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Characterization and Evaluation of Native *Rhizobium* of Groundnut (*Arachis hypogaea L.*) and Soybean (*Glycine max L.)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was conducted to characterize and evaluate native *Rhizobium* isolates of groundnut and soybean to enhance the Legume-*Rhizobium* symbiosis as well as increase sustainable crop production. In this connection, all the native isolates under study were characterized biochemically and tested with N-free sand culture in the glasshouse to select effective native *Rhizobium* isolates through proper screening. 10 isolates of each crop were collected from the culture collection bank of the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya Raipur (Chhattisgarh). Out of 10 isolates, 3 isolates of each crop were selected on the basis of their growth performance and the purity of the culture. The biochemical studies of selected isolates include starch hydrolysis, catalase, urease, citrate utilization, indole production,

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gelatine iron, and methyl red tests, which were further evaluated for nodulation and biological nitrogen fixation in groundnut and soybean under controlled conditions with four treatments for each crop and five replications. The experiment result revealed that the evaluation of native *Rhizobium* isolate no. 1185 of groundnut and isolate no. 1076 of soybean was found to be superior among all the rhizobial isolates taken for study. These isolates accumulated 1.24, 1.44 mg/plant extra amount of atmospheric nitrogen over un-inoculated control plants, respectively. Further, it can be concluded that these native isolates may be the most effective nitrogen fixers for groundnut and soybean over the control.

Keywords: Rhizobium; groundnut; soybean; legumes; nitrogen.

1. INTRODUCTION

India's agricultural sector relies heavily on the pulses crop. According to Pooniya et al. [1], India makes up roughly 25% of the world's total production of pulses. India is the 2nd largest groundnut producer in the world, with 6,857,000 metric tons of production volume per year. Groundnut (*Arachis hypogaea L.*) is known as the "King of Oil Seeds" [2]. Groundnut seeds are a good source of B vitamins and contain 40 to 50% oil and 20 to 30% protein [3]. They also have a significant role in the economies of many countries and can contribute to soil up to 100 kg N/ha [4]. Similarly, soybean (*Glycine max L.*) is the major source of protein and oil. Soybean accounts for 68% of the world's production and is a source of biological nitrogen fixation [5]. It contributes almost 25% of the world's edible oil and contains approximately 20% dry matter oil with 30–50% protein. The beneficial interaction between soybean and *Rhizobium* can fix up to 300 kg N per hectare [6]. Nitrogen is the most essential macronutrient for plant growth and development. Much of this nitrogen is provided to cropping systems in the form of synthetically produced nitrogen fertilizers. Biological Nitrogen Fixation (BNF) is the best alternative to conventional nitrogen fertilizers. BNF is a low-cost and natural process of fixing nitrogen into the soil by microorganisms through symbiosis with plants. Rhizobia is a generic name for a certain Gram-negative group of Alphaproteobacteria and Betaproteobacteria that can form nodules on the root, or in some cases, on the stems, of their hosts and fix nitrogen in symbiosis with legumes as their host plants [7,8]. The symbiotic interaction between rhizobia and legumes is a mutual symbiosis in which both plants and bacteria benefit. In this symbiotic relationship, rhizobia are hosted and supplied with carbon sources by legumes, and in return, legumes receive ammonia provided by rhizobia [9,10]. *Rhizobium* is capable of infecting the roots of legumes and forming root nodules, where nitrogen fixation takes place, which results in the stimulation of plant growth. Biological nitrogen-fixing (BNF) root nodule bacteria like *Rhizobium* are highly beneficial for enhancing the productivity of various legumes, including pulses and oilseeds. The symbiotic relationships between specific soil microorganisms and plants are the most significant contributors to BNF in most terrestrial ecosystems [11]. Peoples et al. [12] reported that a symbiotic relationship with rhizobia is responsible for fixing, on average, on a whole plant basis (shoots and nodulated roots), the equivalent of 30–40 kg of nitrogen. Screening and selection of an effective *Rhizobium* strain and improvement of their quality are most important for nitrogen fixation. The purpose of this present study was to select efficient strains of rhizobia that can be effective for the nodulation of legumes. About 90% of all legume species become nodulated. The Rhizobium strain is highly specific to legume species [13].

