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Functional diversity of rhizobia associated to *Acacia senegal* in different gum basins in Niger.

ABDOU Maman Manssour[1], ELHADJI SEYBOU Djibo[2], ASSOUMANE Aichatou[3], ALZOUMA MAYAKI Zoubeirou[1, 3].

[1] *Département Production Durable des Cultures, Faculté des Sciences Agronomiques, Université Boubakar Bâ de Tillabéri, BP : 175 Tillabéri ; Niger.*
[2] *Département Programmation, Etudes et Prospective, Haut-Commissariat à l'Initiative 3N, BP : 116 Niamey Niger*
[3] *Département de Biologie, Faculté des Sciences et Techniques ; Université Abdou Moumouni de Niamey, BP : 10662 Niamey ; Niger.*

Corresponding author : ABDOU Maman Manssour

Abstract

This study was carried out in six (6) natural gum groves in three gum basins of Niger, namely Kiki and Kokoiye in the western gum basin, Bader Goula and Azzaï in the central gum basin and Malam Mainari and N'Guel Kolo in the eastern gum basin. The objective of this study is to determine the functional diversity of rhizobia associated with A. senegal. For this, the number of rhizobia nodulating A. senegal was determined by the technique of trapping in Gibson tubes and in greenhouse with seeds from six (6) natural gum groves. The infective capacity of soils was tested by inoculating plants with gum grove'soils in Gibson tube. The rhizobial infectious potential of soils was determined using the method of Vincent, (1970). Results showed a variation in nodulation according to type of soil due to the natural presence of rhizobia in soils of gum groves. The soil of N'Guel kolo's had the most nodulation unlike that of Kiki and this as well in greenhouse as in Gibson tube. The distribution of rhizobium populations able to form nodules with A. senegal was correlated with soil's origin, in particular the physicochemical composition and provenance of seeds. This study also made it possible to observe nodulation whatever the combination origin of seed and soil. This will allow to create a local inoculum that can boost young plants growing and promote the success of reforestation programs in Niger.

Keywords: rhizobium, infectious potential, *Acacia senegal*, gum groves, Niger.

I. INTRODUCTION

In wetlands and tropical forests, legumes significantly contribute to nitrogen balance [1], [2], [3] and play an important role in ecology of ecosystems. The adaptation of legumes, like acacias, to different environmental conditions is due to their ability to associate with rhizobia allowing them to fix atmospheric nitrogen [4]. In sub-Saharan Africa, acacias are widely used in reforestation programs [5]. This is the case of *A. senegal* which is of major importance for the reforestation and the recovery of degraded lands in arid and semi-arid zones. Symbiotic bacteria that fix atmospheric nitrogen or rhizobia associated with acacias show great variability depending on their growth speed, their behavior towards legumes, their tolerance to various stresses and their phylogenetic membership. They form a very diverse heterogeneous group both from the phenotypic and genotypic point of view. In fact, rhizobia are taxonomically diverse and form a heterogeneous phylogenetic group divided into alpha-proteobacteria and beta-proteobacteria. In the Sahelian zone, many studies showned a great diversity of rhizobia ([6], [7], [8], [9]; [10]). This diversity would explain the potential for adaptation of acacias to develop on poor and degraded soils in the Sahel. Several genotypic studies have revealed a great genetic diversity in nitrogen-fixing bacteria capable of associating with *A. senegal*. These studies have shown that there's a great genotypic [11], and/or phenotypic [12] diversity in microsymbionts that nodulate *A. senegal*. Other studies showned that *A. senegal* forms nodules with *Rhizobium* and *Mesorhizobium*; *A. senegal* can be nodulated mainly by rhizobium strains of the genus Mesorhizobium ([12], [11]) but also of the genera Ensifer ([7]; [8]), Rhizobium [12] and Bradyrhizobium [13]. In fact, diversity is of great importance in the stability of the functioning of ecosystems because it provides a varied genetic pool which gives better resistance to environmental constraints. [14] reported that the effective exploitation of biological nitrogen fixation to improve agricultural productivity requires that local rhizobia populations be known and adequately characterized. This study's objective is to highlight the functional diversity of nodulant rhizobia *A. senegal* under the different soil and climatic conditions of gum trees plantations in Niger.

