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Potential of *Rhizobium* to Promote Plant Growth for the Development of *Albizia procera* Seedling

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ABSTRACT: Nitrogen and organic matter content in forest soils have decreased as a result of intensive harvesting of forest resources. One of the essential components for the growth and development of plants is nitrogen. Nitrogen is a growth-limiting nutrient in terrestrial and aquatic ecosystems despite its abundance in the atmosphere because, with the exception of nitrogen-fixing bacteria, it is inert and worthless in its gaseous state. Numerous tree species have been investigated to determine how inorganic fertilizers affect the girth, height, and biomass increment of forest tree species. However, the rising costs and unbalanced usage of these chemical fertilizers have an adverse effect on the environment. Species of trees that fix atmospheric nitrogen through their root nodules have been considered over time because of this potential. There is evidence that nodulating plants can improve soil fertility. An affordable and practical way to lessen the need for nitrogen fertilizers and restore damaged ecosystems is through biological nitrogen fixation. Forests are essential for any country's ecological stability and economic growth. Research on the population structure of forest trees under various environmental conditions have shown that genetic and physiographic variances are related to seed location or source. The economical and environmentally beneficial method of biological nitrogen fixation (BNF) involves leguminous plants and nitrogen-fixing microorganisms working together to increase soil nitrogen availability. Since ancient times, symbiotic bacteria found in legumes have been known to improve the soil's nutrient content. In the Microbiology Laboratory of the Department of Basic Sciences, the current study is based on "Improvement of *Albizia procera* Seedling utilising *Rhizobium* Inoculation", was carried out. Out of ten seed sources, the study found that the Baddi seed source in Himachal Pradesh and the FRI, Dehradun seed in Uttarakhand were the best. The *Albizia procera* nodules yielded a total of 66 isolates, including 28 from Uttarakhand and 38 from Himachal Pradesh. *Rhizobium* spp. has been identified in 35 of 66 isolates. The shoot biomass, root biomass, and nodulation status considerably increased over the uninoculated control after the two isolates, BA2 and FA6, were chosen and used as biofertilizer.

Keywords: *Albizia procera,* Leghaemoglobin, seedling parameters, Nitrogen fixation potential, nodulation, biofertilizers, *rhizobium*, plant growth parameters.

INTRODUCTION

One of the main national resources is the forest. Too much reliance on them, negligent use, and indiscriminate deforestation will alter the earth's structure and have an impact on the entire ecosystem. Many scholars are aware of the significance of origin/seed source variation for tree improvement projects (Wieland *et al*., 2011; Yan *et al*., 2014; Zhou *et al*., 2017). Species exist in a vast variety of genetic variants across a broad geographic range. The level of adaptation fluctuates with the relative rates of environmental change or occasionally with abrupt changes in topography and soil, leading to corresponding genetic variances among species. Genetic differences brought on by distinct origins' adaptability to various environmental circumstances and soil types may be the cause of variation between genera (Ginwal *et al.,* 2005). Several researchers have frequently revisited their work on investigating the possibility of such PGPR (Bhattacharyya and Jha 2012; Dwivedi *et al.,* 2015; LeRoux *et al.,* 2016). The term "PGPR" refers to rhizospheric bacteria that enter plant roots and have effects that encourage growth (plant growth-promoting rhizobacteria). By creating plant regulating hormones, improving the uptake of nutrients like nitrogen, or indirectly reducing competition for nutrients and habitats with many phyto-pathogenic species, these bacteria can directly affect plant growth (Brockwell *et al.,* 2011; Sikarwar *et al.,* 2023). A strong

PGPR(s) consortium may choose to use one or more of these techniques.

Investigation and economic exploitation of their interactions with leguminous plants and trees have taken place (Mohite, 2013; Monteiro *et al.,* 2019). The use of rhizobial biofertilizers has the potential to boost the uptake of atmospheric nitrogen and could result in the accumulation of massive amounts of nitrogen. They are good P-solubilizers in addition to being employed for nitrogen fixation (Kaur *et al.,* 2013; Vejan *et al.,* 2016: Dhiman *et al.,* 2022). *Rhizobium* and its closely related genera are among them and are frequently used bacteria in crops with legume roots. The symbiotic relationship between these bacteria and the roots of certain legumes results in nodules, a specialised structure that serves as nitrogen-fixing sites inside legume roots (Fuentes-Ramirez and Caballero-Mellado, 2005; Sharma *et al.,* 2013; Gopalakrishanan *et al.,* 2015).