2. MATERIALS AND METHODS

The glasshouse experiment was conducted during the *rabi* season of 2018–19 at the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya Raipur (Chhattisgarh). India, which is situated at an altitude of 298.58 m above the mean sea level (MSL) at 21°16 N latitude and 81°36 E longitude, The native Rhizobium isolates used in this experiment were collected from the culture collection bank of the Department of Agricultural Microbiology. Revival of cultures was done by streaking in yeast extract mannitol agar medium (pH 7.0). The streaked YEMA medium [14] plates were incubated at 28°C for 24 to 48 h (till colonies developed) in a BOD incubator. Individual colonies of inoculated isolates with distinct shapes and colors were selected for further study, inoculated in YEMA slant, and kept in a BOD incubator for a day at 28°C. The YEMA slant showing well-desired growth was picked and stored at 6–8 °C in a refrigerator for further

use. Sub culturing was done at a regular interval of 15 days. Colony morphological characteristics, viz. shape of the colony, size, margin, elevation, surface, and color, were observed on yeast extract mannitol agar medium. For this, 1.0 ml of an appropriate dilution of *Rhizobium* was transferred into the Petri plates. The plates were incubated at 28°C for 24-48 hours. The phenotype and growth pattern of *Rhizobium* isolates were observed. Further, these isolates were characterized using standard biochemical methods as described by Lowe [15]. Biochemical characterization was done based on different biochemical tests, viz., starch utilization test, catalase test, urine hydrolysis test, gelatin liquefaction test, indole production test, methyl red test, and citrate utilization test. The microscopic analysis after gram staining has shown that all the *Rhizobium* isolates were gram-negative. However, the *Rhizobium* isolates showed differences in their biochemical characteristics. Further, these isolates are evaluated in N-free sand culture and laid out in a completely randomized design with treatments and five replications. The treatments for groundnut include T_1 (control), T_2 (roundnut-1185), T_3 (roundnut-1750), and T_4 (roundnut-1007). Treatments carried out in soybean include T_1 (control), T_2 (Soybean-705), T_3 (Soybean-545), and T_4 (Soybean-1076). For the experiment, fine-graded, washed, and sterilized river sand was filled in the upper part of Leonard's self-irrigating assembly [16] and the lower portion filled with N-free McKnight solution [17]. Healthy and uniform-sized seeds of groundnut and soybean were rinsed with 95% ethanol, and then the seeds were washed thoroughly with double-distilled water several times. Then the seeds were air-dried to remove the excess moisture. Before sowing the seeds, all the glass bottles were irrigated with a N-free McKnight nutrient solution. Three healthy seeds treated with *Rhizobium* are sown out manually at a depth of 3-5 cm in a glass bottle by placing one seed in each hole with the help of sterilized forceps. After seed germination, a population of one plant per bottle was maintained by thinning out the extra seedlings. The glass bottle was properly tagged and labelled. Proper and uniform irrigation was given to all the glass bottles by the N-free McKnight solution. Groundnuts and soybeans are harvested at the prematurity stage after 45 days of sowing. Morphological characteristics, viz. shape of the colony, size, margin, elevation, surface, and color, were observed on yeast extract mannitol agar medium. For this, 1.0 ml of an appropriate dilution of *Rhizobium* was transferred into the Petri plates. The plates were incubated at 28°C for 24-48 hours. The phenotype and growth pattern of *Rhizobium* isolates were observed. 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Groundnuts and soybeans are harvested at the prematurity stage after 45 days of sowing. The following parameters were recorded: height of the plant (cm), fresh weight and dry weight of the shoot (g), number of nodules (plant^{-1}), N content in the shoot (%), N uptake (mg/plant), and biologically fixed amount of nitrogen (mg/plant). The N content (%) in the shoot was determined by the micro-Kjeldahl method as described by Jackson [18] using an auto-digestive and distillation unit. A 0.5-gram sample (shoot) was taken into the digestion tube, and the digested sample was

taken for distillation. The solution collected in the conical flask was titrated by using 0.5 N of H2SO4. The titration value was noted, and the percentage of nitrogen was calculated and expressed as a percentage. For accurate interpretation, every observation made during this experimental study was methodically tabulated. ANOVA was used to statistically analyse the observations, as described by Gomez and Gomez [19]. A value of p less than 0.05 was deemed statistically significant. By taking the mean value of the observed data, statistical analysis was carried out.

