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Research Article

Efficacy of plant growth promoting (PGP) *Rhizobium sp.* adopted from *Arachis hypogaea* nodules and its impact on the host of *Sesbania sp.*

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Abstract

The intense competition for rhizobia in a variety of niches has resulted in their fitness. Screening of endophytic bacteria with plant growth-promoting traits has wider uses in crop yield.The present study aimed to evaluate the cross-inoculation of peanut root nodulating *Bradyrhizobium* sp. on the Sesbania host. The *Arachis hypogea* L. is the most popular legume collected from Madurai region, India, and was selected for Rhizobium isolation using yeast extract mannitol agar (YEMA) agar. The host specificity of isolated Rhizobium was tested on Sesbania sp. by seed priming, followed by qualitative plant growth promotion efficiency of isolates. Totally seven rhizobial strains were screened for plant growth-promoting traits (PGP), and two were found to be effective PGP and selected for the pot experiment. Isolates AH12 and AH15 were phylogenetically identified as *Bradyyrhizobium arachidis* PQ119952 and *Bradyrhizobium japonicum* PQ119954. Data compared the inoculation of seeds with AH12 and AH15 to those without inoculation in Sesbania. Test plants showed high efficiency for growth promotion on both Bradyrhizobial strains. Germination rate (76.6±0.57%), vigour index (547.6 and 552.2), vitality index (206.8 and 214.4), nodulation state, leghaemoglobin (LHB) and chlorophyll (1.48±0.13 and 1.58±0.12) content were increased significantly among the Bradyrhizobium-treated groups. The foliar application of both isolates was found to control the leaf spot *Alternaria* spp. infection. The cross-inoculation studies on Sesbania seeds showed that *Bradyhizobium* spp (multi-traits strains) improved all seed viability as well as the growth of plants and could form fully effective symbioses with strains in the genera.

Keywords: Auxin, Leghemoglobin, *Sesbania spp.*, Siderophore, Vigour index

INTRODUCTION

One of the first partnerships in the environment may be the plant-microbe association. During natural selection, microorganisms, regardless of specific phylogenic identity, primarily or indirectly impact plant growth (Berg *et al.,* 2015). Symbiosis is a significant and well-understood plant-microbe interaction unique to legume plants. Following soybeans, peanuts are the second most extensively grown grain legume worldwide. The allotetraploid, annual herbaceous groundnut (*Arachis hypogaea*) legume family well establishes symbiosis with many microbes. With an average yield of 1068 metric tons per hectare and an overall harvest of 40 million metric tons, it can be grown on around 24 million hectares globally (Kishlyan *et al.,* 2020).The legume *Arachis hypogia* is important to the global agricultural economy and is mostly grown for its protein and vegetable oil (Krishna *et al.*, 2015). In India, one of the most significant cash crops is groundnut. The majority of the literature, particularly *Bradyrhizobium* sp., described Rhizobia nodulation and nitrogen fixation (Wang *et al.,* 2017). Numerous Bradyrhizobium and Rhizobium species are nodulated throughout the native region of this South American native (Muñoz et al., 2011). Groundnut, often known as peanut (*Arachis hypogaea* L.), is a significant dietary legume in tropical and subtropical regions. Rhizobium, an N-fixing soil bacteria, has been proven in several studies to benefit legumes, especially groundnuts (Gouda *et al.,*2018), raising productivity and lowering production costs. Bradyrhizobium, a slow-growing plant, is usually responsible for nodding groundnuts. However, efficient fast-growing strains have also been observed to do so. A widespread and global collection of microsymbionts called Bradyrhizobium can nodulate both non-legume and a wide range of legumes (van Vugt *et al.,* 2018). The two main symbionts for legume plants are Bradyrhizobium and Rhizobium, however new research has revealed that other non -rhizobial bacteria also coexist with rhizobial strains (Cardoso *et al.,* 2018). Most alpha-proteobacteria groups and some beta-rhizobia species are the primary mediators of stem and root nodule development in symbiotic legumes (Sprent *et al.,* 2017). Rhizobial nod factors interact with nodulation and N_2 fixation in legumes. Certain rhizobia can also create major phytohormones during this process, positively impacting plant growth (Hayashi *et al.,* 2014). The positive effects of rhizobia are mediated through the production of several metabolites and enzymes that plants and rhizobia either directly or indirectly trigger during nodule development. The secretion of IAA, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, riboflavin, and phosphatase for phosphate solubilization have all been identified as significant PGPR processes (Subhan*et al.,*2020).

