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## Study on the isolation and characterization of rhizobia strains as biofertilizer tools for growth improvement of four grain legumes in Ngaoundéré-Cameroon

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### ABSTRACT

Increasing the yield and economical efficiency of grain legumes in the Guinea-Savannah zone of Cameroon is a high researcher priority to assist growers in the rural areas. We report here a two-years study in the production process of fertilizer containing rhizobia for a sustainable production of grain legumes in the region. To address the issue, nodulation and rhizobia strains characterization were assessed at Dang and Wakwa, two soils different in trapping rhizobia partners for groundnut, bambara groundnut, cowpea and soybean. This was a prerequisite step in the determination of the most efficient rhizobia strains for biofertilizer production. Results indicate that all the four grain legumes differently formed nodules in their roots system in the two experimental soils. Cowpea and soybean demonstrated a highly significant ( $p \leq 0.001$ ) potential in fixing atmospheric nitrogen through their nodules than groundnut and bambara groundnut. The dry weight of nodules in groundnut and bambara groundnut was significantly lower ( $0.0001 < p \leq 0.038$ ) than that of cowpea and soybean in the two soils. When rhizobia were isolated from root nodules of plant species, the number of isolates ranged from 2 to 4 per grain legumes. All the isolates were convex in elevation, smooth/humid on the surface, with regular edges, except one groundnut colony from wakwa soil. The colonies were either whitish for soybean, whitish/yellowish for cowpea, groundnut and bambara groundnut. The rhizobia biofertilizer produced for each grain legumes were stored in the refrigerator for field tests. This is a step forward for further molecular characterization of isolates to enable production of elite inoculum in the region.

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**Key words:** Grain legumes, nodulation, Rhizobium characterization, Rhizobium biofertilizer.

### INTRODUCTION

Rhizobia are Gram negative bacteria that exist in a symbiotic relationship with several grain legumes as a host plants. In this association, the host plant provides the bacteria symbiont with sugars and a protected environment, while the bacteria fix nitrogen from the air and make it available to the plant in the form of ammonia (FAO, 1985; Kiers et al., 2002). In the Guinea-Savannah zone of Cameroon where the population diet is mostly based on cereals, there is a need to improve the production of common grain legumes such as cowpea, bambara groundnut, groundnut and soybean through inoculation technology, if full benefit from these crops is to be

achieved in terms of maximum yield and soil fertility.

Legumes play a critical role in natural ecosystems, agriculture, and agroforestry, where their ability to fix N in symbiosis makes them excellent colonizers of low-N environments, and economically friendly crop, pasture, and tree species (Jensen and Hauggaard-Nielsen, 2002). In addition to its role as a source of protein in the diet, N from legume fixation is essentially "free" for use by the host plant and by associated or subsequent crops (Kiers et al., 2002). Legumes also have positive effects on agriculture by adding and recycling biologically fixed N<sub>2</sub>, enhancing nutrient uptake, reducing greenhouse gas

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emissions by reducing N-fertilizer use, and breaking non-legume crop pest cycles (Zahran, 1999; Lupwayi and Kennedy, 2007). Legume yields unfortunately continue to lag behind those of cereals. A research orientation that better balances the needs of sustainable oriented agriculture with the breakthrough low-cost technologies such as rhizobial inoculation is required (Hirsch et al., 2001). Most grain legumes produced by small holder farmers are not often inoculated with rhizobia or applied with fertilizers (Ogendo and Joshua, 2001).

To date, there has been only limited commercial use of plant growth-promoting bacteria in agriculture and horticulture (Vessey, 2003). However, with recent progress towards understanding the mechanisms that these organisms utilize to facilitate plant growth, the use of plant growth-promoting bacteria is expected to continue to increase worldwide (Arshad. and Frankenberger, 1993; Cheng, 2003). Generally, grain legumes do not need additional nitrogen if the seed is effectively inoculated at planting. Inoculation with the most efficient N-fixing bacteria for the type of legume is necessary to help maximise nodulation and N-fixing ability (Pal and Shehu, 2001). In fact, rhizobia are randomly distributed in growing soils where they compete with other indigenous strains (Denison and Kiers, 2004), which often form less efficient nodules (Simms and Taylor, 2002). However, the mechanisms which determine the relative competitiveness among strains are still poorly understood (Kober et al., 2004). The study of *Glycine max*-rhizobia association in the Guinea-savannah zone of Cameroon has revealed the efficiency of indigenous strains over the exotic ones (Megueni et al., 2006), although these later showed efficiency in other agroecological zones for *Vigna unguiculata* (Ngakou, 2007). Rhizobia are fairly specific as to which legumes they will infect, form nodules on the roots of, and for which they will fix nitrogen (Lupwayi and Kennedy, 2007). In order to ensure the availability of the correct effective strain specific to a legume plant species, an inoculation practice should be performed, particularly when the legume being planted has not been grown in that field in at least the

