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Nodulation Responses of Four Food Crop Legumes to Cross-inoculation in the Guinea-savannah (Ngaoundere-cameroon) and Sudanian (Sarh-Chad) Zones

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Abstract A cross-inoculation study on groundnut (*Arachis hypogaea* L.), bambara groundnut (*Vigna subterranea* L.), cowpea (*Vigna unguiculata* L.), and soybean (*Glycine max* L.) was carried out to screen potentially hight nodulating and nitrogen fixing *Rhizobium* strains suitable for important crops legumes in the Adamawa region (Cameroon) and middle-Chari region (Chad). The experiment was displayed in a complete Randomised Block with 6 forms of inoculation representing the treatments, each of which was replicated four times. An experimental field consisted of 4 blocks, each rcorresponding to a specific crop legume, which was submitted to each of the following treatments: the control (Ctrl); Groundnut *Rhizobium* (GR); Cowpea *Rhizobium* (CR); Soybean *Rhizobium* (SR); bambara groundnut *Rhizobium* (BR); and the mixture of these 4 rhizobia (MR). The cross-inoculation types consisted of taking *Rhizobium* isolates from each crop legume and coating with each of the seed crop before sowing (GR/cowpea/soybean/bambara groundnut, CR/groundnut/soybean/ bambara groundnut, BR/cowpea/soybean/groundnut, SR/cowpea/groundnut bambara/ groundnut, MR/groundnut/cowpea/soybean/Bambara groundnut). Cross-inoculation significantly (p = 0.001) improved the number, efficiency and dry weight of the nodules in all the crop legumes. The responses to nodule efficiency differed from one treatment or host plant species to another. In our investigation to the speculation whether one rhizobial strain can nodulate several crop legumes, we found out that groundnut can be inoculated with SR/BR in Cameroon, or CR/BR in Chad, whereas CR/BR/MR were the best trains to be associated with cowpea both in Cameroon and Chad. Farmer could also use CR/SR in Cameroon and SR/BR in Chad to inoculate soybean, or inoculate bambara groundnut in Cameroon and Chad with GR/BR. All the four crop legumes are thus considered as are promiscuous since each can form symbiotic associations with rhizobia from many other hosts. Selecting these highly effective rhizobia is an important step toward commercial inoculants production for biological nitrogen fixation research in our developing world context.

Keywords: *Rhizobium, cross-inoculation, crop legumes, nodulation, Chad, Cameroon*

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1. Introduction

Soil contains several types of microorganisms, including rhizobia, which are involved in the symbiotic biological nitrogen fixation, and affect the physical, chemical, and biological properties of soil. Rhizobia can live either free in the soil or within root nodules of crop legumes [1]. These bacteria colonize root nodules of these crops, where they fix nitrogen that the plant absorbs and assimilates in the form of ammonium ions [2]. The presence of root nodules in these plants reveals the existence of indigenous strains of rhizobia in all soils even if the number of spontaneous nodules per plant is very low, or even none in certain varieties [3,4]. The distribution of root nodules throughout the rhizosphere of plant genotypes is due to the fact that the strains of efficient wild bacteria are present both on the surface and in the depths of the soil [3].

Not all legumes have a symbiotic affinity to all strains of rhizobia [5], or may not be uniformly distributed in the soils [6]. To tacle this symbiotic affinity issue of crop legumes with wild strains of rhizobia, the production of bioinoculants with *Rhizobium* isolated from roots crop legumes is necessary [7,8]. To obtain the full benefits of Biological Nitrogen Fixation, it is extremely important to provide farmers with the correct rhizobia for their legume crops. They have categorized rhizobia and their legume partners into cross- inoculation groups. Each of these groups consists of all the legume species that will develop nodules when inoculated with rhizobia obtained from any other member of the same group [9,10]. The comparison of the symbiotic effectiveness of wild rhizobial strains with cultivated strains through cross-inoculation study has been reported [11,12,13]. Recent cross-inoculation research on important agricultural legumes with rhizobial isolates from wild legumes resulted in an increase in dry matter and total nitrogen contents of cross-infected plants [14,15,16]. Prior inoculant production and field trials, assessing cross-inoculation on nodulation is needed to prove the effectiveness of *Rhizobium* isolates in increasing nitrogen fixation ability of cultivated plants. Therefore, the main objective of the work was to screen potentially hight nodulating and nitrogen fixing *Rhizobium* strains suitable for the four most cultivated crop legumes (soybean, groundnut, cowpea, Bambara groundnut) in the guinea savannah zone (Ngaoundere-Cameroon) and sudanean zone (Sarh-Chad). We hypothesized that the presence of specific rhizobiaö associated to cultivated crop legumes varies from one cultivated soil to another, and depends on the affinity between the crop and its microsymbionts. The outcomes of cross-inoculation of these crops on their nodulation and their effectiveness in nitrogen fixation activity are discussed.

