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Morpho-Physiological and Biochemical Characterization of Soybean-Associated Rhizobia and Effect of Their Liquid Inoculant Formulation on Nodulation of Host Plants in the Cameroon Cotton Fields Zone

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Abstract

The aim of this research was to assess the diversity of the Cameroon cotton zone in soybean associated rhizobia in order to formulate the most efficient elite inoculant to boost both the cotton and soybean production. Therefore, soybean associated rhizobia were isolated and characterized morphologically, physiologically and biochemically on YEMA culture media. For each of the two soybean varieties (Houla1 and TGX1910 14F) used, the trials were laid out in two IRAD-fields of North Cameroon (Sanguere-Paul) and Far-North (Soukoundou) respectively, under a complete randomized complete block design, the isolate formulations representing the treatments. The six isolated strains (IS1, IS2, IS3, IS4, IS5, IS6) from which seven liquid inoculant were formulated were revealed to belong to the same slow growing group of rhizobia, with a high level of tolerance to temperature, pH, and salinity, with optimum growth at respectively 28˚C, pH (7 - 9), salt (1% - 5%). Not surprisingly, root nodules were formed by both inoculated and uninoculated soybean plants. However, the most efficient soybean-rhizobia symbiosis for nodulations were isolate IS6 associated to TGX1910 14F variety, and isolate IS5 associated to Houla1variety at Sanguere-Paul. Whereas isolate M was associated to TGX1910 14F variety, Houla 1 variety had affinity with native rhi-

zobia isolates at Soukoundou. The present results suggest the adaptability of rhizobia isolates to a particular soybean variety at a particular cotton fields zone. These findings should be taken into consideration for commercial inoculant formulation.

Keywords

Isolation, Morphologic Characterization, Soybean-Associated Rhizobia, Nodulation, Cameroon Cotton Zone

1. Introduction

Biological diversity is referred to as the diversity of ecosystems, species, as well as the genetic diversity. Between individuals of the same species, several genes are differently expressed, contributing to the emergence several life forms, phenotypes, physical and biological characters. Apart from specific pre-occupations related to bacterial taxonomy, it is often necessarly to identify and differenciate bacteria strains, in order to explore their richness in a study site, as well as their functions such as detoxifyers, nitrifyers, symbiotic nitrogen fixers, or metabolites producers [1]. With the progress in bacteria taxonimy, it has clearly appeared that a host plant can establish symbiosis with several microbial symbionts. As far as soybean is concerned, this host plant can be nodulated by up to six rhizobia isolates, belonging to three different genera namely *Bradyrhizobium* (*japonicum*, *elkanii*, *liaoningense*), *Sinorhizobium* (*fredii*, *xinjiangensis*), *and Mezorhibium tianshanense* [2]. Soybean was reported to establish symbiosis not only with slow growing rhizobia (*Bradyrhizobium* spp.) but also with fast growing rhizobia (*Ensifer fredii*/*Rhizobium tropici*) [3]. For the success of inoculation, the quality of inoculants in terms of competitiveness of the bacterial symbiont and the specificity between the host-rhizobia partners are the driving factors [4]. Inoculation of crop legumes with microbial symbionts adapted to environmental conditions seems to be an efficient approach for nitrogen fixation and growth improvement of crop legumes [5]. Therefore, the isolation and characterization of local rhizobia strains is an important step towards their best utilization for improved monitoring of on-fields experiments. To address the issue, we hypothesized that the cotton field zones of North-Cameroon contain the rhizobia diversity to enable improved host soybean production, and the soil fertility in nitrogen for cotton cultivation. In the present study, the morpho-physiological and biochemical characteristic of soybean-associated rhizobia and their ability to nodulate the host plant are assessed and discussed.

2. Material and Methods

2.1. Study Sites and Their Climatic Characteristics

The cotton zone of North-Cameroon is characterized by a climate of sudanean

type (precipitation is 1200 mm, temperature is 28˚C, evapotranspiration is 168 mm/month, insolation annual is 2800 hours, cycle cultural cycle of 175 days) within the meridional part comprising the Touboro and Garoua regions. In the north to which belong Guider and Maroua regions, the Sudano-Sahelian type of climate is predominant (precipitation is 700 - 850 mm, temperature is 27˚C - 28˚C, evapotranspiration is 173 mm/month, insolation annual is 2800 hours, cycle cultural cycle of 130 days) [6].