past three years or with every planting of a high value crop (Zdor and Pueppke, 1990). Researchers have sought for ways of improving nitrogen fixation and enhancing yield of grain legumes by selecting efficient strains for production of inoculants (Hynes et al., 2001). Vance and Graham (1995) emphasized the importance of understanding the characteristics of indigenous rhizobia populations as the prerequisite for inoculant production. These characteristics would be considered in the selection of strains which would be most adapted in a particular region.

The objectives of this study were to characterize and produce the native population of rhizobia nodulating four grain legumes in two soil types of Ngaoundéré. During this study, the most efficient strains would be selected and used to produce rhizobia biofertilizers. Such fertilizers are expected to improve the growth of grain legumes in the region.

## MATERIALS AND METHODS

### Study areas

Experiments were conducted in Ngaoundéré within the Guinea savannah zone of Cameroon, from April to July in 2006 and 2007, and in two soil types (Dang and Wakwa). The Dang soil has pH 5.45, 0.16g N and 0.04g P per 100g of soil, while at Wakwa, the soil pH was 5.2, with the N and P contents of respectively 0.26g and 0.12g per 100g of soil. In each of the soil types four individual soil samples of 45 kg originated from selected farm fields were collected from the first 20cm soil depth and bulked to form a 180 kg composite samples. Each composite soil sample was packaged into black plastic bags of 2 kg.

### Grain legumes

Seeds used in the trial were cowpea (*Vigna unguiculata* L. Walp.) seeds from Bafia in the Centre region of Cameroon with lifecycle of 85 to 95 days. C5 variety of soybean (*Glycine max* L.) seeds from Maïscam-Ngaoundéré (155 and 160 days), bambara groundnut (*Vigna subterranea* L. Verdc.) seeds from the Far North region of Cameroon (115 to 160 days), and groundnut (*Arachis hypogea* L.) seeds of the GH119-20 variety from IRAD-Maroua (90 to 110 days).

### Experimental design

The experimental design consists of a completely randomised block systems with four treatments (cowpea; bambara groundnut; groundnut; soybean), and two replicates (soils from Dang and soil from Wakwa). For a soil type, each treatment was represented by 20 free-draining plastic bags. Seeds were sown in May 2006, and the experiment repeated in May 2007 during the first growing season extending from April to July. In each of the 2 kg plastic bags containing non-sterilized soil, rhizobia were trapped using four seeds of each grain legume species. After germination, plantlets were thinned to three to increase the surface area of plantlets in plastic bags.

### Nodulation and plant biomass

Nodulation efficiency was evaluated by enumerating the number and assessing the dry weight of viable nodules on 20 plants per treatment at 60 days after planting (Ngakou *et al.*, 2007). Plants were carefully removed from the soil and the root system washed to enable better visualization of nodules. All root nodules collected from each of the 20 plants of the same grain legume were dried in a hot-air oven for 12 hours at 60 °C and weighed separately. To assess the plant biomass, each plant was also dried for 72 hours at 60 °C and weighed (Athar and Johnson, 1996). To test the nitrogen fixation activity, 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 (Linderman, 2008).