Albizia procera, also called "White Siris", is used in tropical and subtropical moist and wet forest types, where annual precipitation ranges from 1000 to 5000 mm. Due to its versatility and potential for nitrogen fixation, this tree species is regarded as a priority species in plantation programmes (Gera *et al.,* 2002; Aruna *et al.,* 2023). Tree species like Albizia procera are widely employed in commercial applications, in contrast to many nitrogen-fixing plant species used in agriculture. A multipurpose tree legume species from the Leguminosae family called *Albizia procera* forms nodules in association with rhizobia. Up to 30 metres of growth and 2 to 3 metres of girth are possible.

It naturally grows in alluvial soil, which is extensively dispersed on the riverbeds in the sub-Himalayan region between the Indus and Assam rivers and Himalayan mountains. It is a highly regarded plantation species since it is used for several wood and non-wood goods (Wang *et al*., 2012; Sharma and Bakshi 2014; Pereyra *et al.,* 2015; Kumar *et al.,* 2016; Sandhya *et al.,* 2021). For microbial inoculation of rhizospheric bacteria isolated from various geographic *Albizia procera* rhizospheres, a favourable environment was maintained in an *Albizia procera* nursery in this work. The *Rhizobium* inoculants were isolated from the same *Albizia procera* nursery's nodules, and the two most effective *Rhizobium* inoculants were made depending on how well they promoted plant development. Furthermore, different quantities of N were used in another nursery to apply these two efficient *Rhizobium* inoculants.

MATERIAL AND METHODOLOGY

Isolation of Bacteria from Soil. The bacteria were isolated using soil dilution and pour plate techniques. Under aseptic conditions, one gramme of rhizospheric soil was added to 9 ml of sterilised distilled water and successive dilutions were made. Dilutions were then poured on the Nutrient agar media plates. After the corresponding incubation period, total numbers of colonies formed were noted.

$$\text{cfu} = \frac{\text{Bacterial plate count}}{\text{Amount of sample plated}} \times \text{Dilution Factor}$$

Sowing of Seeds in Nursery and Growth Characteristics Measurement

Preparation of soil mixture. The mixture of soil, sand and farm yard manure (FYM) in 2:1:1 ratio was prepared and sieved. Each polybag contained 2 kg of soil mixture and the seeds were sown in replication.

Seed treatment and sowing. Mercuric chloride was used to sterilise the surface of the seeds, and any remaining $HgCl_2$ was quickly rinsed off the seeds with distilled water. Before planting in polybags, seeds were stored in cool water for 24 hours. For germination, seeds from various seed sources were stored in a germinator at 25±2°C for germination. After germination of seeds in germinator, these seeds were sown in previously prepared polybags. The broth suspension of native bacteria isolated from soil of respective sites was applied after every 15 days. The data for growth and nitrogen fixing potential of seedlings was taken after 2, 4 and 6 months of seed sowing.

Number of samples: 10

Replication: 3

Statistical design: CRD

Observations:

1. Shoot characteristics:

(a) Shoot height: Length was measured with the help of meter scale from soil level to shoot tip of each individual seedling after 2, 4 and 6 month. Shoot length was expressed in centimeters (cm).

(b) Number of leaflets per seedling: Total numbers of leaves per plant were counted. However, very young and juvenile leaves were ignored.

(c) Leaflet area (cm^2): Leaf area was measured with the help of leaf area meter LICOR LI-3000.

(d) Collar diameter: Collar diameter was measured with Digital Vernier Caliper and the mean of three values was recorded. Collar diameter was expressed in milliimeter (mm).

(e) Shoot fresh and dry weight (g): Shoot fresh and oven dry weight was recorded and expressed in grams (g).

2. Root characteristics:

(a) Root length: Tap root length was measured with the help of meter scale for each individual seedling and expressed in centimeter (cm).

(b) Root fresh and dry weight (g): Root fresh and dry weight was recorded and expressed in gram (g).

3. Nitrogen fixation potential

(a) Number of nodules: Number of Nodule of individual plant representing different seed sources was recorded.

(b) Nodule fresh and dry weight (g): Nodule fresh and dry weight of everyreplication from each treatment was recorded and expressed in gram (g).

