International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB)
Volume 5, Issue 2, 2019, PP 13-18
ISSN No. (Online) 2454-9428
DOI: http://dx.doi.org/10.20431/2454-9428.0502004
www.arcjournals.org

Nodulation and Agronomic Performance of Indigenous Rhizobia Isolated from Bambara Groundnut (Vigna Subterranean L.) Nodules in Dalao, Côte d'Ivoire

GUEI Nassé Kaéda Raissa[1], KONATE Ibrahim[1*], BAKAYOKO Sidiky[1], OUATTARA adama[1], AMANI Kouadio[1], TIDOU Abiba Sanogo[1], Abdelkarim FILALI-MALTOUF [2]

[1]*UFR Agro-forestry and Environment, Department of Biochemistry and Microbiology, Laboratory of Agrovalorization, Jean Lorougnon Guede University, B.P: 150 Daloa, Côte d'Ivoire.*

[2]*Faculty of Sciences, Laboratory of Microbiology and Molecular Biology, University Mohammed V-Agdal, Rabat, Morocco.*

Corresponding Author:** ***KONATE I., *UFR Agro-forestry and Environment, Department of Biochemistry and Microbiology, Laboratory of Agrovalorization, Jean Lorougnon Guede University, B.P: 150 Daloa, Côte d'Ivoire. Email : konatibrahim@yahoo.fr*

Abstract: *In most African's countries of Sub-Sahara, population skyrocketing driven impoverishment and hunger. With the aim of contributing to the strengthening of food security, the economic empowerment of rural populations and the sustainable management of soil fertility, several countries have opted for the cultivation of legume plants capable of fixing atmospheric nitrogen in symbiosis with certain soil bacteria. The aim of our study was to perform an agronomic characterization of rhizobia nodulating Vigna subterranea (Bambara groundnut), a food legume whose culture is marginalized in Côte d'Ivoire. Thus, 14 rhizobia strains isolated from nodules of three varieties of V. subterranea at an experimental plot in Daloa were agronomically analyzed. All strains were re-nodulated the Ci5 variety of V. subterranea and RVC8, RVC13 and RVC14 strains were identified as the most effective and infective than the reference strain S. meliloti ($p<0.05$). Moreover, the yield of the bambara groundnut (number and weight of pods) was improved by indigenous strains RVC1, RVC4, RVC8, RVC13 and RVC14 that are considered most effective than S. meliloti included in this study.*

Keywords: *Vigna subterranea, Rhizobia, native strain, nodules, agronomic characters*

1. INTRODUCTION

Bambara groundnut [Vigna subterranea (L.) Verdc] is an indigenous grain legume grown mainly by female subsistence farmers in drier parts of sub-Saharan Africa [1]. It is a tropical grain legume which plays an important nutritional role in developing countries of the tropics and subtropics. Because of its high protein content (18-25 %) [2]. Groundnut has several agronomic advantages including high nutritional value, drought tolerance, and ability to produce some yield in soils that are too poor for cultivation of other more favoured species such as common beans and groundnuts [3;4]. In fact, soil bacteria collectively called rhizobia can enter into a symbiotic association with vegetables especially Bambara groundnut which leads to the formation of nitrogen fixing root nodules. This symbiotic interaction is of agronomic and ecological importance due to its high nitrogen content in the total nitrogen balance of terrestrial ecosystems [5]. In addition, the soil bacteria belonging to the symbiotic and endophytic bacteria are well known for their beneficial implications to promote directly the growth and indirectly the defense of plants [6;7].

Thus, Bambara peanut contributes to soil nitrogen enhancement for other crops by fixing atmospheric nitrogen by symbiotic action with rhizobium bacteria. This action is very beneficial for crop rotations and inter-cropping [8;9]. Despite its importance, the harvest of Bambara's peanut is neglected and poorly judged.

Admittedly, several scientific studies have been carried out, notably on the botanical, physiological, biochemical and genetic traits of this plant species in Côte d'Ivoire; but none of them were interested in the rhizobial bacteria that are its natural symbiotic partners and origin of its agronomic and ecological

properties. Thus, our work aims to make an agronomic characterization of the symbiotic partner of the peanut of Bambara in order to use it as inoculum and natural fertilizer.