MATERIALS AND METHODS

Isolation of *Rhizobium* sp

Ten *Arachis hypogaea* plants were collected from the five different local sites around Madurai District, Tamilnadu, India in December 2023. Young, healthy plants were randomly selected, and their roots and root nodules were carefully uprooted to acquire undamaged roots. Nodules were placed in a 0.1% mercury chloride solution for 60 seconds, washed with 50% (v/v) ethanol, and rinsed using sterile distilled water. 1 g of surface-sterilized nodules were crushed in 10 ml normal saline and diluted up to a 10^{-8} under a septic condition. One milliliter (10^{-7}) suspension was used for the pour plate method under Yeast extract mannitol agar. Plates were kept in an incubator for five days at 28˚C for 72 h. White mucoid colonies were selected, subcultured and then Gram's stained according to the methodology described by Ma *et al.* (2015).

Plant growth promotion characters

Production of exopolysaccharide was monitored by culturing isolates on Congored agar (CRA) containing 3% sucrose and 1% glucose (Verma *et al.* 2020). Phosphate and zinc solubilization test was performed on Pikovskaya's and zinc oxide agar plates. Zinc solubility of isolates tested on growing on YEMA with 0.1% ZnO medium.The following formula was used to determine the solubilization index.SI=zone diameter - colony diameter/ colony diameter. The production of ammonia among isolates was tested by adding Nessler's reagent to cultured peptone water. Isolates grown on modified YEMA supplemented with 2 g glycine/L used to detect Hydrogen cyanide. The top of the plate contained a Whatman filter paper No. 1 soaked in a solution of 0.5% picric acid and 2% sodium carbonate. The qualitative CAS agar test was used for screening siderophore production.

Plant growth hormone production

Production of auxin, Gibberellic acid (GA3) and Cytokinin were screened by growing strains underYeast extract mannitol broth supplemented with 1 g/l of L-tryptophan 0.01% thiamine, 2 pg of biotin. Following two days of incubation at 28 ± 2 °C the cultures were centrifuged at 5000rpm.The cell free supernatant was used for detection of growth hormones. After 24 hours of fermentation, the cell-free supernatant was combined with ethyl acetate, and the solvent phase was collected and concentrated. Using n-butanol:acetic acid:water (12:3:5 v/v/v) as the mobile phase, samples and references were spotted on thin layer chromatography (TLC) chromatograms. Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) was utilized for IAA determination. Gibberellic acid was quantified with 2,4-dinitrophenylhydrazine solution (DNPH), at 430 nm (Qi *et al.,*2021). cytokinin was measured at 405 nm using the Plant Growth Regulator (PGR) Enzyme Immunoassay Kit (Merck) with standard 6-benzylaminopurine (Nayar Saraswati, 2021).

1-aminocyclopropane-1-carboxylate (ACC) deaminase assay

Intracellular protein was isolated using RIPA buffer for protein extraction reagent and resuspended in 2x sample buffer. Total protein was estimated using Lowry's method, which used BSA as a standard. ACC deaminase activity was determined by the method of Ali *et al*. (2014).Two hundred proteins were incubated with 20 μL of 0.5 M ACC at 30 °C for 15 min. After incubation, 800 μL of HCl (0.56N) and freshly prepared 300 μL of DNPH reagent (0.1 g 2,4- dinitrophenyl hydrazine was added, vortexed and incubated at 30 °C for 30 min. The reaction terminated by 2mL of NaOH (2N) and absorbance was recorded at 540 nm. The μmoles of α-ketobutyrate were determined from a standard graph plot.

Seed germination test

After being injected into nutrient broth, productive rhizobial colonies that produced PGH grew overnight. *Sesbania grandiflora* seeds were surface sterilized with 70% ethanol for two minutes, after which they were treated with 1% sodium hypochlorite and repeatedly washed with sterile water. The seeds were then soaked under 1% starch solution containing rhizobial cell ($1X10^8$CFU) for 30min. Sterilized Petri plates with damp filter paper containing ten seeds of each treatment were spaced equally apart and incubated at 28°C. The seedling emergence and seed germination were calculated by using:

Percentage of seed germination= Number of emerged

seeds/number of tested seeds x 100 Eq.1
Seed vigor index (VI) = Germination percentage (%) x seedling length (cm) Eq.2

Plant growth promotion studies on (Pot trials)

The pots were 14 inches in circumference and could contain 10 kg of soil. The studies were conducted on sterile soil. Three treatments in all, with three replications of each, were used. One hundred milliliters of culture (OD 2.0×10^8 CFU ml^{-1}) was applied to the soil. Ten seeds were prepared with strains of rhizobia. To maintain seed coating the seeds kept an hour in Rhizobial phosphate saline buffer (RPSB) containing the suspension of the *Rhizobium* sp. Ten seeds were planted in a 5 cm depth in each pot. After germination, during 3rd and 8th day, the germination index was calculated as above.