3. RESULTS AND DISCUSSION

Colony morphological characteristics of native *Rhizobium* isolates produced semi-translucent, raised, circular, and mucilaginous colonies that varied in size between 2.00 and 4.00 mm and were whitish in appearance. The results are almost identical to those investigated by some researchers [20,21]. After the gram-staining test, the bacteria gave a pink color, which indicates that they were gram-negative. All the *Rhizobium* isolates were found to be gram-negative. Further, the *Rhizobium* isolates were identified by consultation with Bergey's Manual of Systematic Bacteriology, Volume 1. In biochemical tests, which are presented in Tables 1 and 2, Groundnut isolates no. 1185, 1750, and 1007 showed positive results for the urease test, gelatin iron test, methyl red test, and citrate utilization test and were found negative for the starch hydrolysis test, catalase test, and indole test. Similarly, native isolates of soybean, namely 705, 545, and 1076, were found positive for the urease test, the gelatin-iron test, and the methyl-red test and showed negative results for the starch hydrolysis test, the catalase test, the indole test, and the citrate utilization test. The biochemical characterization shows their extracellular enzymatic activity, which would be helpful for the identification of bacterial species based on the differences in the biochemical activity of different bacteria. The data on average plant height were recorded at two different stages of groundnut and soybean (Tables 3 and 4). The observed data clearly indicate that the observations at 30 and 45 DAS revealed the maximum height of the plant for groundnut (11.02 and 22.42 cm), which are exhibited by isolates no. 1007, respectively. The maximum plant height for soybean at 30 DAS (69.99 cm) was found in isolates no. 1076, and at 45 DAS, the maximum height (77.66 cm) is associated with isolates no. 545. Similar types of studies were also carried out by Rahman [22], Tahir et al. [23], and Deshwal et al. [21], who reported that *Rhizobium* inoculation affected plant growth significantly; the fresh shoot weight of groundnut and soybean was recorded at 45 DAS. The results revealed that the maximum fresh shoot weight of groundnut (2.12 g/plant) was attributed to treatment T_2, where isolate No. 1185 was inoculated. Similarly, the highest fresh shoot weight of soybean (1.82 g/plant) was found in treatment T_4 due to the inoculation of isolates no. 1076. Biswas et al. [24] reported that increased levels of *Rhizobium* inoculum significantly increased shoot weight in groundnut. Shoot dry weight of groundnut and soybean is a good indicator of plant growth, which also determines the crop yield. Shoot dry matter of groundnut and soybean was quantified at 45 DAS of crop growth. The results revealed that the maximum dry weight for groundnut (0.47 g plant^{-1}) was attributed to treatment T_2, where isolates no. 1185 were applied, and in the case of soybean, the highest dry weight (0.33g plant^{-1}) was associated with isolates no. 1076. However, all the treatments taken under study found it efficient to increase the shoot dry weight over control. Similar results were also carried out by Stefanescu and Palanciuc [25], who mentioned that the shoot dry matter of the inoculated treatments was significantly greater than that of the control as a result of increases in nodulation. The nodule count was recorded at 45 DAS of crop growth for both groundnut and soybean. Results revealed the highest nodulation for groundnut (11/plant) and for soybean (14/plant) raised from seeds inoculated with the native isolates 1185 and 1076, respectively. There was no nodule in the un-inoculated control plants. All the isolates taken under study significantly affected the number of nodules over control. Brah-Maprakash et al. [26] and Biswas et al. [24] reported that the number of nodules improved significantly due to the inoculation of *Rhizobium*. Nitrogen content in shoots varied among treatments; selected isolates exhibited a differential influence on shoot N content of groundnut and soybean at 45 DAS. The maximum content of nitrogen in shoots for groundnut (0.97%) and soybean (0.96%) was found in seeds treated with the native isolates 1185 and 1076, respectively. Similarly, results of N-uptake by groundnut and soybean revealed that N uptake in shoots increased significantly over control due to the inoculation of native *Rhizobium* isolates. Maximum N-uptake in shoots for groundnut (4.55 mg/plant) and for soybean (3.16 mg/plant) was associated with

Table 1. Biochemical characterization of native *Rhizobium* of groundnut

Name of Isolates	Starch hydrolysis	Catalase	Urease hydrolysis	Gelatin liquefaction	Indole production	Methyl red	Citrate utilization
Groundnut-1185	-	-	+	+	-	+	+
Groundnut-1750	-	-	+	+	-	+	+
Groundnut-1007	-	-	+	+	-	+	+

Table 2. Biochemical characterization of native *Rhizobium* of Soybean

Name of Isolates	Starch hydrolysis	Catalase	Urease hydrolysis	Gelatin liquefaction	Indole production	Methyl red	Citrate utilization
Soybean-705	-	-	+	+	-	+	-
Soybean-545	-	-	+	+	-	+	-
Soybean-1076	-	-	+	+	-	+	-

Table 3. Effect of native isolates of *Rhizobium* on growth parameters and nitrogen accumulation of Groundnut