2. Materials and Methods

2.1. Presentation of the Experimental Site

The experimental site of Cameroon was located within the university campus at 1090 m elevation. The geographical coordinates are 07°250 North latitude and 13°5326 East Longitude. In Chad, the experimental field was located within the University campus at Sarh in the south of the country, at 375 m elevation. The geographical coordinates are 09°045 North latitude and 18°252 East longitude. Ngaoundere soil has pH 6.10, 0.16g N and 0.04g P per 100g of soil, while at Sarh, the soil pH was 6.5, with the N and P contents of respectively 1.83g and 1.1g per 100g of soil.

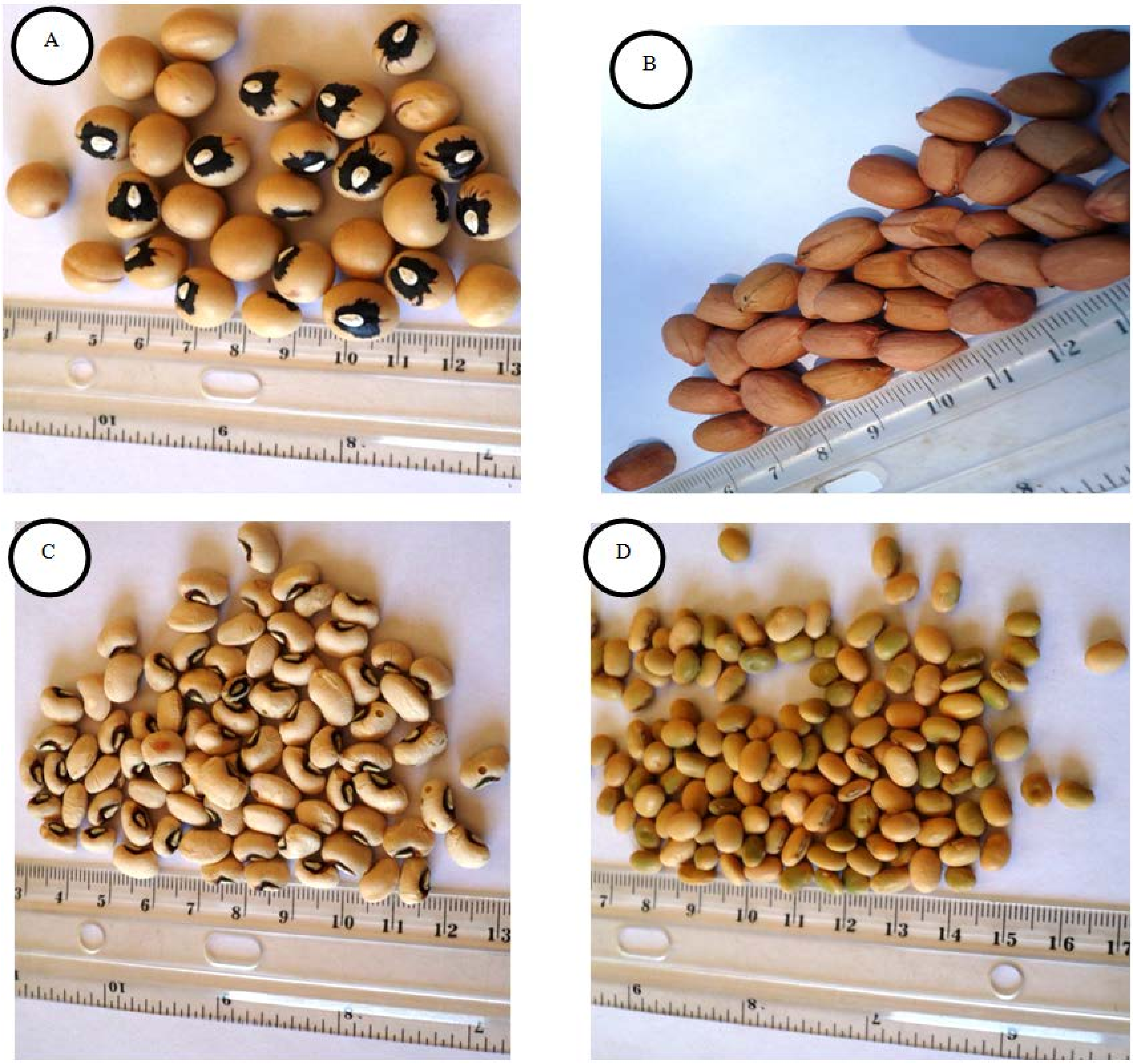


Figure 1. Crop legume seeds used in this study: (A) Bambara groundnut, (B) Groundnut, (C) Cowpea, (D) Soybean

2.2. Biological Materials

Groundnut (*Arachis hypogaea* L.) (Figure 1a) used and provided by the Chadian Institute of Research and Agricultural Development (ITRAD) was flower variety 11 with a life cycle of about 90 days at maturity.