2. MATERIALS AND METHODS

2.1. Symbiotic Characteristic

The study was conducted in the experimental fields of the University Jean Lorougnon Guédé, Daloa, Côte d'Ivoire (6, 91° N, -6, 44° W). Three (03) variety of Bambara groundnut (Vigna subterranea L.) were used for the isolation (Figure 1). However, for the test of authentication, only the variety Ci5of Bambara groundnut was used to estimate the infectivity (ability to re-infect the host plant) and the efficiency (effectiveness in fixing atmospheric nitrogen) of fourteen (14) isolates and one (01) reference strain Sinorhizobium meliloti 1021. The 14 strains isolated in Daloa's soil, were coded RVC (Rhizobia of Vigna of Côte D'Ivoire). Each strain was grown on Yeast Extract Medium (YEM) liquid medium to logarithmic phase and a volume of 1mL of the bacterial broth inoculated to young plant of bambara groundnut old to 12 days, growing in plastic pots (three plants/pot) containing sterilized sand. As control, each block contained two pots (controls) that not inoculated [10]. Plants were supplied with water every two days. Forty-five days after inoculation, number and dry weight (mass) of nodules were recorded for evaluating the infectivity. Also the dry weight (mass) of the aboveground (aerial) part of the plant was recorded for evaluating the effectiveness that expressed in relative index.

Figure1. *Seeds of Bambara groundnut used for this study: (A) Ci5, (B) Ci2 et (C) Ci1*

Relative Index of dry weight increase was calculated according to the described according of Cheriet's method [11]:

$$\text{Relative Index of Dry Weight (RI)} = \frac{\text{Dry weight of inoculated plant}}{\text{Dry weight of uninoculated palnt (control)}}$$

2.2. Agronomic Characteristic

The productivity of bambara groundnut were estimated by two (02) significant parameters: fruit number and dry weigh of fruits. These parameters were evaluated three (03) months after inoculation

2.3. Data Analysis

The data of measured parameters recorded were together and subjected to statistical analysis using the STATISTICA program (7.1). Subjected of Chi2 of Pearson and plant agronomics parameters were subjected by analysis of variance difference between the rhizobial strains evaluated at the 5 % level of HSD test

3. RESULTS AND DISCUSSION

3.1. Authentication and Symbiotic Characteristic

The re-inoculation of the plants with strains isolated from three (03) varieties of Vigna subterrenea and Reference strain (S. meliloti) allowed the re-infection of the host plant (variety Ci5 of Vigna subterrenea) and the formation of the functional nodules on the hand and the increase of the dry weight of the aboveground (aerial) part, the underground (root) part and the nodules on the other hand (Table 1). Osei Yaw et al. [12] showed that rhizobia isolated from cowpea could not nodulate bambara groundnut (V. subteranea) even though cowpea nodulated with rhizobium isolated from these legumes. A large variability infective capacity of the strains was highlighted in this study.

Table1. *Evaluation of symbiotic parameters of the strains tested per plant*

	ADM (g/pl)	RDM (g/pl)	SDM (g/pl)	N nod/pl	NDM (mg)	Relative Index	Infectivity Effectiveness
Témoin	0,93 m	0,51k	1,70 l	0,00j	0g	-	-
RVC1	1,38 h	0,7 h	2,07 g	15defghj	20 e	1,48	Nod+Fix+
RVC2	1,41 g	0,5 k	1,91 j	7hj	0,01 g	1,51	Nod+Fix+
RVC3	1,58 f	1,06 c.d	2,64 d	27,33 bcd	10 e	1,70	Nod+Fix+
RVC4	1,23 j	1,10 c	2,33 f	34 abc	43 b	1,32	Nod+Fix+
RVC5	1,41 g	1,32 b	2,73 c	21 cdefg	7 f	1 ,51	Nod+Fix+
RVC6	1,78 d	0,78 g	2,56 e	22 cdef	10 e	1,91	Nod+Fix+
RVC7	1,10 l	0,57 j	1,61 m	10 ghj	0,01 g	1,18	Nod+Fix+
RVC8	1,94 c	1,70 a	3,63a	42,33 a	110 a	2,08	Nod+Fix+
RVC9	1,71 e	0,91 e.f	2,63 d	27,33 bcd	20 e	1,83	Nod+Fix+
RVC10	1,13 k	0,89 f.g	1,81 k	15 fghj	0,01 g	1,21	Nod+Fix+
RVC11	1,14 k	0,86 g	2,01 h	8 ghj	0,01 g	1,21	Nod+Fix+
RVC12	1,39 gh	0,94 e	2,33 f	26 bcde	37 c	1,49	Nod+Fix+
RVC13	2,31 a	1,33 b	3,64 a	38 ab	110 a	2,48	Nod+Fix+
RVC14	2,00 b	0,94 e	2,95 b	12 fghj	20 d	2,15	Nod+Fix+
S. meliloti	1,94 c	1,03 d	2,97 b	27 bcd	37 c	2,09	Nod+Fix+

Means in columns followed by the same letters are not significantly different at p < 0.05 (Tukey's HSD test).