Germination potential(DAY 3)= seed germinated /n x 100 Eq.3
Germination index (D8) seed germinated /n x100 Eq.4
Vitality index= GI X root weight Eq.5

Plant disease prevention

On 20thday growth stage plants were infected with spores of *Alternaria* sp (2x 10^3 CFU) for 5 days by foliar application. After 5 days, all the infected plants were treated with Rhizobial active strains (CFU=1×10^8) suspended in 1% xanthan gum was applied by foliar application for 5 days, and the disease control was monitored at the end of 20th day of treatment.

Plant growth characters

After 40 days, the crops were taken out and the following metrics were noted: Plant parameters that were assessed were height (cm), root (cm), weight (g) of root nodules, surface are of root nodule, were recorded. The nodules were oven-dried at 60°C for 24hours and weight of nodule was calculated. Plant photosynthetic pigments (µg/ml) were determined at all stages of plant growth using spectrophotometric methods followed by acetone-methanol extraction described by Jiménez-Lao *et al.*(2021).

Carotenoids $=5.02A_{480}$=µg/ml
Chlorophyll a $=10.3A_{663}-0.918A_{644}$=µg/ml
Chlorophyll b $=19.7A_{644}-3.87A_{663}$=µg/ml
Total chlorophylls $=7.04A_{645}+20.27A_{663}$

Leghemoglobin content in nodular tissues

Quantification of LHB content among nodules estimated as described by Singh and Varma (2017).100 mg of frozen nodules were crushed to release leghaemoglobin under 2 mL centrifuge tube along with 0.6 mL of Drabkin's solution. The mixture was centrifuged at 10,000 rpm and 4 °c and the supernatant was collected. The collected aqueous phase was made up to 2 mL and absorption was measured at 540 nm using a UV-visible spectrophotometer. Bovine hemoglobin standard was used with regression coefficient of 0.99.

Molecular analysis

DNA from Rhizobium cells was isolated, followed by the methodology of Jain et al. (2020), which used the Higeno MB (Himedia) kit as per the user manual. Amplification done by 50 µl reaction mixture containing 10 ng DNA template, 1 µl of primer, 1 U of Taq DNA polymerase (Banglore Genei, India) and 100 µM dNTPs. Universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1540 R (5′-AAGGAGGTGATCCAGCC-3′), for 16S rDNA were used. Using the Sanger technique, the PCR products were subjected to sequencing. Partial sequence similarity was compared to DNA database records, and sequences that demonstrated a homology of greater than 95% could be identified through the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information (NCBI) BLAST server (www.ncbi.nlm.nih.gov/BLAST).

RESULTS AND DISCUSSION

A total of seven bacterial isolates isolated from the healthy root nodules of ten *Arachis hypogaea* (AH) plants collected from various sites of Madurai are given in Table 1. Figure 1 represents the growth pattern and exopolysaccharide production among isolates. Among the collected plants, three plant roots did not have any nodulation. The lowest nodule weight was 0.7±0.002 and the maximum of 1.4±0.03 g. All the isolates included white mucoid, and gram-negative rods were designated AH9 to AH15.Isolate AH12 and AH15developed colonies after 48 h and were placed under slow growing strain. Groundnut had already been shown to develop nodulation with slow-growing *Bradyrhizobium* bacteria (Jaiswal *et al.,* 2017); however, Khalid *et al.* (2015) previously documented the presence of fast-growing, effective Rhizobium among peanut nodules. About 71%(n=5)of strains produced exopolysaccharide and AH12 and AH15 showed strong secretion than other positive strains. Similar report on the production of EPS by *Rhizobium*is documented by Sayyed *et al.* (2011). An EPS that has been studied in more detail, namely succinoglycan, was extracted from *Sinorhizobium meliloti* by Sutherland (2001).