2.2. Biological Material and Inoculants Formulation

The tow soybean varieties used in this study were tested and vulgarized by the institute of Agricultural Research and Development (IRAD), and bear the following characteristics (**Table 1**).

Soils used to trap rhizobia in a pot experiment were sampled in the four cotton zones of North-Cameroon represented by fields at Touboro, Garoua, Guider and Maroua. A total of 15 soils samples were collected respectively at Yagoua, Mouhour, kodeck, Zibou for Maroua zone, at Soukoundou, Sorawel, Doundehi for Guider zone, at Gashiga, Adoumri, Pitoa, Sanguere-Paul for Garoua zone, and at Touboro, Ouro-Barka, Vogzom, Sackdje for Touboro zone. Soil samples from the same zone were mixed in a composite sample. Rhizobia were trapped from composite soils by sowing soybean seeds in pots and allowing plants to grow. At 45 days after sowing, plants were removed and the efficient nodules selected for rhizobia isolation [7]. Rhizobia were isolated from deshydrated root nodules on YEMA (Yeast Extract Mannitol Agar) medium composed of Yeast Extract Mannitol Agar (YEMA) medium was prepared as previously described with the following composition [8]: 10 g mannitol; 0.2 g $MgSO_4 \cdot 7H_2O$; 1 g yeast extract; 0.5 g KH_2PO_4/K_2HPO_4; 0.1 g NaCl; pH = 6.8. The medium was supplemented with 0.25% Congo red to restrict possible contaminants. Isolatation of rhizobia was carried out after sterilization and grinding of root nodules in a Petri dish using a sterile carpel, aliquot of the macerate was taken and aseptically struck onto the YEMA solid medium. Inoculated petri dishes were incubated at 28˚C ± 2˚C temperature. Three days after the first streak, different colonies grown separately were sub-cultured onto the YEMA-Congo Red solid medium under the same conditions, in order to screen all the single colonies. Three days after sub-culture, the morpho-cultural characteristics of isolated colonies were described before the study of their physiological and biochemical characteristics [9].

Table 1. Characteristics of soybean varieties used in the study [10].

Varieties	Code	Origin	Growing cycle (days)	Yield (kg/ha)	Growing zones
Houla 1	T4	Cameroon	105	2000	Far-north
TGX 1910 14 F	T12	IITA	120	3000	North

2.3. Screening of Rhizobia Strains

Three days after incubation at 28˚C ± 2˚C on YEMA growth medium in the dark, different typical colonies were identified, counted and their morphological criteria (shape, size, appearance, elevation, surface, color and edge) assessed as reported [11]. Rhizobia strains were screened through Congo red test, Bromothymol blue tests, Gram staining and cell observation under photonic microscope [8].

2.4. Determination of Physiological and Biochemical Characteristics of Isolates

Isolated rhizobia were studied for their resistance to different stresses, by streaking on YEMA media at different NaCl concentrations (1%, 2%, 3%, 5% and 10%), pH (4, 7, 8, 9, 11) and temperatures (28˚C, 30˚C, 35˚C, 38˚C and 42˚C) [8] [11]. After growth of isolates on YEMA solid media at 28˚C ± 2˚C for 3 days, the catalase test was carried out by adding 2 - 3 drops of hydrogen peroxide (3%) on cultures. The formation of gas bubbles was an indication of the presence (+) or the absence (−) of catalase produced by isolates [12]. The ability of rhizobia isolates to hydrolyze starch was assessed by adding 0.2% starch powder to nutrient agar medium plates of each isolates. Labelled petri dishes were then incubated at 28˚C ± 2˚C for 3 days, after which the isolates competences to hydrolyze starch through amylase was detected by flooding plates with Gram's iodine. The presence (+) or absence (−) of halos around the rhizobia colonies was recorded [13]. The triple sugar ion agar test was performed on triple sugar iron agar medium (3 g/L beef extract, 3 g/L yeast extract, 15 g/L peptone, 5 g/L NaCl, 10 g/L lactose, 10 g/L sucrose, 1 g/m dextrose, 0.2 g/L ferrous sulphate, 0.3 g/L thiosulphate, 0.24 g/L phenol red, 15 g/L agar, pH 7.0) to determine the behavior of isolates to use various carbohydrate sources for their growth. The test was carried out to distinguish among rhizobia groups able to ferment glucose, while producing acid and hydrogen sulphide [14]. Ammonia production by isolates was assessed, while adding 2% urea and 0.012% Phenol Red to YEMA medium [15]. Following sterilization by filtration, 10 µL of each isolate was transferred by streaking on the solid medium, and incubated at 28˚C ± 2˚C for 3 days. The shift of the initial medium color from red to pink was an indication of the hydrolysis (+) of urea to form ammonia and carbon dioxide in an alkaline environment, while a yellowish color was a sign of a negative reaction (−) in an acidic environment.