II. MATERIAL ET METHODS

2.1. Rhizobia trapping

The number of rhizobia that nodulate *A. senegal* was determined by the trapping technique in a Gibson tube and in a greenhouse with seeds from 6 provenances (Kokoiyé, Kiki, Bader Goula, Azzaï, N'Guel Kolo and Malam Mainari). The Gibson tube trapping followed the following steps: superficial scarification and disinfection of seeds for 15 min with 96% concentrated sulfuric acid (H_2SO_4) to facilitate their germination and rinsing in sterile distilled water several times. Seeds were aseptically placed under a laminar flow hood in Petri dishes containing sterile agar water (0.9%, w/v). Seeds were sealed with parafilm and incubated in the dark in an oven at 37 °C for a period varying from 24 to 48 h for germination. After germination, the seedlings were cultured under aseptic conditions on a device composed of a Gibson tube containing approximately 30 ml of sterile and inclined agar Jensen's nutrient medium (Figure 24). Tubes were plugged with aluminum foil pierced with two holes: one used for transplanting the seedling through which the radicle descends and the other closed with a rubber stopper allows watering and inoculation. To allow the transplanted seedlings to spontaneously release their integumentary shells and also to prevent the drying of the cotyledons, they were covered with blotting paper soaked in sterile distilled water, then left in a humid atmosphere for 24 h. Plants were kept in a culture chamber for six weeks at a temperature of 28 ° C with a photoperiod of 16 hours day and 8 hours night and a light intensity of 2500-4000 LUX. After one week of growth, young plants in Gibson tubes were inoculated, with 1 ml of soil suspension from each provenance. In each gum tree plantation, it's a composite soil taken under crown to the depth 0-25cm under 10 trees. The soil suspension was obtained by stirring for 1 hour, 10 g of each soil sample in 90 ml of sterile buffered saline pH 7 (NaCl, 0.15 mol l^{-1}; KH_2PO_4, 0.002 mol l^{-1}; Na_2HPO_4, 0.004 mol l^{-1}). Four (4) repetitions were made for each soil. Five (5) weeks after inoculation, fresh nodules were collected and nodulation was assessed. In the greenhouse, seeds of each gum grove were sown on the soil of the same origin. After six weeks of cultivation, the fresh nodules were harvested, counted and dried.

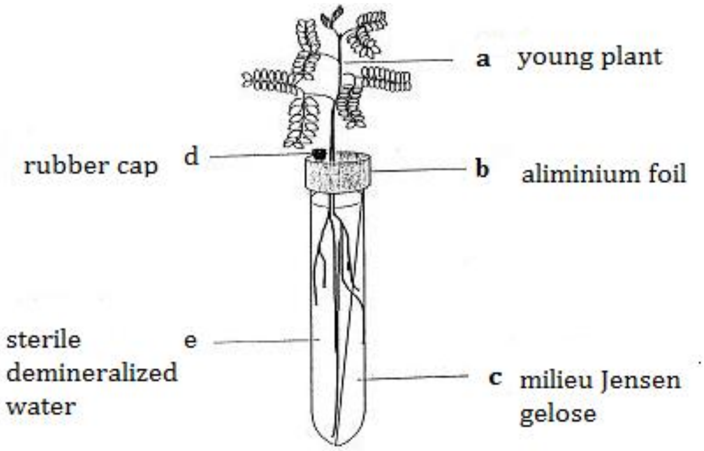


Figure 1: Plant of A. senegal in Gibson tube

2.2 Infectivity test of different sources

The infective capacity of different soils was tested. This test consists in inoculating in a Gibson tube plants of one provenance with the soil of the other provenances. The objective of this test is to determine the most infectious soil. Four (4) repetitions were made for each provenance and with each inoculum. One month after inoculation, nodules were collected and the nodulation was evaluated and compared.