Plant growth promotion (PGP) traits

The frequency of production of NH_3, siderophore, organic acid, HCN, phosphate and zinc solubilization was recorded as 28.5>42.85>28.5>42.85>28.5>14% and is given in Table 2. Two isolates (AH 12 an AH15) were positive for ammonia, siderophore and hydrogen cyanide production (Fig. 2a). Three isolates were identified as positive on organic acid and phosphate solubilization. Among the seven isolates, AH15 alone exhibited zinc solubilizing property. Table 3 represents the growth hormone producers among the isolates. Auxin was produced by strains of AH10, AH12 and AH15 (42.85%), 28.5% (AH12 and AH15) were positive on cytokinin, GA and ACC deaminase activity (Fig.2b). Further, the $R_f$value of Auxin, cytokinin, gibberellic acid of standard and isolated AH12 andAH15 were closely recorded and found to be 2.7, 1.7, 2.52 cm (Table 3). The total intracellular protein 16.4 and 17.56666667 µg/mL showed 55.02218637 and 83.16525481µM of ACC deaminase activity on AH12 and AH15 significant at <0.05 (Fig.3).It was reported that bacteria producing ACC deaminase activity the potential to promote plant growth and development even under stress state(Gupta and Pandey, 2019).

Seed germination and vitality

Strains of AH12 and AH15 over Sesbania seed germination mean values are given in Fig.4a. Percentage of seed germination index was53.3±0.57% control and 76.6±0.57% among *Rhizobium* spp treated seeds. The mean Root length over 10th day growth was cm2.42±0.28, 2.87±0.34 and 2.90±1.46 cm, respectively, on control, AH12 and AH15 (Fig.4b).Likewise, the Shoot height was calculated as 4.02±0.28 4.28±0.48 and 4.31±0.42 cm (Fig.4c). The maximum seedling length was 7.21cm by AH15 inoculate followed by 7.15

Table 1. Growth pattern of Rhizobium spp isolates from *Arachis hypogeal* root nodules

Sample code	Average Nodule weight (mg)	CFU X10^7	Strain code	Growth pattern	EPS
AH1	1.4±0.03	16	AH9	Fast	+
AH2	0.8±0.002	8	AH 10	Fast	-
AH3	0.90±0.001	18	AH 11	Fast	-
AH4	0.92±0.002	12	AH 12	Slow	++
AH5	0.88±0.004	13	AH 13	Fast	+
AH6	0	-	-	-	-
AH7	1.23±0.004	15	AH 14	Fast	+
AH8	0	-	-	-	-
AH9	0	-	-	-	-
AH10	0.7±0.002	6	AH 15	slow	++

Table 2. Plant growth promoting characters among isolated *Rhizobium* spp.

S. Code	NH_3	Phosphate solubilization	Siderophore	Organic acid	HCN	Zinc solubilization
AH9	-	+	-	+	-	-
AH 10	-	-	-	-	-	-
AH 11	-	-	-	-	-	-
AH 12	+	+	+	-	+	-
AH 13	-	-	-	-	-	-
AH 14	-	-	-	+	-	-
AH 15	+	+	+	+	+	+

- Negative ; + positive

Table 3. Plant growth hormone producing *Rhizobium* sp. and its retention factor (R_f)

S. code	IAA	Cytokine	GA	ACC deaminase
AH9	-	-	-	-
AH10	+	-	-	-
AH11	-	-	-	-
AH12	+(R_f 2.7)	+(R_f 1.70)	+(R_f 2.5)	+
AH13	-	-	-	-
AH14	-	-	-	-
AH15	+(R_f 2.7)	+(R_f 1.72)	+(R_f 2.5)	+

- Negative ; + positive

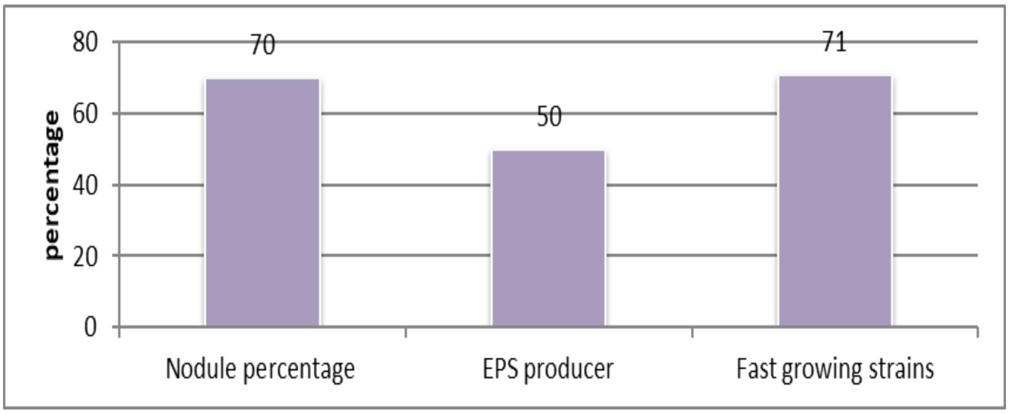

Fig. 1. *Frequency of growth pattern of Rhzobium spp. isolated from A.hypogea (n=7)*

