, A., Amani, K., Tidou, A.S. and Filali-Maltouf, A. (2019) Nodulation and Agronomic Performance of Indigenous Rhizobia Isolated from Bambara Groundnut (*Vigna Subterranea* L.) Nodules in Daloa, Côte d'Ivoire. *International Journal of Research Studies in Microbiology and Biotechnology*, 5, 13-18. <u>https://doi.org/10.20431/2454-9428.0502004</u>
- [27] Alam, F., Bhuiyan, M.A.H., Alam, S.S., Waghmode, T.R., Kim, P.J. and Lee, Y.B. (2015) Effect of Rhizobium sp. BARIRGm901 Inoculation on Nodulation, Nitrogen Fixation and Yield of Soybean (Glycine max) Genotypes in Gray Terrace Soil. *Bioscience, Biotechnology, and Biochemistry*, **79**, 1660-1668. https://doi.org/10.1080/09168451.2015.1044931
- [28] Lamptey, S., Ahiabor, B.D.K., Yeboah, S. and Osei, D. (2014). Effect of *Rhizobium* Inoculants and Reproductive Growth Stages on Shoot Biomass and Yield of Soybean (*Glycine max* (L.) Merril). *Journal of Agricultural Science*, 6, 44-54. <u>https://www.researchgate.net/publication/271826327</u> <u>https://doi.org/10.5539/jas.v6n5p44</u>