2.3 The rhizobial infectious potential of soils (MPN: Most Probable Number)

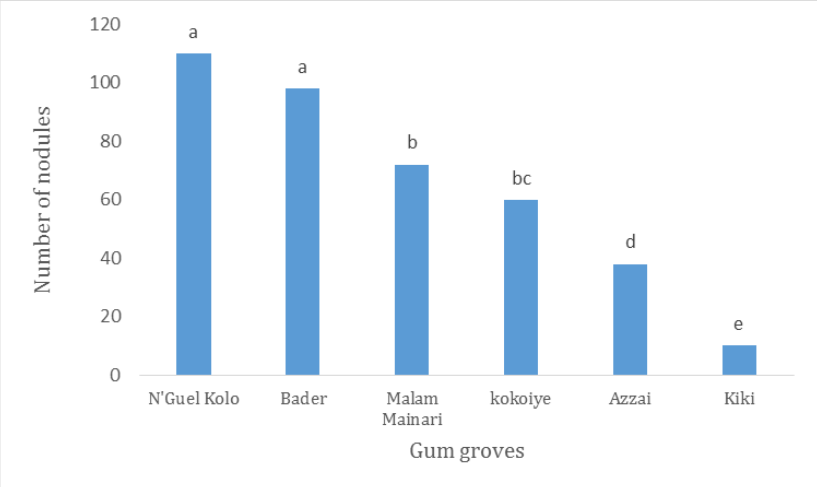
The measurement of rhizobial infectious potential of soils was made by a biological test which consisted in counting the rhizobia present in the soil throw plant infection's technique. The number of rhizobia capable of inducing nodulation in *A. senegal* in soils was evaluated by the MPN (Most Probable Number) method of [15]. This method consists to trap in Gibson tube, strains of rhizobia naturally present in soil's tested. This MPN was carried out on soils of the six provenances, namely: Kokoiyé, Kiki, Azzaï, Bader Goula, N'Guel Kolo and Malam Mainari according to the method described by [16]. For this, *A. senegal* plants were cultivated with a range of soil dilutions from 10^{-1} to 10^{-6}. Thus, 10 g of soil is weighed and suspended in 90 ml of sterile water and stirred for 1 hour. Successive dilutions are made by taking 1 ml of this solution and diluting it in a tube containing 4 ml of physiological water. This tube contains a dilution of 1/50 of the soil sample. This operation is repeated with 1 ml of the contents of this tube and so on. 6 dilutions were carried out 5 in 5: 1/50; 1/250; 1/1250; 1/6250; 1/31250 and 1/156250. From each dilution, 1 ml of the mixture is inoculated per plant. Four plants were inoculated for each level of dilution to obtain four (4) replicates. After six (6) weeks of cultures, results are read, noting tubes in which plants bear one or more nodes. Results are expressed in number of rhizobia per gram of soil.

III. RESULTS

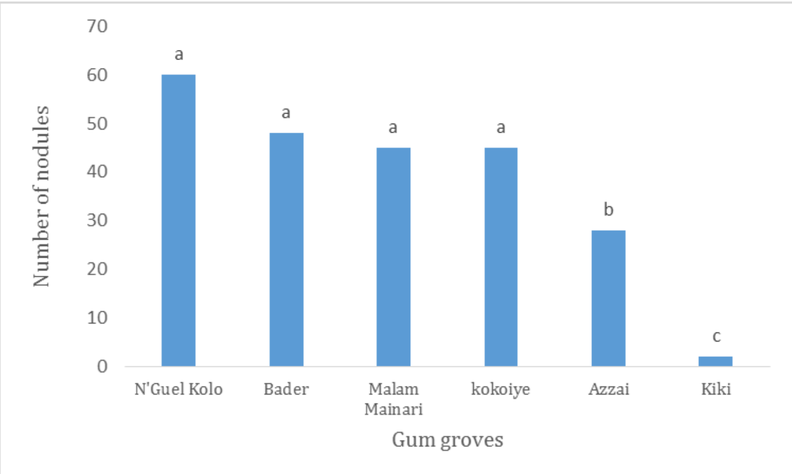
3.1. Nodule trapping

Results show that the nodulation varies between soils (**Figures 2** and **3**). The soil of N'Guel kolo's gum grove was the most nodulated, unlike Kiki's one, both in the greenhouse and in Gibson tubes. In the greenhouse, the comparison test shows a non-significant difference in nodulation between the gum grove of N'Guel kolo and Bader Goula. However, the difference was significant between gum groves of N'Guel kolo and Bader Goula and other gum groves. Between Malam

Mainari and Kokoiyé, the difference in nodulation wasn't significant whereas with the gum groves of Azzai and Kiki, the difference is significant. In Gibson tubes, there isn't significant difference in nodulation between the gum groves of N'Guel Kolo, Bader, Malam Mainari and Kokoiyé. On the other hand, between the latter and the gum groves of Kiki and Azzai, the difference was significant. Also, there is a significant difference in nodulation between Kiki and Azzaï's gum groves.