by AH12 and lowest seedling length 6.42 was observed in control. The 10th day data on seed germination and growth pattern is given in Table 4. Seedling Vigor index (VI) was maximum in AH15, followed by AH12 and control. The calculated VI were 552.2>547.6>345.3. Root weight among control and treated groups revealed that AH15 and AH12 had maximum root weight (2.8 and 2.7 g) compared to the control group (1.6g). Vitality index of the control seed was 85.2, nearly 3fold lower than Rhizobium treated seeds. AH12 treated seed vitality was 206.8 and 214.4 in AH15 treated plants. The present findings agree with the observation of Tarekegn *et al.*(2017) that inoculating *B. japonicum* increased the mean nodule number, germination rate, and vigour index of seeds of legumes.

Vigor index and disease resistance

The growth parameters during 40th day among control and treatment are given in Table 5. The length of root (RL) and shoot (SL) among control was 6.62±1.1/ 31.32±6.85 cm with an average number of 80±1.43 nodules having weight of 142 mg/plant. The AH12 treatment shows 10.04±2.7/41.44±1.73 cm RL and SL with 141.2±12.98 number of nodules with 248 mg weight per plant. The p value of the root length of control and AH12 and between the test was insignificant at >0.05.AH15 group reveals 10.94±2.4/40.54±1.56 cm of RL/SL with 150.2±12.41 nodules having 256 mg /plant (Fig.5).The outcomes corroborate those of Lamptey *et al.* (2014), who stated rhizobium-inoculated plants give more nodules with a greater dry weight than the control. Both Rhizobium treated showed absence of leaf spot disease compared to control. The ability to produce high EPS and HCN helps control the fungal spread and prevent the infection of *Alternaria* sp.

Root plant growth hormones

The growth hormone content in the root of control and Rhizobium-treated plants is given in Table 6. Hormones indole acetic acid (IAA), Gibberlicacid (GA3), Cytokinin and leghemoglobin (LHB) were quantified and the data was found to be statistically significant (Fig.6). The con-

Table 4. Seed germination and vigour index of *Sesbania sp.* on 10th day

Properties	Control	AH12	AH15
Percentage of seed germination index	53.3±0.57	76.6±0.57	76.6±0.57
Root length cm	2.42±0.286356	2.87±0.341216312	2.90±1.46
Shoot height cm	4.02±0.286356421	4.28±0.482367672	4.31±0.422365786
Seedling length	6.42	7.15	7.21
Seedling Vigor index	345.3	547.6	552.2
Root weight(g)	1.6	2.7	2.8
Vitality index	85.2	206.8	214.4
Root IAA Qualitative	+	++	++

+ positive; ++ strongly positive

Table 5. Plant growth promotion of *Rhizobium* spp. on *Sesbania* on 40th day

Group	Root length cm	Shoot length cm	Average nodule number	Weight mg/ plant
Control	6.62±1.1	31.32±6.85	80±1.43	142
AH12	10.04±2.7	41.44±1.73	141.2±12.98	248
AH 15	10.94±2.4	40.54±1.56	150.2±12.41	256

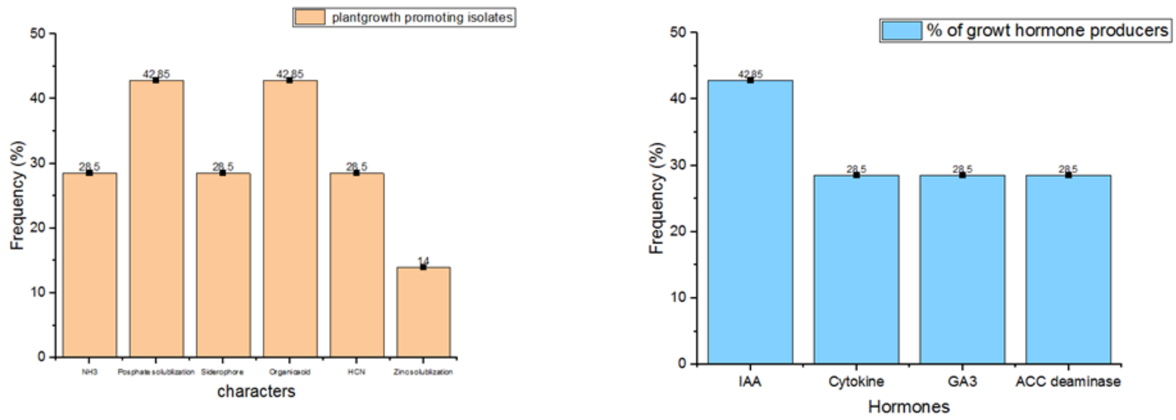

Fig. 2. *Percentage of PGP and growth hormone producing Rhizobium isolates (n=7)*