(c) Leghemoglobin content: Using the Keilin and Wang (1945) method, the leghemoglobin content of root nodules was determined. In a solution containing 50 mM KPO4 (pH 7.4) and 1 mM EDTA, leghemoglobin was extracted from newly obtained nodules (2.0 g). At 4°C for 10 minutes, the mixture was

mixed and centrifuged. Leghemoglobin-bearing supernatant was gathered and dissolved in buffer containing 50 mM K_2PO_4 (pH 7.4). At 710 nm, the colour intensity that emerged was measured spectrophotometrically on a white background. The amount of leghemoglobin was given as mg/g nodule.

Molecular Characterization of Selected Rhizobial Isolates by 16s RDNA Sequencing

Genomic DNA extraction using conventional method (Sambrook *et al.*, 1989). Rhizobial isolates were cultured for 72 hrs at 28±2°C in YEM broth at 200 rpm. The cells were harvested and processed for DNA isolation. The isolated DNA was finally suspended in 100 µl of elution buffer and quantified on 1% (w/v) agarose gel.

Sequence and phylogenetic analysis. Using both forward and reverse primers, the purified fragments were transported to Genei Labs in Bangalore, India for sequencing. By removing unclear and unreadable sequences, both the forward and backward sequences were fixed. Eventually, the full 16S rDNA of a few different bacterial isolates was retrieved based on overlapping areas. A BLAST search was used to connect phylogenetically similar bacteria based on their 16S rDNA sequences (Altschul *et al.,* 1997). Using CLUSTAL W, many alignments with similar taxon sequences were carried out (Thompson *et al.,* 1994). Using MEGA X software, a neighbor-joining phylogenetic tree was created using additional 16S rDNA sequences from similar taxa that were downloaded from the GenBank database.

Isolation of rhizobacteria from the roots of seedlings of *Albizia procera* grown in net house. *Albizia procer*a seedlings' healthy nodules were cut out from their roots and cleaned under running water to get the soil granules off their surface. To get rid of any $HgCl_2$ residue, nodules were submerged in 0.1 percent mercuric chloride (HgCl2) solution for 30 seconds, and then rinsed again eight to ten times with sterilised distilled water. Glass rods were used to crush surface-sterilized nodules in sterilised distilled water to create a milky bacteriod solution. The suspension was streaked onto YEMA medium-filled petri plates and incubated at 28± 2 °C for 2 to 5 days. The colonies were taken and moved to YEMA slant for additional research.

The following treatments were tried under net house conditions:

Treatments:

1. T_1 = Control
2. T_2 = PGPR-I
3. T_3 = PGPR-II
4. T_4 = Nitrogen (20 kg/ha)
5. T_5 = Nitrogen (40 kg/ha)
6. T_6 = Nitrogen (60 kg/ha)
7. T_7 = $T_2 + T_4$
8. T_8 = $T_2 + T_5$
9. T_9 = $T_2 + T_6$
10. T_{10} = $T_3 + T_4$
11. T_{11} = $T_3 + T_5$
12. T_{12} = $T_3 + T_6$

Where,

$PGPR_1$ is *Rhizobium* isolated from Himachal Pradesh seed source

$PGPR_2$ is *Rhizobium* isolated from Uttarakhand seed source

Bacterial isolates : 2

Total treatments: 12

Replication : 3

Statistical design : CRD

Sowing of seeds. Five seeds each treated with a different culture were planted in each pot at a depth of at least 3 cm. Only one plant was kept per container after 15 days of seedling emergence.

Observations:

1. Shoot characters

2. Root characters

3. Nitrogen fixation potential

4. Nitrogenase activity:

By measuring the decrease of acetylene the nitrogenase activity of the *Albizia procera* nodules was given by Hardy *et al.* (1968). To eliminate soil particles, seedlings were repeatedly cleaned in distilled water after being washed in tap water first. The root nodules were weighed and counted before being placed in an Erlenmeyer flask with 10 ml of acetylene (C2H2) added. The flask was then left incubating for an hour. By withdrawing 1 cc of the gas mixture from the flasks and using gas chromatography to test it, the amount of ethylene produced as a result of the reduction of acetylene was determined. After comparison with a standard ethylene gas, the amount of ethylene was calculated from the peak height. In terms of nmole C_2H_4 per plant per h, per nodule per h, per gram of fresh root weight per hour, and per gram of dry root weight per hour, the nitrogenase activity was expressed.

Physico-chemical characteristics, nutritional availability, and microbiological characteristics of a soil mixture (before and after termination of experiment). By using the following standardised protocols, freshly made potting mixture was examined for significant physico-chemical, nutrient availability, and microbiological qualities both before and after the experiment:

pH and electrical conductivity. The pH of the soil was measured in a suspension of soil and water at a ratio of 1:2.5, and the electrical conductivity of the liquid supernatant was measured and expressed in dSm^{-1} (Jackson, 1973).