Cowpea (*Vigna unguiculata* (L.) Walp) variety used was IT99k-573-1-1 and has a life cycle of between 85 and 90 days at maturity. It is a semi-erected variety with rotating root, yellow flower and fruits containing 7 to 17 seeds each. Soybean (*Glycine max* L.) was the TGX 1951-3F variety that has a life cycle extending from 90 to 100 days after planting. It is a plant with purple flowers that display pods with 2 to 3 greenish-yellow seeds. Bambara groundnut (*Vigna subterranea* (L.) Verdc.) was the local variety Djar with a cycle of between 90 to 100 days (Figure 1b), supplied by ITRAD Chad. The seeds are beige black hile, while the plant has yellow flower, trifoliate leaves, giving 1 or very rarely 2 seeds per pod.

All inoculants were produced at the microbiology laboratory of the Institute of Research for Rural Development (IRAD) Ngaoundere. Rhizobia were isolated from the fresh root nodules [17], on Yeast Extract Mannitol Agar (YEMA) medium [18], supplemented with 0.25% Congo Red for examination of possible contaminants. Each *Rhizobium* produced as liquid formulation was prepared in YEMA-Congo Red liquid medium as recently reported [8]. Rhizobia were allowed to grow to 10^6-10^8 cells/ml for 48h under shaking conditions on a magnetic stirrer.

The eight bio-inoculants (Figure 2) derived from *Rhizobium* isolated from root nodules of groundnut, cowpea, soybean and bambara groundnut commonly grown in Chad and Cameroon.

Figure 2. *Rhizobium* formulated as liquid inoculum used at sowing at Ngaoundere (A), and Sarh (B)

2.3. Experimental Field and Treatments

The experimental design adapted for each crop legume either at Ngaoundere or Sarh was a complete Randomised Block with 6 forms of inoculation representing the treatments, each of which was replicated four times. An experimental field consisted of 4 blocks, each representing a specific crop legume which was submitted to each of the following treatments: the control (Ctrl); Groundnut *Rhizobium* (GR); Cowpea *Rhizobium* (CR); Soybean *Rhizobium* (SR); Bambara groundnut *Rhizobium* (BR); and the mixture of these 4 rhizobia (MR). The experimental field had a total of 72 elementary units, each of which was a (3 x 2) m^2 surface area seperated 50 cm apart.

Sowing and Planting was performed on September 10-2015 at Ngaoundere-Cameroon, and on October 25-2015 at Sarh-Chad. Bambara groundnut, soybean, groundnut plants were separated one from another by 25 cm, and 30 cm apart on lines [6]. Cowpea plants were separated each other by 40 cm. Each elementary plot contained 7 rows, each of which had 9 plants, for a total of 63 plants per elementary unit. Different treatments were applied on seeds at sowing time. Inoculation consisted of coating specific *Rhizobium* (100 ml liquid inoculum) to each seed species by mixing 25g of milk Nido powder. Seeds treated this way were sown 1, 1, 3, 3 per plot hole, respectively for groundnut, bambara groundnut, cowpea, and soybean. Cowpea and soybean plants were thinned to 1 per hole two weeks after planting. The rarety of rain was replaced by manual watering of plants in the morning throughout the cropping season in Chad, and as from 45 DAP in the Cameroonian field. Weeding was manually conducted 3 times with hoes, at 12, 26 and 45 days after planting (DAP), to maintain the field weed free.

2.4. Assessment of Nodulation Components

All nodulation parameteres were assessed at 30 days after planting (DAP). Nodules number/plant was assessed following the method described [8]. To test the nitrogen fixation activity known as nodule efficiency or effectiveness, 20 fresh nodules/plant species were cut open with blade and the colour observed. Depending on the grain legume species, the red, pink and/or green colours were recorded as from nitrogen fixing nodules while whitish colours were from non-nitrogen fixing ones. These colorations reflect the colour of leghemoglobine which is an indication within a root nodule that N-fixation is taking place [19]. Root nodules kept in labeled envelopes were dried in an oven (Philips Harris Ltd) at 60°C for 12 h, and weight on an electronic balance (Mettler PC 2000) [8].

2.5. Data Analysis

Results were statistically analyzed using a "StatGraphics plus" program, which performs analysis of variance (ANOVA). Means between treatments were graded using the Duncan multiple range test, while Pearson correlations test was used to establish links betweenn nodulation parameters.