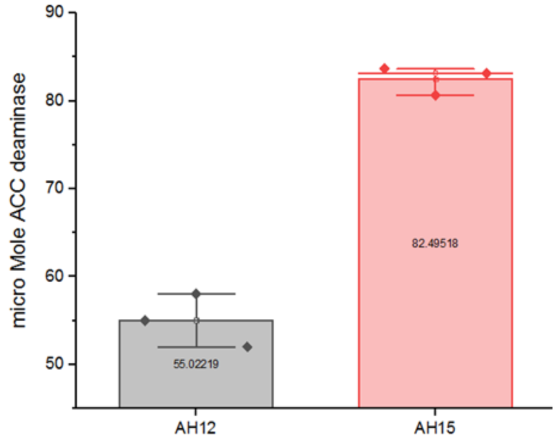

Fig. 3. *ACC deaminase activity of intracellular protein isolated from AH12 and AH15*

centration of IAA was maximum detected in AH15 treated plants and recorded as 12.4±0.1µg/g, whereas moderate in AH12 (8.6±0.1 µg/g) and less among control (6.4±0.2 µg/g). The concentration of GA3 was 1.2±1.2 µg/g in the control 4.6±0.1 µg/g in AH12 group and 6.2±0.05 µg/g in AH15. The GA3 content increased 4-fold in AH12 and 5-fold in AH15 compared to the control. Amount of cytokinin was 3.4±0.4<6.3±0.2<9.2±0.05 µg/g, respectively, among Control, AH12 and AH15. The p-value of triplicate was <0.0001 found to be significant at<0.05. Seneviratne *et al.*(2016) reported similar findings on enhanced IAA production in plants under stress by *B. japonicum.* Increased quantities of bacterial progeny inside the nodule are correlated with elevated GA3 and nodule volume. A similar result by Nett *et al.* (2022) of increasing nodule surface was reported by *Bradyrhizobium* sp infection. In the same way, Xing *et al.* (2022) observed that the administration of *Bradyrhizobium* enhances Gibberlic acid in plants.

Root nodule and leaf content study

Similarly, the LHB content of nodules collected from control and test represents that the *Rhizobium sp* inoculated bags have higher LHB than control. It was estimated that control nodules have 6.2±0.15µg/g, AH12 plant nodules have 10.8±0.2µg/g and 14.2±0.1µg/g in AH15 treated plants were found to be significant at p<0.05.The microscopic observation of the nodules area in control was smaller than the treated sample and calculated surface area was 15,435µm. AH12 treated nodule had 17,783µm surface area and AH15 have 28,161µm (Fig.7).

The impact of the foliar application of AH12 and AH15 on Sesbania leaf was analyzed by estimating pigments (Table 7). The carotenoid of control plant was 34.04 ± 0.5 mg/g, 0.74 ± 0.05 Chla, 0.35 ± 0.03 chlb and 1.09 ± 0 mg/g of total chlorophyll. The AH12 treated plants