ADM: aerial dry mass; RDM: root dry mass; SDM: shoot dry mass; N nod: nodule number; NDM: nodule dry mass; ER: effectiveness relative; Nod+Fix: functional nodules.

The results of this work are consistent with those reported by Maatallah et al. [13] and El-Hilali [10] which authenticated the isolated rhizobia of chickpeas and lupins respectively. Even if each plant is inoculated with 1 ml of bacterial broth, the average number of nodules per plant varies from 7 to 43. Two RVC9 and RVC3 strains showed the same infectious capacity as the reference strain (S. meliloti). While three other RVC4, RVC8 and RVC13 strains showed higher infectious capacity than Sinorhizobium meliloti (Figure 2).

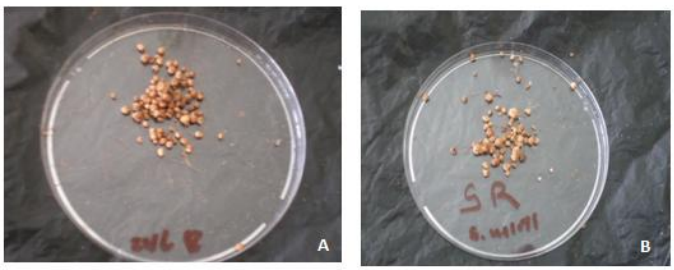

Figure2. *Nodules of plant inoculated with (A) : RVC8 and (B) : S. melilot*

Substantial gains in dry matter were observed in Vigna subterrenea plants grown in semi-sterile soil and inoculated with native Rhizobia from Daloa and S. meliloti. Indeed, more than 57 % of the strains isolated from Vigna subterrenea produced gains in dry matter, in their host plants, of more than 50 % compared to uninoculated plants. The native strains RVC13 and RVC14 were more efficient than the S. meliloti strain, the efficacy of which is similar to that of RVC8. These inoculants also led to a significant development of the root systems of the plants that received them, except for the RVC2 strain which induced a similar effect to that of the controls (Figure 3).

Figure3. *Root systems of plants inoculated with (A) : RVC8 and (B) : Control*

It should be noted that, in general, there was no correlation between the symbiotic parameters evaluated except for two strains RVC13 and RVC8. Several authors have reported similar results [10;13;14]. However, various authors have found strong correlations between aerial biomass, root biomass, and number and mass of nodules [15]. Also, Diagne [16] showed that the introduced Rhizobium strain was more effective than the isolated autochthonous strains of Prosopis.

Following the study of the symbiotic factors, nine strains with a good relative effectiveness and a strong infective capacity were identified. These strains are: RVC1, RVC3, RVC5, RVC6, RVC8, RVC9, RVC12, RVC13 and RVC14. In addition, the symbiotic parameters analysis performed by Amani et al. [17] showed that Daloa's soil contains native Rhizobia that can boost soybean (Glycine max L.) growth and yield compared to imported inoculums.

3.2. Performance Evaluation

In terms of yield, all isolates of Vigna subterrenea produced the same effects in plants as the reference strain S. meliloti except the RVC13 and RVC8 strains which significantly increased the production of the host plant (Table 2). Furthermore, the plant inoculated with the RVC3 strain gave pods which had a much higher dry weight than the plants inoculated with the other strains (Figure 4). Also, four strains RVC1, RVC3, RVC4 and RVC13 induced the production of pods with higher dry weights than pods whose production is stimulated by the strain reference S. meliloti. It should be noted that the obtaining of good yields is intimately linked to the strains which have proved to be good in fixing nitrogen by the formation of functional nodules. Okogun and Sanginga [18] and Tefera et al. [19] found a strong association between biomass and grain yield. The production of large pods in terms of quantity can be stimulated by the strains RVC1, RVC3, RVC4, RVC8 and RVC13. Some of these strains produced good yields (on average 7 pods / plant) and others have been more effective in stimulating the production of pods whose dry weight far exceeds those induced by the S. meliloti reference strain.