2.5. Assessment of Plant Growth Promoting Traits

Ammonia production by rhizobia isolates was assessed on 0.5 mL Peptone broth medium (1 g peptone, 0.5 g NaCl, 0.5 g potassium nitrate, and 1000 mL distilled water), dispersed in each labelled test tubes with a control. Following incubation for 3 days, 1 mL of Nessler's reagent was added to each of the rhizobia culture in the test tubes, then development of color from orange to brown was recorded as

an indication of the presence (+) of ammonia [16]. The qualitative analysis of Indole Acetic Acid (IAA) production by rhizobia isolates was carried out in tubes culture in each of which was added 50 µg/mL filtered tryptophan broth solution. Following incubation at 28˚C ± 2˚C for 3 days of tubes containing different isolates including the negative control, 4 mL of the reagent was added to 1 mL of the supernatant, and the solution was thoroughly mixed and incubated for 30 min to allow development of pink color as an indication of IAA production (+) by isolates [17].

2.6. Formulation of Rhizobia Inoculants

From the six rhizobia strains isolated from pot experiment, seven rhizobia inoculants namely S0, IS1, IS2, IS3, IS4, IS5, IS6 and M were prepared as liquid formulations, 1 mL of each containing averagely 1×10^9 cells [7].

2.7. Experimental Design in the Field

The experimental fields were established at Soukoundou, and Sanguere-Paul, belonging respectively within the Guider and Garoua cotton field zones. The fields trials were established in a completely randomized block design for each of the soybean Houla1 (V1) and TGX1910 14F (V2) varieties, each comprising 7 treatments represented by the 7 rhizobia formulations IS0, IS1, IS2, IS3, IS4, IS5, IS6 and M. IS0 was the negative control, while M was a mixture of the six rhizobia inoculants at equal volume. Inoculation at sowing consisted of introducing two seeds in a sowing hole, and wet it with 2 mL of the corresponding inoculant. Seed of the control treatment were sown without inoculation.

2.8. Assessment of the Responses of Soybean to Nodulation

At 35 days (at flowering) 60 days (at podding) after sowing, 30 randomly selected plants per treatments were carefully removed from soil, and their total root nodules counted. The efficiency of a nodule to fix atmospheric nitrogen was assessed by cutting with a laser blade all root nodules from a plant to observe the color of the leghemoglobin that respectively indicates if the host plant is not fixing (whitish) or is fixing (greenish) atmospheric nitrogen [7]. The dry weigh of root nodules was obtained after measurement on an electronic balance.

2.9. Statistical Analysis

The effects of different treatments were analyzed by One-way ANOVA, using the plant weights and the nodule weights as variables. Means between treatments were graded using the Duncan multiple range test, through the STATGRAPHICS 5.0 program.

3. Results and Discussions

3.1. Differences in Morphological Traits of Rhizobia Isolates

At the end of the 72 hours incubation at 28˚C on YEMA medium of struck cul-

ture, 16 rhizobia colonies (Figure 1) were identified and grouped into 6 isolates based on their morphological characteristics, of which isolates IS1, 1S2, 1S3 and IS4, IS5, IS6 were respectively from Houa1 and TGX 1910 14F rhizospheres (Table 2). Isolate 1 (IS1) was a composite of three (circular, translucid and convex) colonies representing 18.75% of all the colonies. Isolate 2 (IS2) was represented by three (circular, white and convex) colonies (18.75%). Isolate 3 (IS3) was composed of six colonies (37.5%) circular, milklish and convex. Isolate 4 (IS4) was made up of two (12.5%) ovoid, milklish and convex colonies. Isolate 5 (IS5) and 6 (IS6) were represented each by only one colony, respectively ovoid, yellowish, flat or circular, whitish and flat.