### Isolation of rhizobia strains from fresh nodules

Petri dishes were cleaned with tap water, rinsed with distilled water and allowed to dry at room temperature. Cleaned dishes were sterilized in the dry oven at 105 °C for 2 hours. The Yeast Extract Mannitol Agar (YEMA) medium was prepared as described by Vincent (1970) with the following composition: 10 g mannitol; 0.2 g

MgSO<sub>4</sub>·7H<sub>2</sub>O; 1 g yeast extract; 0.5 g KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>; 0.1 g NaCl; distilled water 1L; pH = 6.8. The medium was supplemented with 0.25% Congo Red for examination of possible contaminants. Rhizobia were isolated from the fresh root nodules as described by Somasegaran and Hoben (1985). Root nodules were immersed in 95% alcohol for 30 s, and then in 0.1-0.2% HgCl<sub>2</sub> for 5 min. Control nodules were whitish and were expected to contain no rhizobia. They were then sterilized in 70% alcohol for 1 min and rinsed again with distilled water to remove all traces of alcohol. Sterilized nodules were ground in a Petri dish using a sterile scapel. A loopful of ground nodule was sampled and struck onto the YEMA solid medium. Petri dishes inoculated for each soil type and each grain legume were incubated at room temperature. One week after the first streak, different colonies formed separately were sub-cultured onto the YEMA-Congo Red solid medium under the same conditions, in order to screen all the single colonies. One week after subculturing, the morpho-cultural characteristics of isolated colonies (number, elevation, surface, colour, aspect, edges) were determined by observation under a stereomicroscope (Seeley and VanDemark, 1989).

### Liquid culture and inoculum production

In the YEMA-Congo Red liquid medium, agar was reduced from 15 to 1.5g/l. The carrier substrate was made of ground charcoal, palm oil peat, vermiculite in the ratio 1:1:1 (w:w:w), and macroelements (N, P, K) in micro-quantities. Isolated colonies from grain legume species (cowpea, soybean, bambara groundnut, groundnut) under growth on solid medium were transferred into Erlenmeyer flasks containing 200 ml YEMA-Congo Red liquid medium. Rhizobia were allowed to grow to 10<sup>6</sup> - 10<sup>8</sup> cells/ml for 48h under shaking conditions on a magnetic stirrer. The carrier substrate was weighed (20 g) in heat-resistant white and transparent plastic sheets, wetted with 10 ml distilled water and autoclaved at 121 °C for 25 minutes. The sterilized substrate was cooled down under the flux laminar hood where 20 ml liquid culture was thoroughly mixed with it and the package sealed immediately to

prevent contamination. The rhizobia inoculum thus obtained was weighed and labelled as a specific grain legume inoculum.

#### Statistical analysis

Data were statistically subjected to analysis of variance (ANOVA) using a Statgraphic Plus, version 5.0 (SIGMA PLUS) computer program. Means were compared between treatments using the Duncan Multiple range test at 5% level.

### RESULTS

Differential Nodulation and growth of grain legumes in Dang and Wakwa soils during the 2006 and 2007 cropping seasons

Data on nodulation efficiency of the four grain legumes at Dang and Wakwa soils are presented in tables 1 and 2. All the four grain legumes formed nodules on their roots during the two growing seasons in Dang soil (Table 2). Whereas bambara groundnut and cowpea significantly produced more nodules than soybean and groundnut in 2006, the number of nodules produced by bambara groundnut was significantly lower ( $p < 0.0001$ ) than that of cowpea, soybean and groundnut in 2007. Root nodules were red or pink for cowpea, groundnut and bambara groundnut, and greenish for soybean. Cowpea and soybean showed significantly ( $p < 0.0001$ ) greater potential in fixing atmospheric nitrogen through their nodules than groundnut and bambara groundnut. Among the four grain legumes, soybean had a significantly higher biomass than cowpea or groundnut at 60 days after planting during the two growing seasons in Dang soil.

In Wakwa, soybean and groundnut produced more nodules than bambara groundnut and cowpea in 2007, while the reverse situation occurred in 2006 (Table 2). The dry weight of nodules in groundnut and bambara groundnut was once more lower than that of cowpea and soybean during the two growing seasons. Soybean was the only grain legume able to fix more nitrogen than the other three in both soils.