Values followed by the same letter are not statistically different at the 5% threshold of the Newman-Keuls test.
Figure 2: Nodules obtained by trapping in greenhouses in soils of gum groves.



Values followed by the same letter are not statistically different at the 5% threshold of the Newman-Keuls test.
Figure 3: Nodules obtained by Gibson tube trapping in gum groves soils.

3.2. Rhizobial infectious potential of gum groves soils

Table 1 presents the most probable number (MPN) of rhizobia in gum groves soils.

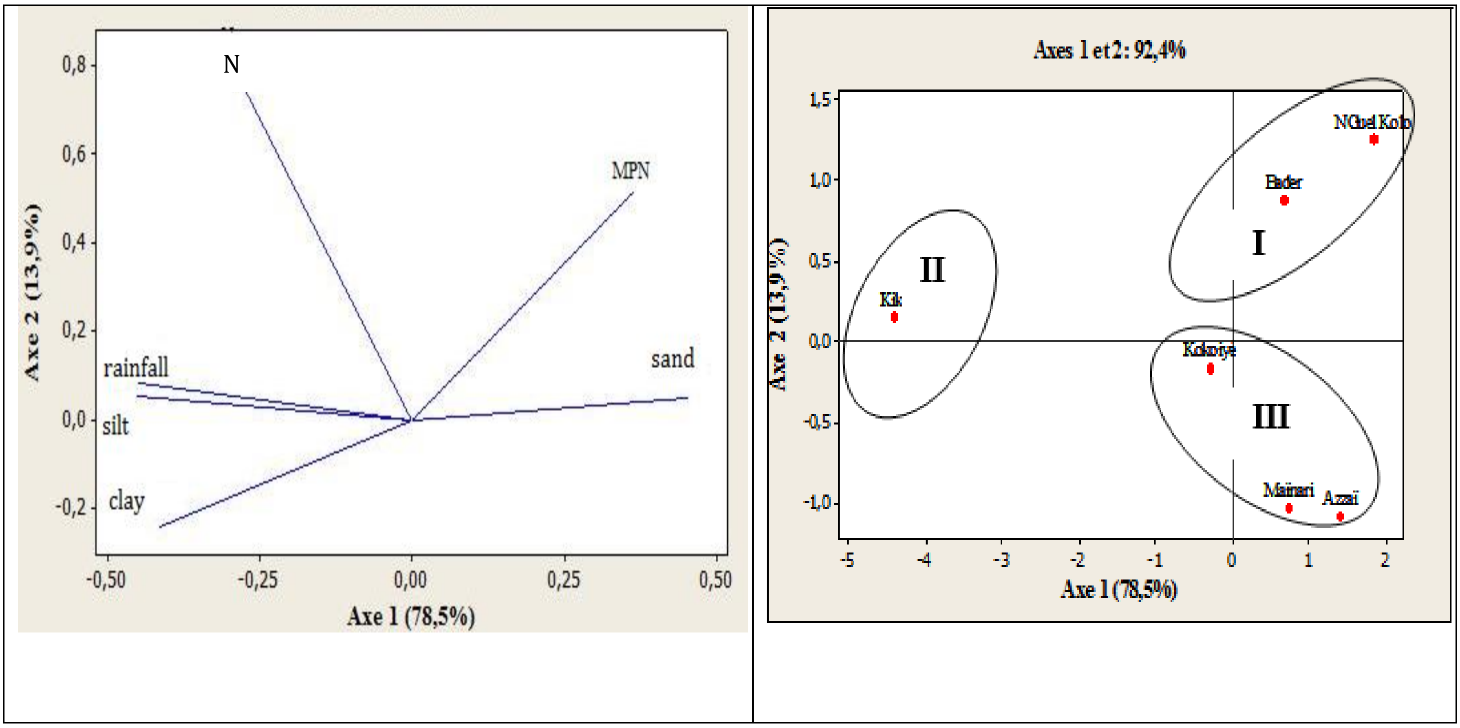
Table 1 : MPN of rhizobia in gum groves soils.

Gum groves	MPN (rhizobia/g)	superior limit	lower limite
N'Guel Kolo	1090	2834	419,2
Bader Goula	1090	2834	419,2
Malam Mainari	820	2132	315,4
Kokoiye	710	1846	273,1
Azzaï	490	1274	188,5
Kiki	113	293,8	43,5

Result shown that soils of N'Guel Kolo and Bader is richer in rhizobia/g of soil (1090 one both) compared to Kiki's one (113 rhizobia/g).