Of all the sixteen identified colonies, 81.25% were circular, whereas 87.5% were convex in elevation. Translucid colonies were less represented. These morphological characteristics referred to as slow growing rhizobia colonies, which were reported to be mostly circular and convex, and rarely translucid [18]. The average size of colonies ranged from 2 to 7 mm after 3 days of incubation on YEMA medium. The present size range of colonies was higher than the one previously pointed out, which reported the size of slow growing rhizobia colonies between 1 and 2 mm after 3 - 5 days of incubation [18] [19].

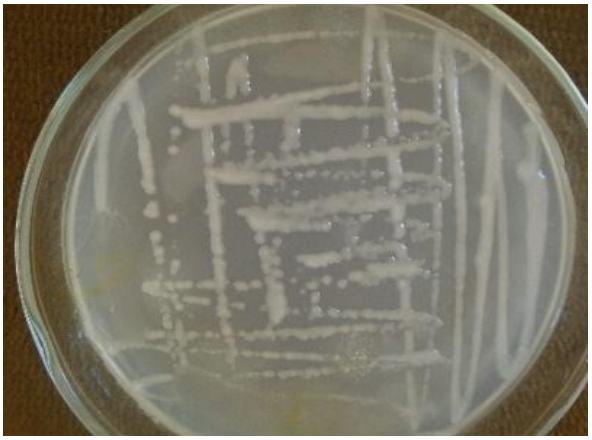


Figure 1. Rhizobia colonies growing on YEMA medium.

Table 2. Number and morphology of rhizobia isolates.

Isolates	Form	Color	Elevation	Number of colonies	Diameter of colonies (mm)	Sampling sites
IS1	Circular	Translucid	Convex	3	2.3	TB, GA, GD
IS2	Circular	White	Convex	3	2.0	GD, MA
IS3	Circular	Milklish	Convex	6	2.5	GA, GD, MA
IS4	Ovoid	Milklish	Convex	2	7.0	TB, GA
IS5	Ovoid	yellowish	Flat	1	3.0	TB
IS6	Circular	whitish	Flat	1	6.0	TB

TB: Touboro; GA: Garoua; GD: Guider; MA: Maroua.

3.2. Tolerance of Isolates to Different Temperatures, pH and Salt Ranges

The six identified rhizobia isolates were incubated for 3 days at 25˚C, 28˚C, 30˚C and 42˚C each, to test their ability to resist to heat (Table 3).

All the isolates grew well at 28˚C, confirming previous reported results indicating that optimal growth of rhizobia occurs at between 25˚C - 30˚C [20]. The optimal growth of isolates IS1, IS3 and IS5 was still maintained at 25˚C, compared to that of isolates IS2, IS4 and IS6. Above 28˚C, rhizobia isolates maintained a good growth, except isolate IS4 for which a weak growth was observed at 42˚C, in accordance to other results from which some rhizobia strains were able to display growth at between 40˚C - 45˚C [21]. Moreover, slow growing rhizobia were reported to be more thermo-tolerant than fast growing rhizobia [22]. When rhizobia isolates were tested for growth at different pH, no growth was observed at pH 3. Although all isolates did grow well at pH 4, the maximum growth was observed at pH 7 for all the isolates, confirming the neutroplilic behavior of most of the isolated rhizobia [20]. In contrast, the optimum growth of rhizobia found in *Calycotome spinose* rhizosphere was said to be located between pH 4 - 6.8 [23]. These differences could be ascribed not only to the intrinsic characters of isolates, but also to the environmental condition factors. In this study, all the isolates expressed growth for a wide pH spectrum extending from

Table 3. Variation of growth between rhizobia isolates at different physiologic parameters.