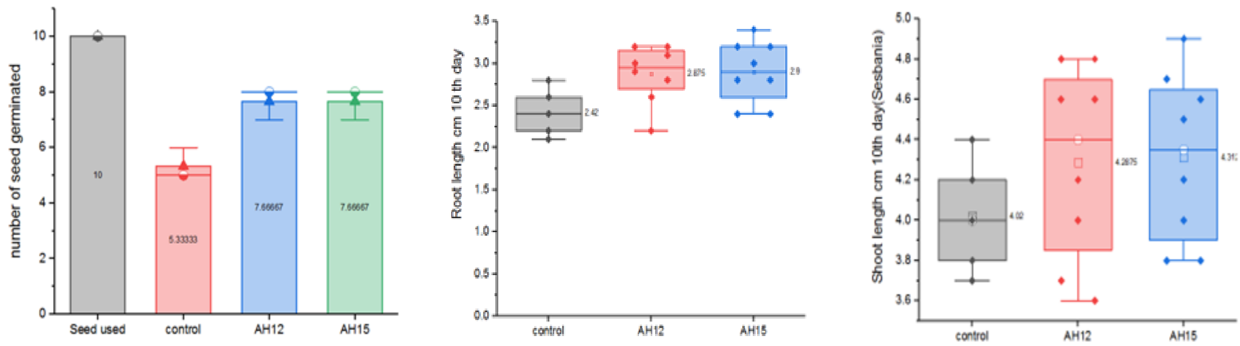

Fig.4. *Seed germination and growth on 10th day*

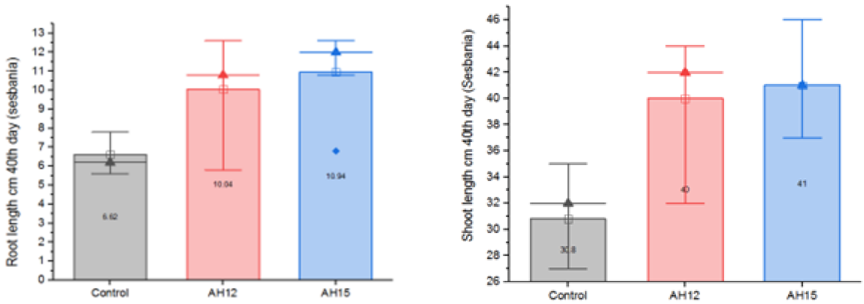

Fig.5. *Root and shoot length of Sesbania on 40th day growth*

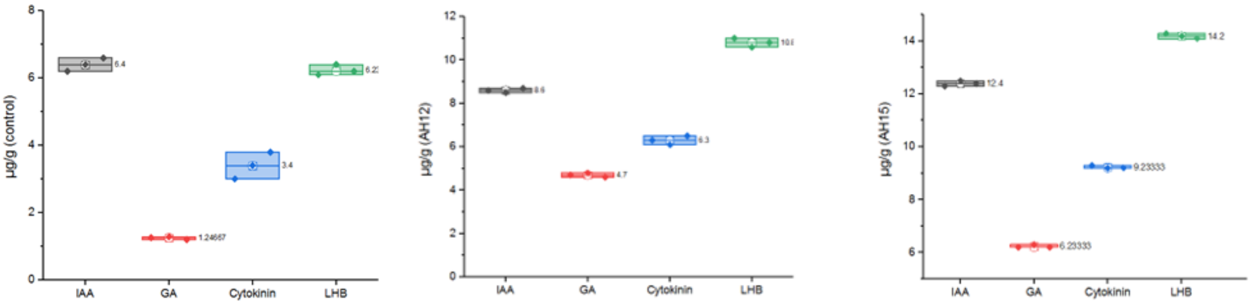

Fig.6. *Plant growth hormones estimated among root nodule of sesbania among control and treated groups*

Fig. 7.*Microscopic observation of nodule Size of Sesbania sp*

Table 6. Matured root nodule hormone analysis

Group	IAA μg/g	GA μg/g	Cytokinin μg/g	P value	LHB μg/g	P value
Control	6.4±0.2	1.2±1.2	3.4±0.4		6.2±0.15	
AH12	8.6±0.1	4.6±0.1	6.3±0.2	<0.0001	10.8±0.2	<0.0001
AH15	12.4±0.1	6.2±0.05	9.2±0.05		14.2±0.1	

had 52.2±1.05 mg/g of carotenoids and a total chlorophyll content 1.48±0 mg/g with 1.04±0.14 and 0.44± 0.02 mg of Chl a/b. The AH15 treated plants had 54.6± 0.4 mg/g of carotenoids, 1.14±0.14 mg of Chla, 0.44± 0.02 mg Chl b and 1.58±0 mg/g total chl(Fig. 8). At 0.05 levels the population of mean of treated and control data are statistically significant. The p values among AH12 and AH15 were not significant at 0.05. Increases in chlorophyll, plant height, shoot length and root length among Rhizobium-treated *Sesbania grandiflora* were recorded earlier by Karuppasamy *et al.*(2012)

Molecular characterization

Both the isolated Rhizobium strains (AH12 and AH15) were phylogenetically identified by 16s rDNA sequencing and the sequence query at NCBI reveals AH12 97.19% related to *Bradyrhizobium arachidis* (1347bp) and 96.88% with *Bradyrhizobium sp.* showing the inter-specific p-distance ranged from 0.94%, and the genbank accession was PQ119952 (Fig.9) and AH15 (1449bp) showed 98.35% similarity to *B. japonicum* and 98.28% with *B. liaoningense* with a p-distance of 0.92%(Fig. 10).The sequence was submitted to gen-