During the 2006 growing season, the ability of bambara groundnut ( $p = 0.34$ ) and soybean ( $p = 0.66$ ) to fix atmospheric nitrogen did not significantly differ between Dang and Wakwa soils. Conversely, cowpea nodules ( $p$

$= 0.027$ ) and groundnut nodules ( $p = 0.007$ ) were more efficient in fixing nitrogen in Wakwa than in Dang soil. All the four grain legumes grown in Wakwa significantly ( $p \leq 0.044$ ) produced more root nodules than those raised from Dang soil. However, the dry weights of nodules were the same for cowpea and bambara groundnut in the two trapping soils. The dry weight of nodules on soybean and groundnut were significantly ( $p \leq 0.037$ ) greater in Wakwa than in Dang soils. Just like the number of nodules, the biomass of grain legumes at 60 days after planting was significantly lower ( $p \leq 0.047$ ) in Dang than Wakwa soils.

During the 2007 cropping season, the number and dry weight of nodules per plant, the capacity of plant species to fix nitrogen, and the plant biomass did not significantly differ ( $p > 0.05$ ) between the two soils for cowpea and soybean. However, all these parameters were significantly different ( $p \leq 0.038$ ) between the two soils for bambara groundnut and groundnut, except for the number of nodules in groundnut ( $p = 0.17$ ).

There was a positive and significant correlation between the number of nodules and the biomass of plants for groundnut grown in Dang soil ( $r = 0.80$ ;  $P = 0.005$ ), while for bambara groundnut, this correlation was observed between the plant biomass and the efficiency of nodules ( $r = 0.80$ ;  $P = 0.005$ ) in Wakwa soil. Similarly, a significant correlation occurred between the number and dry weight of nodules in Dang ( $r = 0.78$ ;  $p < 0.01$ ) and Wakwa ( $r = 0.84$ ;  $p < 0.05$ ) soils for groundnut. For cowpea and soybean, there was also a positive and significant correlation between the number and efficiency of nodules with ( $r = 0.63$ ;  $p < 0.05$ ) and ( $r = 0.64$ ;  $p < 0.05$ ) respectively.

#### Morphological Characterization of rhizobia colonies

Rhizobia isolated from each of the four grain legumes were characterized on YEMA-Congo Red solid medium by comparing the number, elevation, aspect, edge, surface and colour of colonies (Table 3). The number of colonies was either the same or differed from one grain legume to another and from one soil type to another. In cowpea and bambara groundnut two and three colonies were

**Table 1:** Differential growth and nodulation of bambara groundnut, cowpea, groundnut and soybean in Dang soil.

Treatments	Number of Nodules (plant <sup>-1</sup> )		Nodules dw (g.plant <sup>-1</sup> )		Efficiency of nodules (%)		Plant dw (g.plant <sup>-1</sup> )	
	2006	2007	2006	2007	2006	2007	2006	2007
Cowpea	10.1±0.40b	103.1±15.26ab	0.55±0.05b	0.71±0.02b	62.44±2.44b	72.25±2.86b	5.51±0.68a	5.87±0.60a
Bambara	9.9±0.37b	9.1±2.07a	0.38±0.02a	0.51±0.07a	57.79±3.90ab	51.38±7.41a	10.32±0.77b	11.07±3.52ab
Groundnut	7.9±0.27a	148.1±18.59b	0.30±0.02a	0.45±0.03a	51.21±2.61a	45.74±3.23a	4.99±0.45a	5.61±0.51a
Soybean	7.2±0.41a	143.8±20.31b	0.61±0.03b	0.75±0.06b	82.63±2.72c	84.66±3.11c	11.18±0.73b	17.98±2.54b
P-value	< 0.0001	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0009

**Table 2:** Differential growth and nodulation of bambara groundnut, cowpea, groundnut and soybean in Wakwa soil.

Treatments	Number of Nodules (plant <sup>-1</sup> )		Nodules dw (g.plant <sup>-1</sup> )		Efficiency of nodules (%)		Plant dw (g.plant <sup>-1</sup> )	
	2006	2007	2006	2007	2006	2007	2006	2007
Cowpea	19.3±1.94b	93.4±7.29b	0.5±0.03b	0.62±0.03a	66.12±4.20a	62.33±3.14a	7.65±0.73a	9.39±1.38
Bambara	20.3±2.88b	15.6±3.20a	0.34±0.02a	0.61±0.08a	66.30±3.15a	61.60±8.22a	15.03±0.80b	15.96±1.86b
Groundnut	12.3±2.01a	196.2±28.91c	0.47±0.05b	0.66±0.04ab	70.67±3.70b	66.99±4.85ab	16.79±0.12b	12.15±1.35b
Soybean	11.5±1.10a	103.0±7.23b	0.47±0.03b	0.88±0.01b	84.29±2.58c	88.99±1.79b	21.94±0.71c	23.81±2.39c
P-value	0.006	< 0.0001	0.038	0.001	0.001	0.001	0.06	0.002

Values in the same column of a cropping season followed by the same letter for each parameter are not significantly different at 5% level. Data are mean ± se (standard error of individual) of 20 samples; Nodules dw: Nodules dry weight; Plant dw: Plant dry weight; g: gramme.