Tests	Ranges	IS1	IS2	IS3	IS4	IS5	IS6
Temperature	25˚C	+++	++	+++	++	+++	++
	28˚C	+++	+++	+++	+++	+++	+++
	30˚C	++	++	++	++	++	++
	37˚C	++	++	++	++	++	++
	42˚C	++	++	++	+	++	++
pH	3	–	–	–	–	–	–
	4	++	++	+++	++	+++	++
	7	+++	+++	+++	+++	+++	+++
	9	++	++	+++	+++	++	++
	11	++	++	++	++	++	++
Salt	1%	+++	+++	+++	+++	+++	+++
	2%	+++	+++	+++	+++	+++	+++
	3%	+++	+++	+++	+++	+++	+++
	5%	+++	+++	+++	+++	+++	+++
	10%	–	–	–	–	–	–

(+++): very good growth; (++): Good growth; (+): weak growth; (–): no growth.

4 - 11, thus close to pH range 4 - 10 reported to be tolerated by some rhizobia [24]. Fast growing rhizobia were revealed to be more sensible to acidity than slow growing rhizobia [12]. As far as tolerance to salt concentrations is concerned, all the identified rhizobia isolates showed a good growth from 1% to 5% NaCl, but an alteration of growth at 10% NaCl, in agreement with results of other authors [25].

3.3. Means of Identification of Rhizobia Isolates

Isolated bacteria were characterized on YEMA medium, supplemented either with Bromothymol Blue (BBT) or Congo Red (CR) as shown on **Table 4**. After BBT reaction, all the isolates shifted the medium from green (BBT color) to blue, indicating that they were all not acid producers, thus were slow growing rhizobia [26]. These results obtained from a different agro-eco-zone are opposite to those of Gomoung *et al.* [27], who isolated fast growing rhizobia (acid producers) associated to soybean in the guinea-savannah zone of Cameroon. These results indicate the ability of soybean to establish symbiosis either by slow/or fast-growing rhizobia or both. The microscopic obervations of cells after Gram staining revealed all the isolated rhizobia to be of rod-shape, and pink color, indicating that they were Gram negative bacteria [7] [8]. These results line with those recently reported and indicating Gram negative rhizobia-associated to nodulation of *Senegalia senegal*, *Vachellia seyal*, *Cajanus cajan L.* and *Vigna unguiculate.* [28].

Table 4. Identification criteria of rhizobia isolates.

Isolates	RC	BBT	Cells color	Cells form	Gram staining types
ISI	-	Blue	Pink	Rod-shape	-
IS2	-	Blue	Pink	Rod-shape	-
IS3	-	Blue	Pink	Rod-shape	-
IS4	-	Blue	Pink	Rod-shape	-
IS5	-	Blue	Pink	Rod-shape	-
IS6	-	Blue	Pink	Rod-shape	-

RC: Congo Red; BBT: Bromothymol Blue; Gram staining types. The negative (−) signs for CR signify that isolates absorb very weakly RC and are Gram negative.

3.4. Variation of Biochemical Characteristic between Rhizobia Isolates

Isolates IS2 and IS4 used starch, whereas IS3, IS4 and IS5 were the only isolates able to utilize triple sugars for their growth (**Table 5**). Citrate was used for growth by almost all the six rhizobia isolates, except isolate IS6. All the isolates were unable to produce catalase for detoxification of hydrogen peroxide, but were able to produce ammonia (NH3). Isolates IS1, IS3 and IS5 were able to provide a good production of the growth regulator IAA, against a weak production by isolates IS2, IS4 and IS6.

Table 5. Differential uses of carbon and nitrogen by isolates.

Isolates	catalase	amylase	Triple sugars	Citrate	IAA	NH_3
IS1	−	−	−	+	+	+
IS2	−	±	−	+	±	+
IS3	−	−	+	+	+	+
IS4	−	+	+	+	±	+
IS5	−	−	+	+	+	+
IS6	−	−	−	−	±	+