3.3. Principal component analyzes between MPN, rainfall and physicochemical parameters of gum groves soils.

Figure 4 shows the principal component analysis (PCA) carried out from MPN, physico-chemical parameters and rainfall of gum groves. The physico-chemical parameters used are nitrogen, clay, silt and sand. Axe 1 and axe 2 explain 92.4% of data's total variance.



(a) (b)

Legend: **N** = nitrogen, **MPN** = Number of rhizobia per g of soil.

Figure 4: PCA carried out on MPN, nitrogen, clay, silt, sand and rainfall.

(a): the correlation circle of variables, axis 1 represents 78.5% and axis 2 represents 13.9%.

(b): projection of the gum grove on the factorial plane formed by the axis 1 and 2.

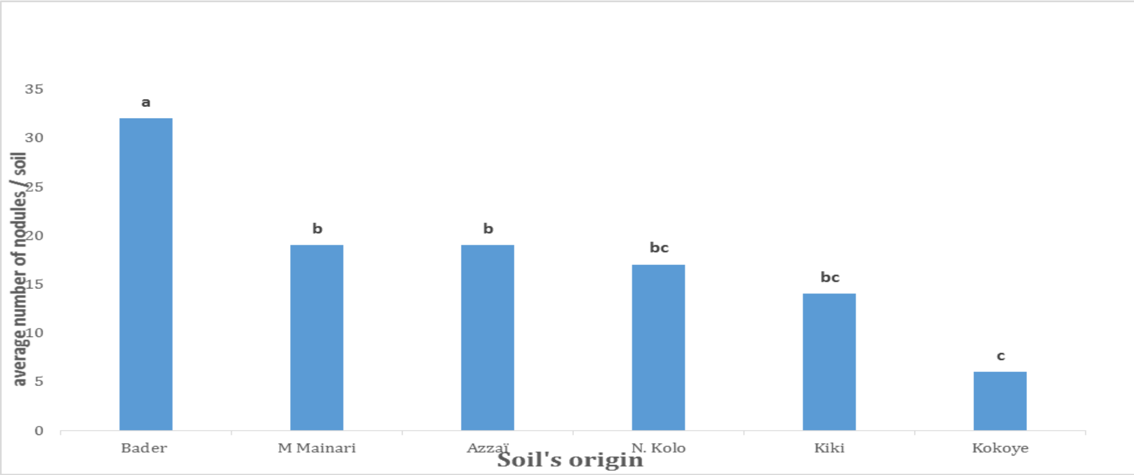
Three groups were formed through this PCA (figure 4b):

- Group I formed by N'guel Kolo and Bader Goula's soils where MPN is high associated with a high sand content;
- Group II formed by Kiki's soil where MPN is low associated with high contents of nitrogen, silt, clay and rainfall;
- The third one formed by soils of Kokoiye, Azzaï and Malam Mainari where the MPN is average associated with average contents of nitrogen, silt and sand.

3.4. Soils infective capacity in Gibson tube

Figure 5 shows the average number of nodules per type of soil obtained in Gibson tubes. Results show that provenances have nodulated with all soils inoculates. The comparison test of means shows significant results for the number of nodules obtained in Bader Goula's soil than other soils. Concerning Malam Mainari and Azzai's soils, numbers of nodules are not significant between them but signifiant with N'Guel Kolo, Kiki and Kokoiye's soils. As for soils of N'Guel Kolo and

Kiki, the number of nodules obtained wasn't significant between these two gum groves but significant with Kokoiye's one. Thus, whatever the combination of seed and soil origin, plants all nodulated and the number of nodules obtained in Gibson tube was greater with Bader's soil and lower with Kokoiye's one.