3. Results and Discussion

3.1. Effect of Cross-Inoculation on Root Nodules Formation in four Crop Legumes at Ngaoundere-Cameroon and Sarh-Chad

Groundnut

At Ngaoundere, the number of root nodules formed by groundnut (Table 1) was significantly ($p < 0.0001$) higher in plants cross-inoculated with BR than in other treatments. All *Rhizobium* inoculants stimulated production of moderate number of nodules, except the mixture of *Rhizobium* (MR) for which the number of nodules remained low. This finding was previously reported after inoculation of *Vigna mungo* with rhizobia from tree legumes under arid conditions [19]. Moreover, a given *Rhizobium* strain was reported to be associated with only a few legume crop species [20]. This lines with another report for which strains of rhizobia isolated from another host plant was able to infest lupine [21]. There was no significant difference between the nodules number formed on groundnuts roots inoculated with CR, BR on one hand, and between GR and the control. These results suggest that groundnut-*Rhizobium* symbiosis is moreless broad, as far as the affinity to the four *Rhizobium* strains (CR, GR, BR, SR) are concerned.

Table 1. Root nodules per plant in four crop legumes as impacted by different cross-inoculations at Ngaoundere-Cameroon and Sarh-Chad

Legume crops	Treatments						P-Value
	Ctrl	GR	CR	SR	BR	MR	
			Ngaoundere				
Bambara	61.9±9.78^a	83.7±12.57^b	98.2 ±12.26^c	54.4±11.49^a	106.8±9.85^c	54.2±18.15^a	0.0001
Soybean	1.8±1.61^a	1.4±0.84^a	5.2±1.81^c	3.1±1.19^b	1.8±1.22^a	1.6±0.69^a	< 0.0001
Cowpea	6.8±1.54^a	6.8±1.47^a	9.3±2.83^b	6.0±0.94^a	15.5±3.34^d	11.8±2.69^c	< 0.0001
Groundnut	48.5±4.88^b	46.8±3.58^b	60.6±8.32^d	55.1±5.21^c	61.3±3.30^d	38.3±6.34^a	< 0.0001
			Sarh				
Bambara	45.7±16.06^a	82.4±15.35^b	50.9±21.17^a	93.7±22.18^{bc}	105.2±22.89^c	82.3±24.94^b	< 0.0001
Soybean	0.5±0.52^a	0.6±0.51^a	1.0±1.15^a	4.9±1.52^c	3.6±1.50^b	4.1±1.91^{bc}	< 0.0001
Cowpea	10.7±2.0^b	8.3±1.33^a	8.2±0.91^a	11.8±3.39^b	14.6±1.17^c	16.7±1.7^d	< 0.0001
Groundnut	41.81±11.76^a	42.5±10.54^a	59.1±10.42^c	47.9±14.93^{ab}	54.6±18.28^{bc}	43.6±10.54^a	0.017

Within each study site and for each crop legume on a raw, values affected with the same letter are not significantly different at the indicated level of significance.

The supply of water during dry conditions would have contributed to alleviating the water stress leading to the increased nodulation, since water stress has been indicated to reduced nodulation [15]. The elevated number of nodules on control plant roots may be due to the fact that the soil contains indigenous strains of bacteria which are efficient for groundnut [6], compared to SR administered to groundnut. This result is close to enhanced nodule number in untreated compared to *Bradyrhizobium* inoculated soybean plants previously reported [3]. At sarh, the number of nodules (59) from CR treatment was greater than the rest of the nodules of the different treatments. There was no significant difference between the number of nodules from CR and GR inoculated plants. The equal number of nodules between GR inoculated and the control plants suggests that the indigenous strains were more competitive for the host plant. When nodulation takes place, the number of nodules that develop on the root system of crop legume is proportional to the nitrogen requirements of plant for its growth [22]. The contribution of an exogenous inoculum has been reported not to have the same effects on different plants [5].

Cowpea

At Ngaoundere, the number of root nodules per cowpea plant inoculated with Bambara groundnut *Rhizobium* (BR) was 10 folds higher than that of plants inoculated with soybean *Rhizobium* (SR), and two folds that the control. These findings are below recent results reported in Burkina Faso (90 nodules per plant) [23], and at Dang Ngaoundere-Cameroon (93 nodules) during the wet cropping season [8]. The low number of nodules formed following inoculation of cowpea with SR compared to that of the control could be attrinuted to the close affinity that SR has for its host soybean, and not for other crop legume species. Responses to inoculation may depend on several factors, of which the seed variety, the rhizobial strain, the soil water availability and the environment are the most important [24]. At sarh, the number of nodules per plant was high (p = 0.0001) in the mixture of *Rhizobium* (MR) compared to other treatments. In plants inoculated in pairs with fast-growing and slow-growing strains, more nodules were formed, indicating the synergism between *Rhizobium* strains and the host plant under controlled conditions [25]. Plants inoculated with BR had a higher number of nodules than those treated with CR. This high number of nodules in the control compared to CR treatment suggests that the indigenous strains of rhizobia at this stage have greater affinity to the host plant compared to in coming strains [1]. It is convenient to notice that the highest number of nodules from MR treatment could be due to increased chance that one of this mixture strains has to establish an affinity with cowpea [20].