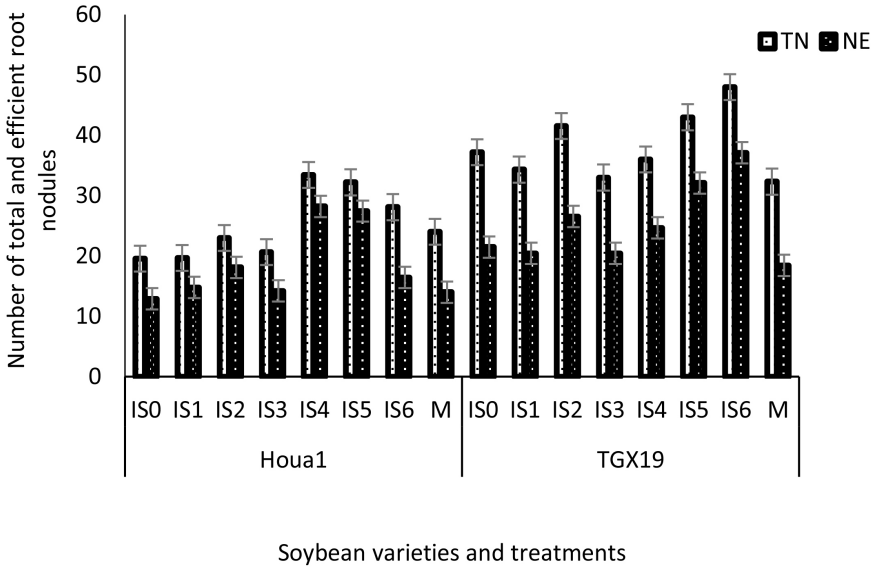
(+): good production; (±): weak production; (−): no production, NH3: Ammoniac; IAA: Indole Acetic Acid.

3.5. Nodulation Responses of the Two Soybean Varieties to Inoculation with Rhizobia Isolates as Liquid Formulations

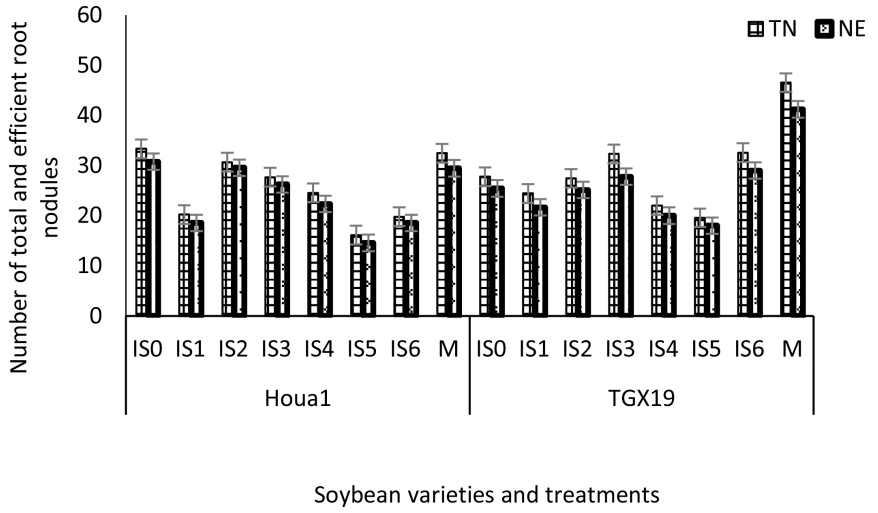
Not only all inoculated plants with rhizobia formulations were stimulated to produce root nodules, but also control plants that were not inoculated (IS0), respectively at Sanguere-Paul (**Figure 2(a)**) and Soukoundou (**Figure 2(b)**). The formation of root nodules by uninoculated plants was an indication of the presence of native rhizobia strains which could be specific and efficient or/not to the soybean varieties [7] [29]. The greatest efficiency of nodules expressed by uninoculated plants would indicate the high competitiveness of indigenous isolates compared to the introduced ones [30]. Defined as the capacity of a bacteria strains to form root nodule with its host partner, the infectivity was measured by the total number of nodules found on roots. In contrast, the efficiency of isolates was their ability to effectively fix atmospheric nitrogen, and was measured in this case by the greenish color of the leghemoglobin pigment inside the root nodules [31] [32].