Table 7. Descriptive statistics of chlorophyll estimated

Chlorophyll	Group	Mean	Standard Deviation	SE of mean	Sum	Minimum	Median	Maximum
	Control	0.74	0.05292	0.03055	2.22	0.7	0.72	0.8
Chl a	AH12	1.04	0.14	0.08083	3.12	0.94	0.98	1.2
	AH15	1.14667	0.14468	0.08353	3.44	0.98	1.22	1.24
	Control	0.35	0.03	0.01732	1.05	0.32	0.35	0.38
Chl b	AH12	0.44	0.02	0.01155	1.32	0.42	0.44	0.46
	AH15	0.44	0.02	0.01155	1.32	0.42	0.44	0.46
	Control	1.09	0.03606	0.02082	3.27	1.05	1.1	1.12
Tot chl	AH12	1.48	0.13856	0.08	4.44	1.4	1.4	1.64
	AH15	1.58667	0.12858	0.07424	4.76	1.44	1.64	1.68

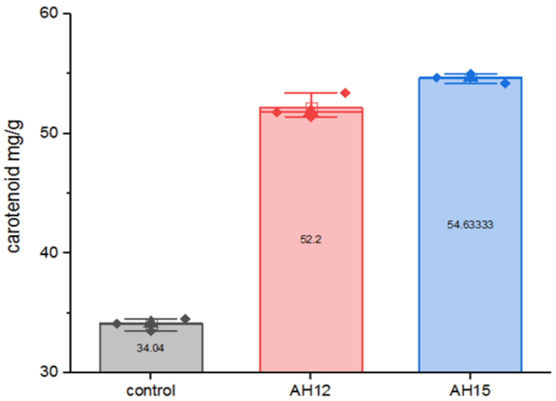

Fig.8. *Amount of carotenoid estimated among treated and control Sesbania leaves*

bank and the accession number is PQ119954.Both the strains come under alpha proteobacteria with 97 and 68 leaves. Pair-wise Sequence Alignment (PSA) by the Smith-Waterman algorithm revealed the identity among *B. arachidis* and *B. japonicum* was 90.0% % with a gap of 8.9%.The presence of *B. arachidis* strain determinate root nodules of peanut was reported by Chen *et al.* (2023).Colonization of *B. japonicum* from root nodules of *Arachis hypogaea* was also reported by Wang *et al.*(2013)

Conclusion

The present study concluded that *Bradyyrhizobium arachidis* (PQ119952) and *Bradyrhizobium japonicum* (PQ119954) isolated from *Arachis hypogaea were* found to produce EPS, Auxin, GA, cytokinin, siderophor, ammonia, HCN, ACC deaminase and mineral solublization. Both strains could infect other legumes and promote seed vitality, growth, nodulation, and control of fungal infection. Therefore, they can be considered effective biofertilizers and biofungicides in nature.

Conflict of interest

The authors declare that they have no conflict of interest.

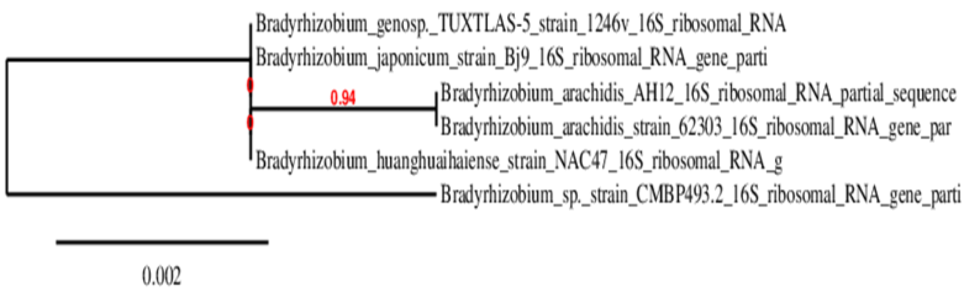

Fig. 9. *Phylogenetic relatedness of AH12 strain –Neighbour-joining method*

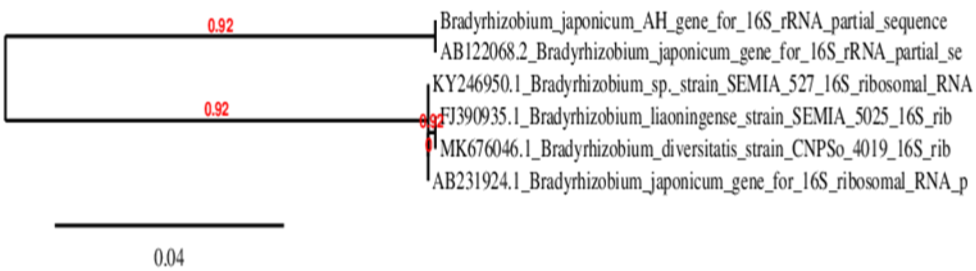

Fig.10. *Phylogenetic relatedness of AH15 strain –Neighbour-joining method*

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