Organic carbon, available nitrogen, phosphorus and potassium. Using Walkley and Black's chromatic acid titration method, organic carbon was measured (1934). Using accepted techniques, the amounts of available nitrogen, phosphorus, and potassium in soil samples were determined.

Viable microbial count. By dissolving 1 g of soil in 9 ml of distilled water blank then diluting the soil suspension ten times, the soil was examined for the presence of viable microorganisms. The microbial count was then calculated using the conventional pour plate technique on various media, according to Subba Rao (1999). Colony forming units per gramme of soil (cfu/g soil) were used to express the count.

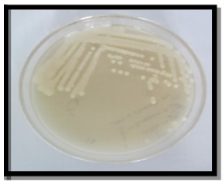
RESULTS AND DISCUSSION

Isolation of Rhizobia from Root Nodules of different Seed Sources *Albizia procera* Seedlings. From the root nodules of six-month-old *Albizia procera* seedlings cultivated in net houses, rhizobia was identified. CFU/g root growth on YEMA media was measured.

Rhizobium isolated from nodules of *Albizia procera,* produced translucent, nearly round and sticky colonies that varied between 1.5 and 2.00 mm in size. The morphological features of Rhizobium colony can also be seen in *Albizia procera*-Plate-1. After the color of the gram, the bacteria took on a red color indicating that it is a gram-way strain. Gram staining of cultured isolates was performed to provide information as to the presumptive tests of isolates.

Seedling with root nodules

Root Nodules of *Albizia procera* seedlings



Growth on YEMA medium

Plate 1: Isolation of rhizobial isolates of *Albizia procera.*

From tree shoots grown in a net house, root nodules were harvested. *Albizia procera* seedlings cultivated beneath the net house had their rhizobial population and the number of isolates from the root nodules counted. The maximum number of rhizobial isolates (11) and rhizobial population (84.1 × 10^2 cfu/g soil) were obtained from Baddi. Seedlings in Himachal Pradesh sample, whereas maximum number of rhizobial isolates (8) and rhizobial population (85.8 × 10^2 cfu/g soil) in Uttarakhand were obtained from seedlings from FRI, Dehradun.

Seed collection from a suitable location can enhance the germination process and thus accelerate plantation programmes, as the genetic makeup of these seed sources can select for superiority. Bacteria isolated from rhizospheric soils of *Albizia procera* collected from selected sites in Himachal Pradesh and Uttarakhand varied from 150 × 10^4 to 24^3 × 10^4 cfu/g soil. Purwaningsih (2004) reported that the number of bacteria from soil was 0.6 × 10^5 CFU/g soil and ranged from 11.6 × 10^5 CFU/g soil.

The process of introducing microbes into the soil in a forest nursery can be an effective method for achieving high growth and establishment of tree species at reforestation sites. Rhizobia diversity exists in specific biological systems as a result of the association between biotic and abiotic components of rhizobia, their host legumes, environment (Yan *et al.,* 2014). In addition to nitrogen fixation, growth-promoting rhizobacteria are known to benefit plants in a number of other direct and indirect ways.

Molecular Characterisation. The bacterial isolates displayed every PGP characteristic discovered by molecular methods based on 16S rDNA sequencing, up to the species level. Using the traditional technique, DNA was retrieved (Sambrook *et al.*, 1989). Using universal bacterial primers, purified DNA was amplified, yielding an amplicon of the predicted size, or 1400 bp. A PCR extraction kit was used to purify the obtained PCR product before it was sent for further sequencing.

Rhizobia isolated from *Albizia procera* root nodules belonged to *Rhizobium* sp., according to Blast sequences of BA2, with 99% similarity. Sequence submission to GenBank NCBI USA yielded the entry number BA2: MK643828.

The isolates MK 643828 and FA6 were tentatively identified as *Rhizobium* sp. based on their morphological, physiological, and biochemical properties and Burgi's Manual of Systematic Bacteriology (Claus and Berkeley 1986). Wang *et al.*, 2006; Rasool *et al.* (2015); Dipta (2018) revealed similar morphological, physiological, and biochemical features of rhizobial isolates.