Soybean

At Ngaoundere, the number of root nodules (Table 1) formed by soybean plants inoculated with CR was significantly greater (p < 0.0001) than that of other inoculants. This suggests that *Rhizobium* isolated from cowpea may have a strong symbiotic affinity with soybean. However, the number of nodules formed by SR inoculated plants was more elevated than that of GR, BR and MR inoculated plants. The equal number of nodules in BR-treated and the control plants could be due to the non efficiency of this strain to soybean as previously reported [1]. The number of nodules formed after inoculation of soybean with GR, BR, MR and the control did not differ from each other. The contribution of an exogenous inoculum was revealed not have the same effect on plants [5].

At Sarh nodules were considerably improved by SR and MR inoculants (Table 1). These two inoculants increased by 7 to 10 folds the number of nodules on plants roots treated respectively with MR and SR with respect to the control. There was no significant difference between the number of nodules in the control plants and that of GR treated plants. The number of nodules from SR and MR treated plants was greater than 3 nodules per plant at 30 DAP [26], but was lower than between 27 and 51 nodules per plant obtained after inoculation of soybean with *B. japonicum* in the field at Kombe-Brazzaville [27]. Soybean inoculated with CR or SR was shown to yield results with significant differences in numbers, of active, fresh and dry weight of nodules [28].

Bambara groundnut

Table 1 indicates that BR inoculum positively improved (p = 0.0001) the number of nodules formed on bambara groundnut roots at Ngaoundere, that was twice the one encountered on SR and MR inoculated plants (54), and more important than the one previously reported [29]. MR will still remain efficient in cultuvated bambara groundnut soil. Some strains of *Bradyrhizobium japonicum* inoculated to soybean were reported to remain in the soil for several months [27]. At Sarh, bambara groundnut inoculated with BR improved the number of nodules by 130.19% compared to uninoculated plants (Table 1). An equal number of nodules was observed on GR/MR treated plants, as well as on CR and the control plants, although it was lower than 117 to 172 nodules counted per plant in the rainy season [6]. These results confirm the changes in nodulation of the same crop legume grown in different agro-ecologies.

3.2. Variation in Nodule Efficiency of Four Crop Legumes in the Study Sites Following Cross-inoculum of Seeds at Sowing

Groundnut

Root nodules from CR and MR inoculated groundnut displayed a relatively similar, high and significant (p = 0.0001) ability to fix nitrogen expressed as nodule efficiency than other inoculation groups at 30 DAP (Figure 3). However, the efficiency of nodules from GR plants was greater than that of control, SR and BR inoculated plants. This efficiency was higher than between 62.44-72.25% reported in pots experiment at Dang-Ngaoundere [8]. At Sarh, nodules efficiency (Figure 3) from BR treated plants was higher than that of plants inoculated with the mixture of *Rhizobium* (MR). Nodules formed by GR, CR, SR, MR and control plants had the same level of efficiency that was not statistical different.

Cowpea

The most effiecient root nodule in Ngaoundere was obtained when cowpea was cross-inoculated with GR (Figure 3A). There was no difference between the efficiency of nodules from GR, CR, BR, MR treated plants. Introduced *Rhizonium* strain may express more nitrogen fixation ability on one host plant than the other [5]. At Sarh, nodules from CR treated cowpea plants were more efficient than those of the control and other treatments (Figure 3B).