Treatment details	Plant height (cm)		Fresh shoot weight (g/plant)	Dry shoot weight (g/plant)	Nodule number (plant^{1})	Nitrogen content (%)	N-uptake (mg/plant)	BNF (mg/plant)
	30 DAS	45 DAS	45 DAS	45 DAS	45 DAS	45 DAS	45 DAS	45 DAS
T_1	8.88	12.74	1.76	0.37	0	0.85	3.31	-
T_2	10.40	15.74	2.12	0.47	11	0.97	4.55	1.24
T_3	10.74	16.52	2.01	0.40	9	0.91	3.72	0.41
T_4	11.02	22.42	1.85	0.42	8	0.93	3.44	0.13
SEm±	0.20	0.64	0.07	0.01	0.61	0.01	0.30	-
CD (p=0.05)	0.63	1.94	0.23	0.04	1.85	0.03	0.93	-

Table 4. Effect of native isolates of *Rhizobium* on growth parameters and nitrogen accumulation of Soybean

Treatment details	Plant height (cm)		Fresh shoot weight (g/plant)	Dry shoot weight (g/plant)	Nodule number (plant^{1})	Nitrogen content (%)	N-uptake (mg/plant)	BNF (mg/plant)
	30 DAS	45 DAS	45 DAS	45 DAS	45 DAS	45 DAS	45 DAS	45 DAS
T_1	60.74	67.57	1.14	0.21	0	0.82	1.72	-
T_2	66.61	76.49	1.60	0.28	11	0.92	2.57	0.85
T_3	63.93	77.66	1.62	0.29	12	0.91	2.66	0.94
T_4	69.99	74.52	1.82	0.33	14	0.96	3.16	1.44
SEm±	0.60	0.88	0.13	0.01	0.70	0.01	0.10	-
CD (p=0.05)	1.83	2.67	0.39	0.04	2.12	0.03	0.32	-

seeds inoculated by native isolates 1185 and 1076, respectively. Similar findings were reported by Biswas et al. [24] who mentioned that increased levels of *Rhizobium* inoculum significantly increased nitrogen accumulation in legumes. Biologically fixed amount of nitrogen also varied among treatments; inoculated isolates exhibited a differential influence on the fixed amount of nitrogen for groundnut and soybean. The results revealed that the maximum fixed amount of nitrogen for groundnut (1.24 mg/plant) and for soybean (1.44 mg/plant) was found in the seeds inoculated with native *Rhizobium* isolates no. 1185 and 1076, respectively. The symbiotic association between *Rhizobium* and legume plants usually leads to the initiation and development of root nodules [27], resulting in an increased amount of nitrogen fixation [28,29].

4. CONCLUSION

The study found that BNF (Biological nitrogen fixation) parameters like an accumulation of biomass and nitrogen, performance of native *Rhizobium* isolate no. 1185 of groundnut, isolate no. 1076 of soybean were found to superior among all rhizobial isolates taken for study. Further, it can be concluded that these isolates accumulated 1.24 and 1.44 mg/plant extra amount of atmospheric nitrogen over un-inoculated control plants, respectively. However, the result is indicative and required further experimentation to arrive at a more consistent result.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pooniya V, Choudhary AK, Dass A, Bana RS, Rana KS, Rana DS et al. Improved crop management practices for sustainable pulse production: An Indian perspective. Indian J Agril Sci. 2015;85:747-58. 9.
2. Kumar IS, Venkataravana P, Marappa N. Genetic divergence of new germplasm and advanced breeding lines of groundnut (*Arachis hypogaea L*.) studied under late kharif situation, Legume Research. 2010; 33(2):124-127.
3. Pardee WD. Groundnut, Encarta Encyclopedia, Microsoft Corp, USA; 2002.
4. Degefu T, Wolde-meskel E, Ataro Z, Fikre A, Amede T, Ojiewo CO. Groundnut (*Arachis hypogaea* L.) and cowpea (Vigna unguiculata L. Walp) growing in Ethiopia are nodulated by diverse rhizobia. African Journal of Microbiology Research. 2018; 12(9):200-217.
5. Ansari PG, Rao DLN. Soybean rhizobia in Indian soils: populations, host specificity and competitiveness. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2014;84(3): 457-464.
6. Kapembwa R, Mweetwa AM, Ngulube M, Yengwe J. Morphological and Biochemical Characterization of Soybean Nodulating Rhizobia Indigenous to Zambia. Sustainable Agriculture Research. 2016; 5(526-2016-37804).
7. Garrity G, Bell J, Lilburn T. Class I. *Alphaproteobacteria* class. nov. In Bergey's Manual® of Systematic Bacteriology. Brenner D, Krieg N, Staley, J. (eds). New York, NY: Springer US. 2005;1–574.
8. Sprent JI. 60Ma of legume nodulation. What's new? What's changing? J Exp Bot. 2008;59:1081–1084.
9. Bonaldi K, Gourion B, Fardoux J, Hannibal L, Cartieaux F, Boursot M. Large-scale transposon mutagenesis of photosynthetic *BradyRhizobium* sp. strain ORS278 reveals new genetic loci putatively important for nod-independent symbiosis with Aeschynomene indica. Mol Plant Microbe Interact. 2010;23:760–770.
10. Rogel MA, Ormeno-Orrillo E, Romero EM. Symbiovars in rhizobia reflect bacterial adaptation to legumes. Syst Appl Microbiol. 2011;34:96–104.
11. Russelle MP. Biological dinitrogen fixation in agriculture: Nitrogen in agricultural systems. 2008;281-359.
12. Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, et al. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural system, symbiosis. 2009;48:1-17.