**Table 3:** Morpho-cultural characteristics of rhizobia isolates for each grain legumes.

Characteristics of colonies	Soil types	Soybean	Cowpea	Graoundnut	Bambara groundnut
Number of colonies	Dang	2	3	2	2
	Wakwa	3	3	4	2
Elevation	Dang	convex	convex	convex	convex
	Wakwa	convex	convex	convex	convex
Surface	Dang	smooth/humid	smooth/humid	smooth/humid	smooth/humid
	Wakwa	smooth/humid	smooth/humid	smooth/humid	smooth/humid
Colour	Dang	white	White green	or White or green	white
	Wakwa	white	White green	or White or green	White green or
Edges	Dang	regular	regular	regular	regular
	Wakwa	regular	regular	regular (many) irregular (few)	regular
Incubation time	Dang	72 h	72 h	72 h	72 h
	Wakwa	72 h	72 h	5 days	72 h

observed respectively in Dang and Wakwa soils. There were two and three different colonies for soybean respectively in Dang and Wakwa, while four were observed for groundnut in each of the soil type. The elevation was convex for all the isolated colonies. Their surface was convex, smooth or humid. The edges of colonies were regular for all the colonies, except for one groundnut colony from Wakwa soil which had irregular edge. The colonies were either whitish for soybean in the two soils, whitish or yellowish for cowpea and groundnut, whitish in Dang soil, whitish or yellowish in Wakwa soil for bambara groundnut. All the colonies took 72h to show growth on YEMA-Congo Red solid medium, except for groundnut colonies which were observed only 5 days after incubation. These results suggest that the symbiotic partners associated with groundnut are slower growing than those of other three grain legumes.

#### Rhizobia biofertilizer

All the selected rhizobia isolated from grain legume species *Glycine max* (L.) *Arachis hypogea* (L.), *Vigna unguiculata* (L.) Walp, *Vigna subterranea* (L.) Verdc were mixed to the carrier substrates to form bio-inoculants as shown in Figure 1. These fertilizers were stored in 70 g transparent white plastic bags, and kept at room temperature for field tests as alternative to nitrogen fertilizers for improving grain legumes production in the region.

#### DISCUSSION

Rhizobia including *Rhizobium* and *Bradyrhizobium* are best known as biological nitrogen fixers in the root nodules of legumes (Kiers *et al.*, 2003; Denison and Kiers, 2004). In the Guinea-savannah zone of Cameroon, the number of nodule formed in cowpea was already reported to be low (Ngakou, 2007), with nodules starting to degenerate as from 50 days after planting. Soybean showed the highest ability to fix atmospheric nitrogen





**Figure 1:** Rhizobia fertilizers for grain legumes production in Ngaoundéré.  
A: Soybean (70g); B: Groundnut (70g); C: Cowpea (70g); D: Bambara groundnut (70g).



through its greater nodule efficiency in both Dang and Wakwa soils suggesting the abundance of rhizobia strains able to express a relatively high affinity to this grain legume in the soil. Native rhizobia partners were recently found to be more competitive than a mixture of inoculants within these soils (Megueni *et al.*, 2006). The greater soybean nodules biomass in the two experimental soils was attributed to the large size nodules formed by this grain legume, similar to recent findings reported in cowpea (Ngakou *et al.*, 2007). These results are in agreement with those of Thao *et al.* (2002) who reported 671mg dry weight of nodules in the local «MTD-176» soybean variety in Vietnam, but contradict those of Ames *et al.* (1991), Daniels-Hylton and Ahmad (1994) who attributed increased nodules dry weight to increased nodules number. The number of grain legume nodules obtained in this study was not as high as the abundant nodulation reported in soybean (Hungria *et al.*, 2001), and Pisium (Ballard *et al.*, 2004). This can be attributed to the fact that our experiment was conducted in plastic bags whereas those of Hungria and Ballard were carried out under field conditions. In plastic bags, the growing area of the root system seems to be restricted and this has a direct negative effect on nodule production, thus on the plant growth.