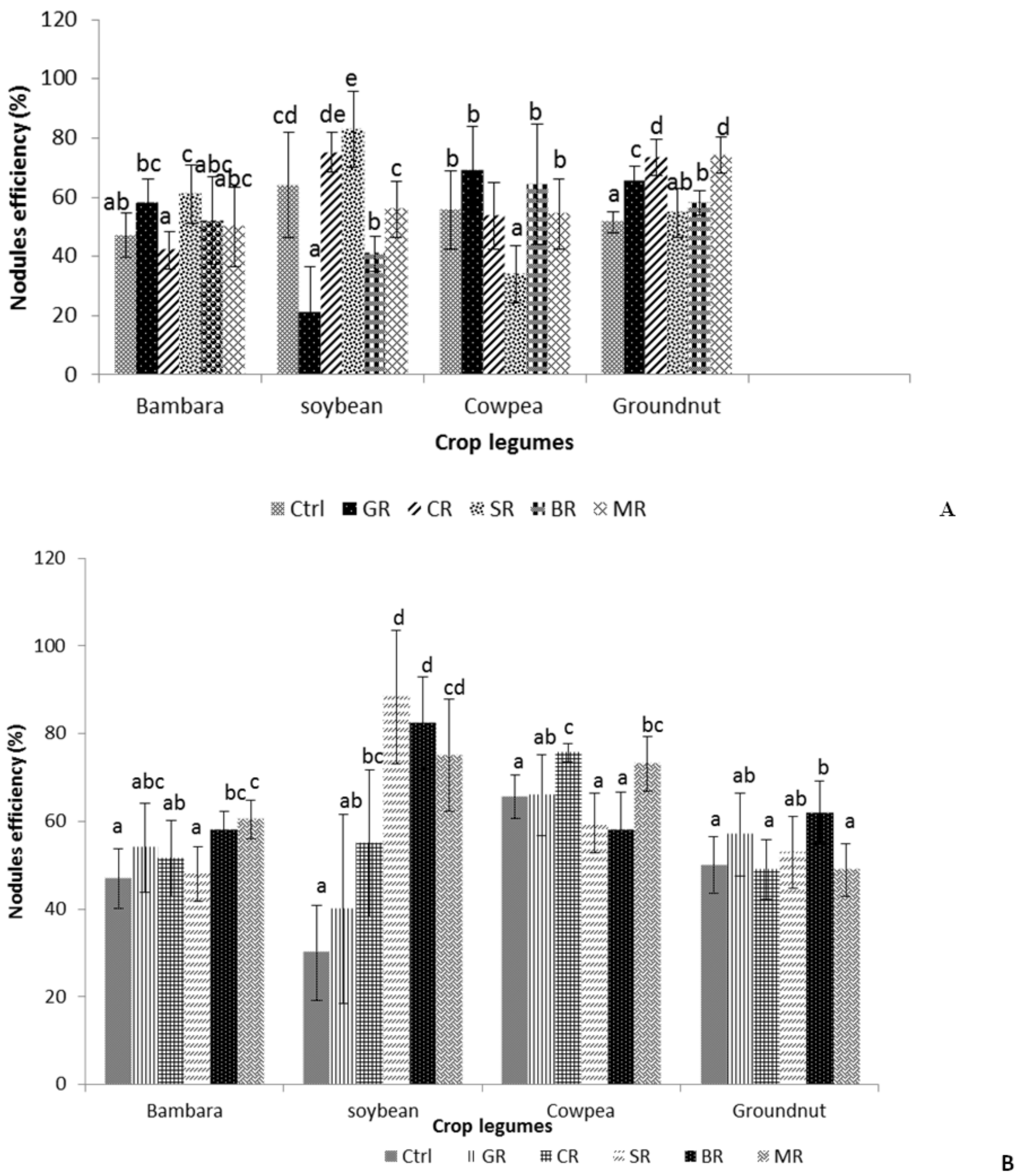
This result could be attributed to enhanced atmospheric nitrogen fixation by specific *Rhizobium* strains. The greatest efficiency of nodules expressed by uniniculated plants would indicate the high competitiveness of indigenous *Rhizobium* vis-a-vis the introduced strains associated to cowpea [30].

Soybean

The efficiency of nodules harvested from soybean plants inoculated with SR or CR in Ngaoundere was 82.83 % and was significantly ($p < 0.0001$) the greatest of all the other treatments, including the control. For soybean, the specificity of symbiosis was shown to be restricted, a given strain of rhizobia being able to associate only with very few crop legumes [20]. At Sarh, root nodules from SR, BR and MR treated plants showed efficiency of respectively 88.33, 82.43 and 75.16% (Figure 3) at 30 DAP. These rhizobia contributed to an improvement of nodules efficiency by 150.33 to 194.43% over that of uninoculated plants. This increment indicates that *Rhizobium* acting as biofertilizers have fixed the atmospheric nitrogen during the symbiotic association with its host plant [6,31,32].

Bambara groundnut

At Ngaoundere, bambara groundnut inoculated with CR and BR formed nodules that were significantly ($p < 0.0001$) improved the efficiency of nodule more than in other treatments. Biological nitrogen fixation was reported to be linked to photosynthetic activity and varied with physiological stages of the plant [33]. At sarh, the most efficient bambara groundnut nodules were those harvested from roots of MR inoculated plants (Figure 3B) at 30 DAP. These nodules were 60.5% efficient, with 28.72% increment compared to the control. This efficiency was lower than the one obtained the field as the response to dual inoculation *Rhizobium*-mycorrhiza [6].