13. Al-Mujahidy SMJ, Hassan MM, Rahman MM, Mamun-Or-Rashid ANM. Isolation and characterization of *Rhizobium* spp. and determination of their potency for growth factor production. International Research Journal of Biotechnology. 2013; 47:117-123.
14. Vincent JM. A manualfor the practical study of the root-nodule bacteria. Blackwell, Oxford; 1970.
15. Lowe GH. The rapid detection of lactose fermentation in paracolon organism by demonstration of 6-D-galactosidase. J. Med. Lab. Technol. 1962;19:21–25
16. Leonard LT. A simple assembly for use in the testing of cultures of rhizobia. Journal of Bacteriology. 1943;45(6):523-527.
17. McKnight T. Efficiency of isolates of *Rhizobium* in the cowpea group, with proposed additions to this group. Queensland Journal of Agricultural Science. 1949;6:61–76.
18. Jackson ML. Soil chemical analysis, prentice hall of India Pvt. Ltd., New Delhi. 1973;38-56.
19. Gomez KA, Gomez AA. Statistical procedures for agricultural research, 2edition, John Wiley and Sons, New York. 1984;8-13.
20. Arora NK, Kang SC, Maheshwari DK. Isolation of siderophore producing strains of *Rhizobium* meliloti and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curriculum Science. 2001;81: 673-677.
21. Deshwal VK, Dubey RC, Maheshwari DK. Solation of plant growth promoting strains of *BradyRhizobium* Arachis sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut, Curriculum Science. 2003;84(3): 443-448.
22. Rahman MA. Effect of calcium and *BradyRhizobium* inoculation of the growth, yield and quality of groundnut (*Arachis hypogaea L.).* Bangladesh J. Sci. Indust. Res. 2006;41:181–188.
23. Tahir MM, Abbasi MK, Rahim N, Khaliq A, Kazmi MH. Effect of *Rhizobium* inoculation and NP fertilization on growth, yield and nodulation of soybean (*Glycine max* L.) in the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. African Journal of Biotechnology. 2009; 8(22):6191-6200.
24. Biswas P, Hosain D, Ullah M, Akter N, Bhuiya MAA. Performance of groundnut (*Arachis hypogaea* L.) under different levels of bradyrhizobial inoculum and nitrogen fertilizer. SAARC J. Agric. 2003;1: 61–68.
25. Stefanescu M, Palanciuc V. Efficiency of bacterial inoculation and mineral nitrogen and phosphorus fertilization in rainfed soybean. Roma. Agric. Res. 2000;13-14: 75-83.
26. Brahmaprakash GP, Girisha HC, Navi V, Laxmipathy R, Hegde SV. Liquid *Rhizobium* inoculant formulations to enhance biological nitrogen fixation in food legumes. J. Food Legumes. 2007;20(1): 75–79.
27. Tirichine L, James EK, Sandal N, Stougaard J. Spontaneous root-nodule formation in the model legume Lotus japonicus: a novel class of mutants nodulates in the absence of rhizobia. Molecular Plant- Microbe Interactions Journal. 2006;19(4):373-382.
28. Askari-Khorasgani O, Hatterman-Valenti H, Flores Pardo FB, Pessarakli M. Plant and symbiont metabolic regulation and biostimulants application improve symbiotic performance and cold acclimation. Journal of Plant Nutrition. 2019;42(17):2151–2163.
29. Osei O, Abaidoo RC, Ahiabor BD, Boddey RM, Rouws LF. Bacteria related to *Brady Rhizobium* yuanmingense from Ghana are effective groundnut micro-symbionts. Applied Soil Ecology. 2018;127:41-50.

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