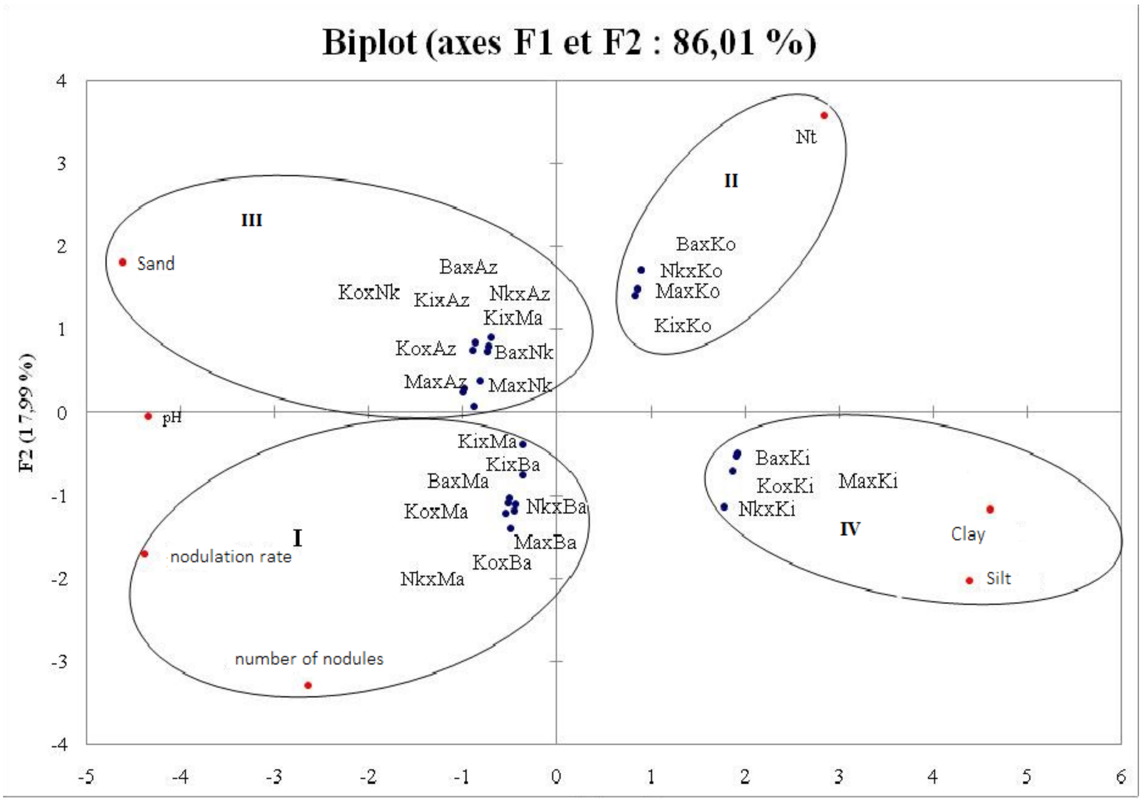
Values followed by the same letter are not statistically different at 5% threshold of Newman-Keuls test.
Figure 5: Number of nodules obtained by type of soils.

3.5. Principal Components Analysis (PCA) between parameters of nodulation, physicochemical characteristics and sources of seeds and soils.

PCA was used to study correlations between parameters of nodulation, physicochemical characteristics of soils and origins of soils and seeds. Axes 1 and 2 explained 86% of total variance of data (figure 6).

Four (4) groups have been identified:

- The first one (I) represented by soils of Malam Mainari (Ma) and Bader (Ba) where the nodulation rate and number of nodules were the most important and have low total nitrogen (Nt) contents;
- The second group (II) represented by Kokoiye's soil (Ko) whitch has a higher nitrogen content and lowest rate of nodulation and nodules;
- The third one (III) represented by soils of the N'Guel Kolo (Nk) and Azzai (Az) where nitrogen and sand contents, nodulation rate and number of nodules are average;
- Group IV represented by Kiki's soils (Ki) that has higher contents of nitrogen, clay and silt and lower rate of nodulation rate and nodules.



***Ba** : Bader, Nk : N'Guel Kolo, Ki : Kiki, Ma : Malam Mainari, Ko : Kokoiye and Az : Azzaï.*
*Example of reading pairs's letters: **KoxNk** means that plant whose seeds come from **Ko** was inoculated with **Nk**'s soil.*
Figure 6: Projection of parameters on axes F1 and F2 of PCA.