At Sanguere-Paul, soybean plants of the variety Houla 1 inoculated with IS4 and IS5 isolates were revealed to significantly ($p = 0.019$) bear more efficient root nodules than control plants, meaning that these two strains were more competitive than the native strains, thus able to improve biological nitrogen fixation following inoculation. When inoculated with IS6 isolate, soybean variety TXG 1910 14F formed more efficient root nodules than the native isolates IS0, or IS1, IS2 and the mixture of isolate (M). These findings suggest that the ability of a rhizobia isolate to efficiently fix atmospheric nitrogen may differ for the same host plant from one host variety to another, and from one rhizobia isolate to another even for the same host variety. At Soukoundou**,** soybean varieties Houla 1 and TGX 1910 14F were very much affected by inoculation with rhizobia isolates.

For soybean variety Houla 1, the native rhizobia were more infective and efficient as the mixture of rhizobia isolates (M) than the other isolates. As far as soybean variety TGX 1910 14F is concerned, the rhizobia isolates mixture significantly increased the number of root nodules per plant ($p = 0.0001$), as well as



(a)



(b)

Figure 2. Effects of rhizobia liquid inoculant formulations on plant nodulation of soybean varieties Houla 1 and TGX 1910 14F at Sanguere-Paul (a) and Soukoundou (b). TN: Number of total root nodules per plant; EN: Number of efficient root nodules per plant root nodules per plant.

the effective root nodules per plant ($p = 0.002$), compared to the control and other inoculated plants. This increment would indicate that rhizobia acting as biofertilizers have fixed the atmospheric nitrogen during the symbiotic association with its host plant [29] [33]. It looks like the synergistic action of isolates was the main factor when several isolates were fused together before inoculation of the soybean variety TGX 1910 14F, instead of competition. This mixed infection with several rhizobia isolates was previously revealed with the host *Vigna unguiculata* or other crop legumes [34]. The mixed infection is in line with the theory of nodule occupancy which determines the different rhizobia isolates that

are found within the same root nodule [35]. Surprisingly, rhizobia isolates IS5 and IS6 that were more competitive for the two soybean Houla1 and TGX 1910 14F varieties at Sanguere-Paul became less performant at Soukoundou. The fact that these tow isolates originated from Touboro cotton zone, which is close to the Sudano-guinea savannah zone, justifies the differences in the pedo-climatic conditions at Soukoundou (belonging to the Sudano-sahelian zone) that can affect the behavior and efficiency of isolates. These results revealed that the isolates promiscuity, defined as the symbiotic affinity of a host plant to at least two rhizobia strains and attributed to several cultivated crop legumes [27], was less pronounced for the Houa1 soybean variety at Soukoundou. Moreover, the specificity of symbiosis was shown to be restricted in soybean, a given strain of rhizobia being able to associate only with very few hosts [36]. In a previous study, root nodule efficiency was shown to be correlated to nitrogen fixation and soybean seed yield at harvest [37].

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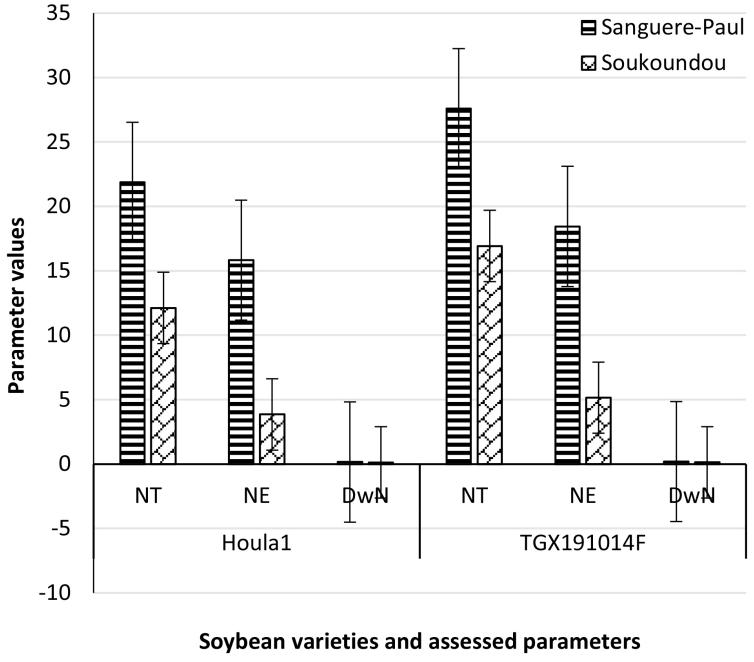
At flowering (**Figure 3(a)**), inoculated soybean of the variety Haoua 1 significantly formed more efficient root nodules ($p = 0.0001$), increased the nodule dry weight ($p < 0.0001$) at Soukoundou than at Sanguere-paul. There was no significant difference on the total number of root nodules of the variety Haoua 1 or the number of efficient nodules of the variety TGX1014F whether plants were cultivated at Soukoundou or Sanguere-Paul. Conversely, the total number of root nodules was significantly very high ($p = 0.0002$) at Sanguere-Paul, whereas the Soukoundou site mostly and significantly ($p < 0.0001$) enhanced the dry weight of root nodules of soybean variety TGX1014F. At podding (**Figure 3(b)**), Sanguere-Paul site was significantly favorable to the root nodule formation efficiency and dry weight of nodules ($p < 0.0001$) compared to Soukoundou site when both soybean varieties Haoua 1 and TGX1014F were sown. The salt stress of the growing site has been reported to negatively influence the symbiotic nitrogen fixation through inhibition of nodulation by reducing the infection sites [38]. We therefore are pointing out the differences in the soil salt concentrations Soukoundo and Sanguere-Paul sites as the causes of the various results obtained.