Within each study site and for each crop legume, bars afected with the same letter are not significantly different at the indicated level of significance

Figure 3. Changes in the efficiency (%) of root nodules of four crop legumes under cross-inoculation at Ngaoundere-Cameroon (A) and Sarh-Chad (B)

3.3. Changes in Root Nodules Biomass as Influenced by Cross-inoculation in Four Crop Legumes

Groundnut

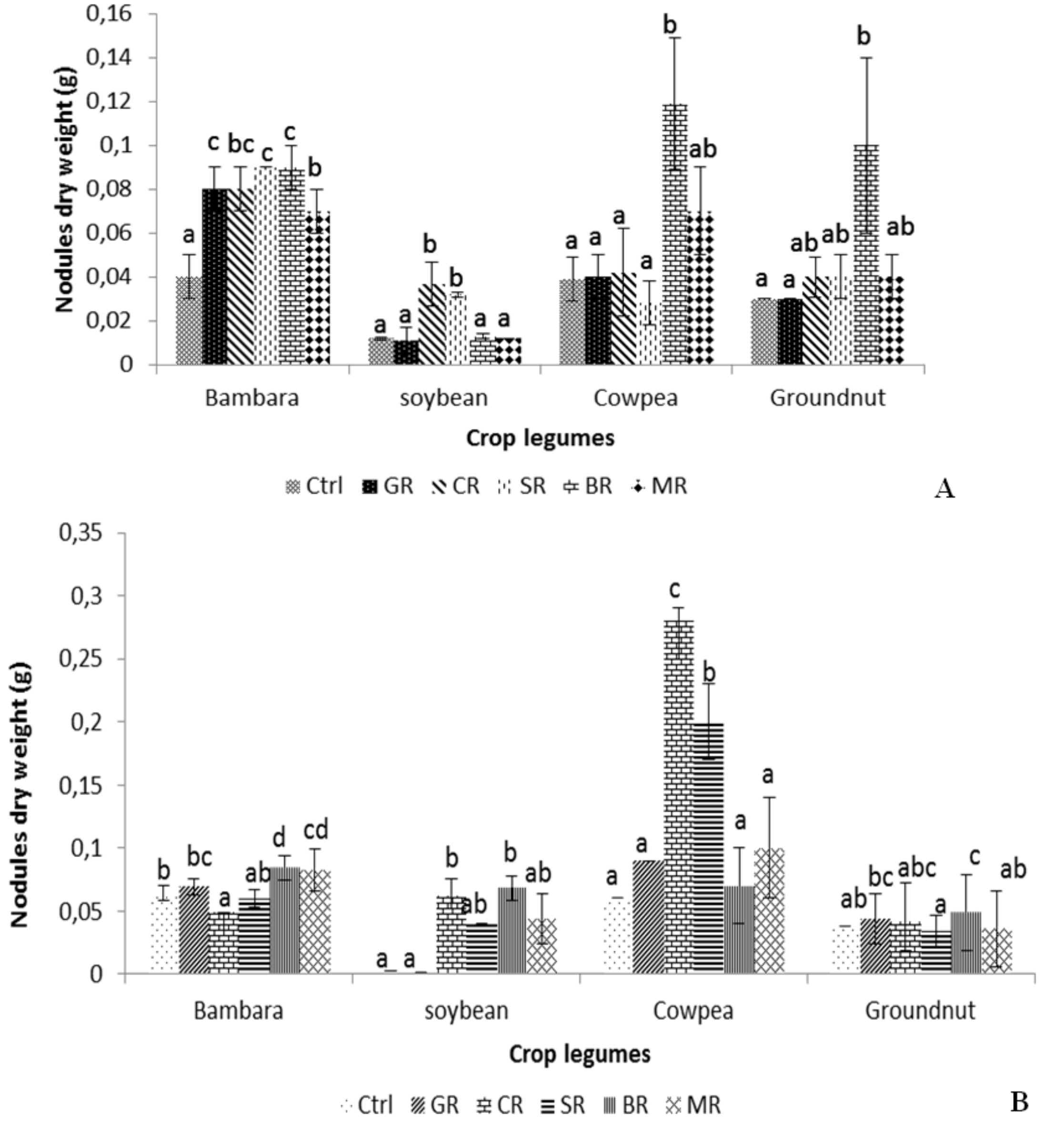
At Ngaoundere, the dry weight of nodules from BR-treated groundnut plants was higher than that of the other treatments and the control (Figure 4A). This elevated nodule biomass was ascribed to enhanced nodules counted on groundnut roots after cross-inoculation with BR. However, there was no significant difference ($p = 0.24$) between the nodules dry weight of control and each of CR, GR, SR, MR inoculated plants, indicating that the efficiency of nodules did not impact their biomasses. At Sarh, nodules from Groundnut plants treated with BR displayed elevated dry weight compared to that of CR treated plants (Figure 4B).