The positive correlations between nodule and plant dry weight was similar to those reported by other authors (Thiagarajan *et al.*, 1992; Hungria *et al.*, 2001). The efficiency of nodules varied from 46 to 89% for all the four grain legumes, within the range of 40-90% obtained in Lutus (Baraibar *et al.*, 1999). There was a significant correlation between the efficiency of nodules and the plant biomass, supporting the improved nitrogen fixation potential of the host grain legume that usually lead to increased soil fertility. Through their symbiotic abilities, grain legumes can also play a crucial role in colonizing disturbed ecosystems, including those that are fire-prone (Arianoutsou and Thanos, 1996). They account for 27% of the world's primary crop production, contributing alone for 33% of the dietary protein nitrogen (N) needs of humans (Vance *et al.*, 2000). Grain legume seeds generally contain 20% to 30% protein, are lysine-rich, and

complementing the nutritional profiles of cereals and tubers in the diet (Duranti and Gius, 1997). All these attributes can account for their increased growth.

Inoculation is important to all legumes, especially under planting situations where soil has not previously grown the specific legume. Another factor to be considered is the long time interval (six months to one year) between the planting of successive legume crops. During this period, soil erosion might have removed or depleted bacterial populations or levelling might have exposed lower soil profiles which contain low bacterial populations (Thompson, 1983). The factors influencing the grain legumes-rhizobia symbiosis have been reported to include nutrient constraints particularly nitrogen (N) and phosphorus (P) deficiency, soil acidity, including aluminium (Al) and manganese (Mn) toxicity, and drought. All these factors affect strain variation in nodulation and nitrogen (N<sub>2</sub>) fixation, host strain interaction (Graham *et al.*, 2003). Organic matter appeared critical for maintenance of high populations of indigenous rhizobia in the soils (Mapfumo *et al.*, 2000). This can fully account for the different responses observed between the two soils on the one hand and grain legumes on the other.

The morpho-cultural characteristics of colonies observed in this study confirm that all isolates belong to the genus rhizobia (Larpent and Larpent-Gourgau, 1985). Each grain legume was infested by at least two different isolates, and might suggest the multi-strains interactions with the host plant species. Only one strain isolated from groundnut was found to show growth on YEMA solid medium 5 days after inoculation, suggesting the slow-growth nature of these rhizobia. Conversely, the other rhizobia isolates from bambara groundnut, cowpea and soybean were considered as fast-growing strains with growth occurring 72 h after inoculation. Similar results were obtained by Mbenoun (1992) on cowpea and soybean. Some of the claimed advantages of using fast-growing strains for grain legume inoculation include the facility of commercial production, easier establishment in soil, and displacement of indigenous strains (Buendia-Clavenia *et al.*, 1994). A similar inoculum produced on whey

as carrier substrate (Bissonnette et al., 1986), and a mixture of inoculum for legumes (Nwaga, 1997) were previously reported. Rhizobia inoculum are generally very sensitive and unable to live long in dry conditions, so, our grain legume species specific rhizobia inoculum were stored in a cool place, preferably in the refrigerator at 4-5 °C, until needed for re-inoculation assays in order to confirm the nodulation ability of the isolates.

### Conclusions

In the Guinea-Savannah zone of Cameroon, farmers still ignore the inoculation technology and this appears to explain the low yield they obtain from crops. Fertilizer containing rhizobia can be considered as a cheaper and environmentally friendly alternative to the resource poor farmers who cannot afford the expensive artificial N fertilizer. However, further research is needed to determine whether rhizobia isolated within nodules of the same grain legume species belong or not to the cross-inoculation groups. This information will increase our knowledge on the establishment of the mutual interactions between grain legumes and rhizobia in the region. Re-inoculation of plant species under controlled conditions will confirm these interactions through Koch's postulates, then, identification of isolates will refine characterization of strains as the prerequisite for manufacturing elite commercial inoculum production to grain legumes in the region.

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