IV. DISCUSSION

This study highlighted a variation in nodulation according to soil's type. Results demonstrate that nodulation depends on the number of rhizobia present in the soil. Similar results have been highlighted by [17] in Senegal on different types of soil. These results show that environmental conditions could modulate plant nodulation by influencing the number of rhizobia in the soil. MPN analysis showed that rhizobia's number was higher in N'Guel Kolo and Bader soils and lower in Kiki one. Hence a higher nodulation rate in N'Guel Kolo and Bader compared to Kiki. This could be due to physicochemical characteristics of soils. For [18] & [19], rhizobia are associated with aggregates, which protection them against disturbance by environmental and biotic factors.

Results of the PCA showed that rhizobia's distribution was correlated with physicochemical characteristics of soil. Thus, the spatial variation of nutrients and the type of soil can influence microorganisms. Differences in nodulations observed between soils could be due to environmental conditions such as physicochemical characteristics of gum groves's soils. Similar results were found by [17] on *A. senegal* & [20] on *Phaseolus vulgaris*. This indicates a higher diversity of rhizobia when physicochemical conditions of soil are favorable for bacteria's development in particular when nitrogen content is low in the soil. However, [21] noted a higher variety of rhizobia nodulating cowpea under limited water conditions in Senegal. As for [22] & [23], they didn't find a clear relationship between diversity of rhizobium strains and their ecogeographic origin. Overall, the soil of Bader Goula gave the best nodulation rate with all sources. This high proportion of plants to form nodules with Bader Goula's soil could be attributed to a co-evolution between strains of rhizobia and the origin of Bader Goula. [17] found similar results in Senegal on different types of soil with *A. senegal*. However, the best plant nodulation was obtained when using soil and seeds from the same gum grove as in the case of N'Guel Kolo for this study. Results corroborate those found by [24].

These authors concluded that a legume has better nodulation capacity in original soils than in soils in which it was established. This confirms the work of [25] on peas (*Pisum sativum*) which have demonstrated the coexistence of genotypes and their specific rhizobia strains within the same locality.

Thus, nodule's observation can be used to differentiate the infectivity of rhizobia strains in the soil. What's consistent with [26] works who show the existence of a significant degree of host preference for rhizobia strains compared to the nodulation of different cultivars of *Phaseolus vulgaris*. Results also showed that nodulation varies with soil's origin, suggesting that *A. senegal* doesn't respond uniformly to infection by rhizobia. Several authors have shown considerable intraspecific variations in woody legumes regarding their symbiotic characteristics. Observations made by [27] on many woody legumes and [28] on *Gliricidia sepium* confirm these results.

Results of this study showed that nodulation was higher in soils of N'Guel Kolo and Bader and low in Kiki's one. This is due to the higher nitrogen concentration in soil, which is higher in Kiki's soil. These results suggest that nodulation decreases when nitrogen is high in the soil indicating that the self-regulation of nitrogen begins before this fixation in the nodules. [29] has shown that nitrogen compounds like nitrate affect nodulation regardless age, size or inoculation status of plant. In fact, plants use the nitrogen available in the soil and only use nitrogen fixation through the nodes to supplement the amount of nitrogen they need. These results are consistent with those of [30] who demonstrated that the high level of nitrogen available in soil eliminates the inoculating effect of rhizobium. However, [31] & [32] demonstrated that the response to inoculation with rhizobia occurs especially when densities of local populations of rhizobia are less than 50 rhizobia g^{-1} of soil. Indeed, the MPN of Kiki's soil is 113 rhizobia g^{-1} and is above this threshold. Consequently, the low nodulation observed in kiki's soil cannot be explained by the number of local rhizobia present, but rather by the amount of nitrogen in soil. In addition, local strains of N'Guel Kolo have shown good nodulation compared to exogenous strains. Which suggests that diversity of local rhizobia can be affected by competition from exogenous strains introduced by inoculation ([33], [34]). It's thus preferable to inoculate in some cases with effective indigenous strains than with exogenous strains as has been demonstrated by [35].

V. CONCLUSION

This study highlight a natural nodulation in *A. senegal* due to natural presence of rhizobia in gum grove's soils. The distribution of rhizobia able to form nodules with *A. senegal* is correlated with soil origin, in particular physicochemical composition and seeds origin. This study also made it possible to observe a nodulation in gum grove's soils whatever the combination origin of seed and soil. Thus, a rigorous identification and selection of the most efficient rhizobial strains will allow to establish a local inocula. This could be used to inoculate *A. senegal* species evolving in ecoclimatic conditions similar to formulated inocula and to favor success to the reforestation programs notably the Great Green Wall.

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