Cowpea

At Ngaoundere, the dry mass of nodules from cowpea plants treated with BR was consistently higher ($p = 0.03$) than that of the rest of the cowpea plots (Figure 4A), and was proportional to the number of nodules. These results line with findings showing enhanced nodule biomass following inoculation of cheackpea plants at sowing with two *Rhizobium* strains [31]. At sarh, cowpea nodule biomass was important in CR inoculated plants compared to other treatments (Figure 4B). Whereas nodules from SR inoculated cowpea were less efficient than that of other treatments, their dry biomass was highly important due to their big size.

Soybean

The dry weight of the nodules from CR and SR treated soybean plants (Figure 2) at Ngaoundere was the highest, and significantly differed ($p < 0.0001$) from one treatment to another. At Sarh, the dry weight of soybean nodules was 3 folds higher in BR and CR treated plants than in the control plants, and 6 folds that of GR inoculated plants. Our results suggest that the dry weight of the nodules depends not only on their number, but also on other factors including their size.



Within each study site and for each crop legume, bars afected with the same letter are not significantly different at the indicated level of significance

Figure 4. Variation of nodules weight of four crop legumes grown at Ngaoundere-Cameroon (A) and Sarh-Chad (B)

Table 2. Significant correlations between nodulation parameters within study sites

Crop legumes	Parameters	Correlations (p-values)	Study sites
Groundnut	Nodules number-nodules weight	0.294* (p = 0.02)	Sarh
Cowpea	Nodules number-nodules weight	0.467*** (p < 0.0001)	Ngaoundere
	Nodules number- nodules efficiency	0.364** (p = 0.004)	Ngaoundere
	Nodules number-nodules weight	0.297* (p = 0.02)	Sarh
	Nodules weight-nodules efficiency	-0.318* (p = 0.01)	Sarh
Soybean	Nodules number- nodules efficiency	-0.617*** (p < 0.0001)	Sarh
	Nodules number-nodules weight	0.384** (p = 0.002)	Ngaoundere
Bambara groundnut	Nodules number-nodules weight	0.516*** (p < 0.0001)	Sarh

*significant, ** highly significant, *** very highly significant.

Bambara groundnut

At Ngaoundere, nodules from inoculated bambara groundnut globally had twice more dry weight compared to the uninoculated plants (Figure 4A). BR inoculated plants significantly (p= 0.0001) improved the weight of nodules as compared to other treatments. At Sarh, the dry weight of the nodules from plants treated with BR or MR was higher than that of the control (Figure 4B), and was proportional to the number of nodules. Inoculation of plants with two *Rhizobium* strains was reported to increase nodule biomass, but only slightly improved the amount of atmospheric nitrogen in the soil during the dry season [31].

Correlation between nodulation parameters

There was a positive and significant correlation between the number and dry weight of nodules at Ngaoundere for cowpea ($r = 0.297$; $p = 0.02$), soybean ($r = 0.384$; $p = 0.002$), and at Sarh for bambara groundnut ($r = 0.297$; $p = 0.003$) and groundnut ($r = 0.516$; $p < 0.0001$). Whereas the nodule number was positively correlated to their efficiency for cowpea at Ngaoundere, it was instead negatively correlated for soybean at Sarh. This negative correlation indicates that not all the nodules formed were able to biologically fix nitrogen. Nodule weight was only correlated to nodule efficiency for cowpea at Sarh (Table 2).

4. Conclusion

At the end of this study, it appears that at Ngaoundere, the inoculum improved the number and dry weight of the nodules of inoculated plants. For some crop legumes such as soybean, nodule bimass was influenced both by the number and size of nodules. Nodule number was not correlated to their efficiency at Sarh, indicating that not all nodules formed were able to biologically fix nitrogen. Although cross-inoculation improved the number and dry weight of nodules, BR was the most indicated inoculum for bambara groundnut, cowpea and groundnut at Ngaoundere, whereas at Sarh, BR could be used to boost nodulation of bambara groundnut, soybean and groundnut. This cross-inoculation study has revealed that the four cultivated crop legumes have a symbiotic affinity with at least two *Rhizobium* strains and are thus considered as promiscuous. This is of great importance to the poor farmers who will opte to use a *Rhizobium* strain of the same cross-inoculation group to successfully and efficiently inoculate several of his crop legumes and obtain the return outcome in yield.

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