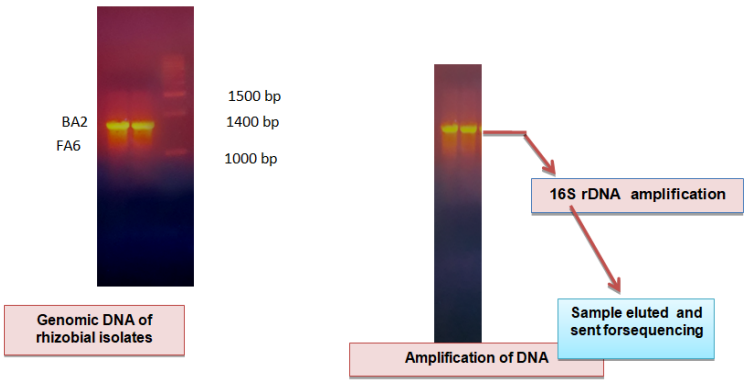


Plate 2: 16S rDNA amplification-based molecular identification of bacterial isolates.

Outcome of Rhizobial Inoculation and Chemical Fertilizers on *Albizia procera* Growth (Net House condition)

Outcome of Rhizobial Inoculum on Plant Growth Parameters. *Albizia procera's* growth characteristics, such as root length, seedling diameter, collar size, number of leaves, and recess area, increased when chemical fertilisers and rhizobial isolates were applied together (100% suggested PK). The signs of plant development with various treatments are shown in Plate 3.

(a) Seedling parameters. According to the shoot parameter data in Table 3, the usage of chemical fertilisers with rhizobia improved growth compared to the control. Under treatment T_7 (PGPR1 + 20Kg/ha N), the maximum shoot length (50.91cm), number of leaves (31.29), leaf area (35.5cm^2), collar diameter (4.23mm), and shoot dry weight (4.88g) were observed. All other therapies that were attempted paled in comparison to therapy T_7. It was determined that treatment T_{10}, or (PGPR2 + 20Kg/ha N), was similar to T_7. Treatment T_1 did, however, record the smallest shoot length (25.79 cm), number of leaves (23.08), leaf area (24.82 cm), collar diameter (2.19 mm), and shoot dry weight (2.39 g) (control).

Treatment T_7 (PGPR1 + 20Kg/ha N) produced the largest root length (35.10cm) and root dry weight (3.71g). It was discovered that this treatment was superior to other therapies. Under treatment T_1, minimum root length (19.17cm) and dry weight (2.08g) were noted (control).

(b) Nitrogen fixing capacity. With the application of T_7 (PGPR1 + 20 Kg/ha N), which was discovered to be better than the other tried treatments, reduced maximum nodule number (27), nodule fresh weight (3.97 g), nodule dry weight (2.81 g), leghemoglobin content (9.61 mg g^{-1} nodule), and nitrogenase activity (671 N mol C_2H_2 g^{-1} fresh weight) H-1 were recorded. Uninfected controls showed no nodule development (Table 1, Plate 3).

T_1=Control T_2=PGPR1 T_3=PGPRII
T_4= 20kg/ha Nitrogen T_5= 40kg/ha Nitrogen T_6= 60kg/ha Nitrogen
T_7= PGPR1+20kg/ha Nitrogen T_8= PGPR1+40kg/ha Nitrogen T_9= PGPR1+60kg/ha Nitrogen
T_{10}= PGPRII+20kg/ha Nitrogen T_{11}= PGPRII+40kg/ha Nitrogen T_{12}= PGPRII+60kg/ha Nitrogen

Plate 3: Effect of rhizobial inoculation along with N fertilizer on *A. procera.*

Table 1: Effect of rhizobial inoculation and chemical fertilizer on seedling growth and nodulation behaviour.