Table2. *Evaluation of performance parameters*

	N G /pl	PDM (g/pl)	PlDM(g)
Témoin	1,33 b	0,10c	2,3 bc
RVC1	6 ab	2,96 ab	1,87 c
RVC2	5,33 ab	2,26 b	2,03 c
RVC3	5,67 ab	4,55 a	5,61a
RVC4	5,67 ab	2,58 ab	4,32 ab
RVC5	3 ab	1,16bc	2,03 c
RVC6	2,33 ab	1,55 bc	2,09 c
RVC7	6 ab	2,43 b	2,62 bc
RVC8	6,67 a	2,31 b	1,5 c
RVC9	4 ab	1,52 bc	1,87 c
RVC10	4 ab	1,76 bc	2,77 bc
RVC11	5 ab	1,75 bc	1,86 c
RVC12	6 ab	2,49 b	2,3 bc
RVC13	6,67 a	2,54ab	2,11 c
RVC14	5,67 ab	2,18 b	3,63 abc
S.meliloti	3,67 ab	2,26 b	1,96 c

Means in columns followed by the same letters are not significantly different at p < 0.05 (Tukey's HSD test). NG: number pods, PDM: pods dry mass, PlDM: plant dry mass

Figure4. *Pods produced by plants inoculated with (A) : RV13 and (B) : RVC1*

4. CONCLUSION

In total, fourteen (14) bacterial strains coded RVC were isolated in vitro from the nodules of Vigna subterranea plants (bambara peas) grown in an experimental space at the University of Daloa (Côte d'Ivoire). All strains were authenticated with the Ci5 variety of Vigna s. and showed agronomically high infectivity and efficacy. Thus, the strains RVC8, RVC13 and RVC14 were identified as the most efficient and infectious as the reference strain Sinorhizobium meliloti ($p<0.05$). In addition, the analysis of parameters related to infectivity, symbiotic efficiency and yield of host plants in pods allowed the selection of five strains RVC3, RVC4, RVC8, RVC13 and RVC14 considered being more effective than the international strain Sinorhizobium meliloti. These strains can be constituted as well as local inoculums in the improvement of Vigna s. production in West Africa

REFERENCES

[1] Ntundu W. H, Inga C. Bach, Jørgen L. Christiansen and Sven B. Andersen. 2004, Analysis of genetic diversity in bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces using amplified fragment length polymorphism (AFLP) markers. *African Journal of Biotechnology*, 3 (4), pp. 220-225. https://www.ajol.info/index.php/ajb/article

[2] Brough S.H., S.N.Azam-Ali and Taylor A.J. (1993). The potential of Bambara groundnut [*Vigna subterranea* (L.) Verdc.] in Vegetable milk production and basic protein functionality systems. *Food chemistry.* 47: 227-283. https://doi.org/10.1016/0308-8146(93)90161-8

[3] Anchirinah VM, Yiridoe EK and Bennett-Lartey S.O (2001). Enhancing sustainable production and genetic resource conservation of bambara groundnut: a survey of indigenous agricultural knowledge systems. Outlook on Agriculture 30: (4) 281-288. https://doi.org/10.5367/000000001101293788

[4] Jorgensen S. T., Liu F., Ouédraogo M., Sarrazin J. and Christiansen J. L. (2010). Drought Responses of Two Bambara Groundnut (*Vigna subterranea* L. Verdc.) Landraces Collected from a Dry and a Humid Area of Africa. Journal of Agronomy and Crop Science, 196(6): 412-422.https://onlinelibrary .wiley.com /doi/pdf/10.1111/j.1439-037X.2010.00435.x

[5] Puri R.R., Dangi S.R., Dhungana S.A. and Itoh K. (2018). Diversity and Plant Growth Promoting Ability of Culturable Endophytic Bacteria in Nepalese Sweet Potato. *Advances in Microbiology*, 8, 734-761. https://doi.org/10.4236/aim.2018.89049

[6] Postgate J (1998). Nitrogen fixation. Cambridge University Press,Cambridge, UK.