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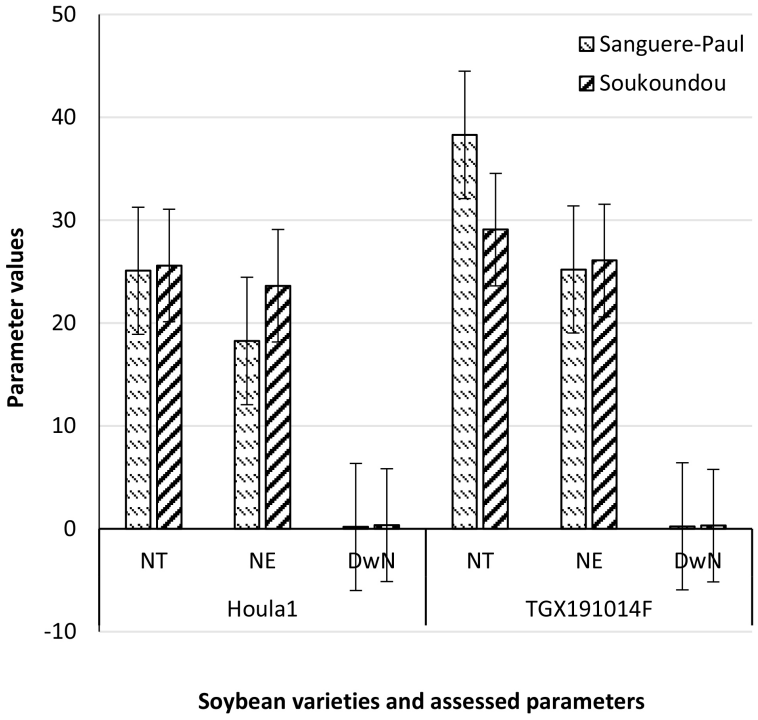

(b)

Figure 3. Sites effects of soybean varieties on nodulation parameters at flowering (a) and and podding (b).

4. Conclusion

At the end of the characterization of soybean-associated rhizobia, 6 isolates were identified from 16 purified colonies on YEMA medium. These isolates were

recognized as not able to produce acids, an indication that they were slow growing rhizobia, presumably of the genus *Bradyrhizobium.* In addition, they were highly tolerant in temperature, pH and different salt concentrations. The symbiotic test revealed efficient rhizobia-soybean combinations such as isolates IS4 or IS5-Houla 1 variety, whereas isolate IS6 was the best symbiont for the TGX 1910 14F soybean variety at the Sanguere-Paul study site (Garoua). On the other hand, the mixture of isolates (M) was the best efficient inoculant indicated for growth of TGX 1910 14F soybean variety at Soukoundou (Guider), while Houla1 variety had more affinity with the native rhizobia isolates. Our further research will focus on molecular identification through 16S rRNA method of the native rhizobia isolates found in Sudano-Sahelian soils, to increase the efficient isolates bank for a sustainable soybean-cotton intercropping.

Conflicts of Interest

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

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