Treatments	Shoot Characteristics						Root Characteristics			Nitrogen Fixation Potential				
	Shoot Length	Shoot Fresh Weight	Shoot Dry Weight	Number of Leaves	Leaf Area	Collar Diameter (mm)	Root Length	Root Fresh Weight	Root Dry Weight	Nodule Number	Nodule Fresh Weight	Nodule Dry Weight	Leghaemoglobin Content (mg g^{-1} nodule)	Nitrogenase Activity (n moles C_2H_2 reduced g^{-1} fresh weight h^{-1})
T_1	25.79	4.98	2.39	23.08	24.82	2.19	19.17	2.96	2.08	10	2.29	1.16	5.91	501
T_2	42.47	6.21	3.70	28.91	31.46	3.85	33.51	4.66	3.0	21	3. 67	2.11	8.65	611
T_3	42.81	5.98	3.81	29.34	31.60	3.68	32.86	4.49	2.94	19	3.46	2.07	8.53	616
T_4	33.71	5.23	3.19	27.59	30.26	2.91	29.94	3.69	2.49	17	3.39	1.71	8.19	597
T_5	38.71	5.57	3.36	29.01	29.76	3.01	2961	3.81	2.61	20	3.59	1.95	8.27	601
T_6	28.85	4.86	2.75	23.19	27.05	2.35	25.71	309	2.19	13	2.41	122	7.62	511
T_7	50.91	6.89	4.88	3129	35.5	4.23	35.10	5.19	3.71	27	3.96	2.81	9.61	671
T_8	44.96	5.74	4.20	31.06	33.19	4.07	32.95	4.86	319	23	3.71	2.17	8.83	643
T_9	30.65	5.09	3.04	25.87	27.61	2.71	27.81	3.49	2.36	16	2.81	1.25	7.76	547
T_{10}	50.73	6.91	4.92	31.6	33.96	4.19	33.91	5.07	3.61	24	3.72	2.75	9.66	660
T_{11}	43.17	6.60	3.96	30.87	31.72	3.96	33.41	4.91	3.09	24	3.84	2.15	8.78	626
T_{12}	30.17	5.02	2.98	25.95	27.02	2.31	27.56	3.15	2.29	13	2.69	1.20	7.89	521

T_1=Control; T_2=PGPR1; T_3=PGPRII; T_4= 20kg/ha Nitrogen; T_5= 40kg/ha Nitrogen; T_6= 60kg/ha Nitrogen; T_7= PGPR1+20kg/ha Nitrogen; T_8= PGPR1+40kg/ha Nitrogen; T_9 = PGPR1+60kg/ha Nitrogen; T_{10}= PGPRII+20kg/ha Nitrogen; T_{11}= PGPRII+40kg/ha Nitrogen; T_{12}=PGPRII+60kg/ha Nitrogen

Numerous workers reported using rhizobia to boost the productivity of forest trees including Albizia, Acacia, and Dalbergia at the nursery level (Sahgal *et al.,* 2004; Thatoi *et al.,* 1993; Guleria *et al.,* 2014). Table 1 showed that the interaction of treatment and plant age showed variation in shoot, root and nodulation behavior. PGPR1 +20Kg/ha N(T_7) has the highest seedling properties which are equal to PGPR2 +20Kg/ha N(T_{10}). By accelerating root development and boosting the plant's enzymatic activity, microbial inoculation is most likely to blame for the increase in seedling parameters.

The increase in growth by biofertilizers can be attributed to improved nutrient availability, especially for planting by direct mechanisms of N (symbiotic N fixation), P (P solubility) and S (siderophore

production). Application of rhizobial inoculum to the rhizosphere may improve plant metabolism leading to increased root development and suppression of plant pathogens. Our findings support those of (Guleria *et al.,* 2014; Dhiman *et al.,* 2022), who found that *rhizobium* isolated from *Albizia lebbeck* and *Acacia catechu* performed better results in comparison to other strains in terms of germination percentage, Seedling Vigor Index, root and shoot length, number of leaves, secondary root formation, and total biomass yield.

Rhizobium inoculation on *Acacia catechu* and *A. mollissima* had a beneficial symbiotic impact, increasing seedling development and biomass in contrast to uninoculated seedlings, according to Banyal *et al.* (2001). The best mixed strain for Acacia catechu development, according to their advice. Forest trees including *Albizia, Acacia*, and *Dalbergia* have been observed to produce more fruit when given microbial biofertilizers at the nursery level (Thatoi *et al.,* 1993; Sehgal *et al.,* 2004; Gupta *et al*., 2021).

The genetic makeup of a tree species and its surroundings have an impact on its nodulation activity (Hardy *et al.,* 1968; Pokhriyal *et al.,* 1991; Boakye *et al.,* 2016). Rhizobia's ability to fix nitrogen, however, frequently varies dramatically depending on the host plant species. Inoculating the plant with rhizobial strains is the most practical technique to give the nitrogen necessary for legumes to grow to their full potential (Kanna, 2020).

CONCLUSIONS

It is important to use different seed pre-treatments and locations to produce quality seedlings with the lowest costs and labor. For tree improvement programs quality seed source play important role. The application of rhizobial isolates BA2+ and FA6+, along with eco-friendly methods for producing quality seedlings of *Albizia procera*, is advised based on the aforementioned study. These methods could significantly reduce the dependence on chemical fertilizers and play a significant role in afforestation cultural practices and programmes.

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Conflict of Interest. None.

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