[7] Birhanu B. (2019). Plant Growth Promoting Bacteria and their Prospective Role for Crop Improvement. *5(1): 16-27. International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB)* https://www.arcjournals.org/pdfs/ijrsmb/v5-i1/3.pdf

[8] Murukumbira LM, 1985. Effects of rate of fertilizer nitrogen and previous grain legume crop on maize yields. *Zimbabwe Agriculture Journal* 82(6): 177-179.

[9] Karikari SK, Chaba O and Molosiwa B (1999). Effects of intercropping bambara groundnut on pearl millet, sorghum and maize in Botswana. Afr. Crop Sci. J. 7:143-152. https://www.ajol.info/index.php/acsj /article /view/27765

[10] El-Hilali. 2006. la symbiose rhizobium-lupin : biodiversité des microsymbiotes et mise en evidence d'une multi-infection nodulaire chez lupinus luteus. Thèse de doctorat, Faculté des Sciences, Université Mohammed V, Rabat, Maroc. https://toubkal.imist.ma/handle/123456789/184

[11] Cheriet D., 2016. Etude des bactéries symbiotiques de la luzerne (*Medicago ciliaris* L.) fixatrices d'Azote. Thèse de doctorat, Université Badji Moktar de Annaba (UBMA).http://www.secheresse.info /spip. php? article67493

[12] Osei Y.A., Emmanuel O.A., Bjorn S. and Mette M.S. (2008). Host range, symbiotic effectiveness and nodulation competitiveness of some indigenous cowpea bradyrhizobia isolates from the transitional savanna zone of Ghana. *African Journal of Biotechnology*, 7 (8), pp. 988-996. https://academicjournals.org /journal/AJB/how-to-cite-article/829B2ED8412

[13] Maâtallah, J., E. B. Berraho, S. Munoz, J. Sanjuan, and C. Lluch. (2001). Phenotypic characterization of rhizobia isolated from chickpea (*Cicer arietinum* L.) growing in Moroccan soils. *Agronomie*. 22, 321-329. https://pdfs.semanticscholar.org

[14] Belay Z. and Fassil A. (2010). Symbiotic and phenotypic diversity of *Rhizobium leguminosarum* bv. *viciae* from Northern Gondar,Ethiopia. *African Journal of Biotechnology*, 10(21), pp. 4372-4379.https: //www. ajol. info/index.php/ajb/article/view/93631

[15] *N'gbesso M.F.D.P., Fondio L., Coulibaly N.D. and Kouame N.C* (2017). Efficacité symbiotique de cinq souches locales de rhizobiums sur les paramètres de croissance du soja. International Journal of Biological and Chemical Sciences, Vol 11 n° 5. https://www.ajol.info/index.php/ijbcs/article/view/164775

[16] Diagne O., (1992). Isolement et caractérisation de souches de rhizobium de prosopis provenant de différentes zones écologiques, rapport chercheurs associes ORSTOM. Direction des Recherches sur les Productions Forestières ; Ministère du Développement Rural et de l'Hydraulique, Institut Sénégalais de Recherches Agricoles. http://intranet.isra.sn/aurifere/opac_css/docnum/FT9300365.pdf

[17] Amani K., Konate I., N'gbesso M.F.D.P., Attien Y.P., Fondio L., Filali-Maltouf A. and Tidou A.S. (2019). Phenotypic and symbiotic diversity of Rhizobia isolated from root nodules of soybean [*Glycine max* (L.) Merrill] in Côte d'Ivoire. . *Int. J. Curr. Microbiol. App. Sci*, *8*(3): 766-774. https://doi.org/10.20546/ijcmas.2019.803.094

[18] Okogun J.A. and Sanginga N. 2003. Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria? *Biology and Fertility of Soils*, 38: 26–31. https://link.springer.com/article/10.1007%2Fs00374-003-0611-8

[19] Tefera, H., Kamara, A.Y., Asafo-Adjei, B., and Dashiell, K.E. (2009). Improvement in grain and fodder yields of early- maturing promiscuous soybean varieties in the Guinea savanna of Nigeria. Crop Science, 49: 2037-2042. https://naldc.nal.usda.gov/download/37344/PDF

Citation: *KONATE I., et.al, "Nodulation and Agronomic Performance of Indigenous Rhizobia Isolated from Bambara Groundnut (Vigna Subterranean L.) Nodules in Dalao, Côte D'ivoire", International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB), vol. 5, no. 2, pp. 13-18, 2019. Available: DOI: http://dx.doi.org/10.20431/2454-9428.0502004*