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Characterization and Seed Priming Effect of Plant Growth Promoting Bacteria Isolated from Wild Cotton Rhizosphere

Moanaro Ong [a], Rajkumar B. K. [b*], Preeti R. Parmar [b], H. R. Ramani [b], Meet Dobariya [c], Diya Patel [c] and Dukare Nikhil [a]

[a] N.M. College of Agriculture, Navsari Agricultural University (NAU), Navsari 396 450, Gujarat, India.
[b] Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athawa Farm, Surat - 395007, Gujarat State, India.
[c] Shree Ramkrishna Institute of Computer Education and Applied Sciences, MTB College Campus, Athwalines, Surat- 395001, Gujarat, India.

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 undertaken to explore the untapped potential of the rhizospheric bacteria associated with wild cotton, *Gossypium aridum*. All the 13 isolated rhizospheric bacterial isolates were screened for ten parameters comprising plant growth promoting (potash, phosphorus

**Corresponding author: Email: rajkumar@nau.in;*

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and zinc solubilization; IAA and GA_3 production) and plant protection (chitinase, cellulase, and protease production; siderophore and HCN production). Four rhizospheric bacteria *viz.,* NAU-RPM-4, NAU-RPM-9, NAU-RPM-1 and NAU-RPM-11 of wild cotton species recorded statistically significant plant growth and protection activities and thus chosen to study their seed priming effect on early cotton seedling growth of cultivated cotton (*G. hirsutum*). Bacterial isolate NAU-RPM-1 and NAU-RPM-11 significantly improved the growth parameters such as germination percent, shoot length, root length, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root and root-shoot ratio as compared to absolute control. However, seed biopriming with NAU-RPM-4 and NAU-RPM-9 isolates also improved the early seedling growth parameters but this improvement was comparable to control. NAU-RPM-1 and NAU-RPM-11 were further characterized using 16S rDNA technique and preliminary identified as *Enterobacter mori* NAU-RPM-1 and *Bacillus cereus* NAU-RPM-11, respectively. Our results highlight the importance of rhizosphere bacteria isolated from wild cotton species (*G. aridum*) in improving the growth and resilience of cultivated cotton, especially in the context of utilizing PGPR for long-term sustainable agricultural practices.

Keywords: PGPR; wild cotton rhizosphere; seed priming; seedling growth; Gossypium aridum; Gossypium hirsutum.

1. INTRODUCTION

Gossypium aridum (Rose & Standl.) Skovst is a wild cotton species known for its tolerance to arid conditions and resistance to certain nematodes (Baez-Gonzalez *et al.,* 2022); and may act as reservoir of the most favorable attributes required under climate change scenario. In addition to that, the rhizosphere of cotton known to harbor the diverse group of microbes with multifarious plant growth promoting traits like phytohormone production, mineral solubilisation along with plant protection activities like lytic enzyme and antimicrobial traits. These multifarious activities of microbes directly or indirectly support plant growth under normal and stressed condition (Hakim *et al.,* 2021). Qiao *et al*. (2017) reported that Acidobacteria, Actinobacteria, Bacteroidetes, Planctomycetes, Proteobacteria, and Verrucomicrobia are the six bacterial phyla dominantly found in cotton rhizosphere. However, soil of the wild cotton plant rhizosphere and its PGP activities are rarely studied. Thus, present study aimed to isolate bacteria from cotton rhizosphere of wild cotton *Gossypium aridum*.

Frequent and injudicious use of chemicals (fertilizers, insecticides, and pesticides) for plant growth, development and protection in agriculture not only affected the soil health, nutrient utilization and microbiome but also heavily impacted the environment. Wide range of rhizospheric bacteria known to solubilised varied minerals, produce phytohormones like auxins, cytokinine, gibberellic acid, Indole-3-acetic acid *etc*., that play a key role in plant growth and development (Olenska *et al*., 2020). Fahimi *et al*. (2014) reported that bacterial strains *viz*., *Bacillus amyloliquefacians* and other *Bacillus* species can promote and protect the cotton plant due to its phosphate solubilisation, phytohormones, siderophore and nitrogen fixation ability. Rhizosphere bacteria-derived bio-molecules like siderophores, hydrogen cyanide (HCN) and enzymes (chitinase, cellulase, protease and ACC deaminase) can directly or indirectly protect the plant against varied pest, insects along with biotic and abiotic stress. Plant growth-promoting rhizobacteria (PGPR) can also induce systemic resistance (ISR) against pathogen infections (Zhu *et al.,* 2022). Thus, harnessing the biologicals or microbes presents an eco-friendly and effective approach to enhancing plant growth and protection. Perhaps, application of these potentials microbes is a difficult task in agriculture. Among varied methods used to apply the biologicals, seed biopriming is one of the simple and eco-friendly approaches used to improve seed germination, seedling vigor, abiotic stress as well as biotic stress management (Fahsi *et al.,* 2021; Ahmad *et al.,* 2021). Earlier, Vishnuveni *et al.* (2016) reported improved germination in cotton cotton variety MCU 12 seed primedg with *Azotobacter* at 3%. Yao *et al.* (2010) bioprimed cotton seeds with Rs-198 recorded increased germination rate, plant height, fresh weight and dry weight by 23.8%, 12.8%, 30.7% and 10.0% respectively as compared to the control. Ragadevi *et al*. (2021) also reported maximum root length (6 cm) and maximum shoot length (12cm) in cotton seeds treated with *Azospirillum* sp7 strain. Recently, Zhao *et al.* (2024) reported that cotton plants

treated with potassium solubilizing rhizobacteria (KSR) *Pseudomonas aeruginosa* A 10 increased the shoot and root growth of the plant, the ratio of root to shoot decresed from 52% to 50%.

The present study implies to isolate and identify the bacteria from the soil of wild cotton rhizosphere and screen for plant growth and protecting traits; and study effect of potent PGP rhizobacterial strains on early seedling growth of cultivated species of cotton was attempted thorough seed priming.

2. MATERIALS AND METHODS

2.1 Isolation of Rhizospheric Bacteria

Rhizospheric soil sample of wild cotton species (*Gossypium aridum*) was collected from the nursery of Main Cotton Research Station, Navsari Agricultural University, Surat, Gujarat, India (latitude 21º10'11.3"N and longitude 72º48'17.7"E). Rhizospheric soil was collected as a composite sample by mixing of rhizospheric soil samples around the root and surface of the root from the depth of 5.0 cm from the ground soil. The samples were labeled properly, placed into sterile polythene bags and kept at 4ºC until used for further investigation. The bacterial colonies were isolated by serial dilution and spread plate technique on sterile nutrient agar plate.

2.2 Plant Growth Promoting (PGP) Activities

The present study assessed each of the rhizospheric bacterial colonies for its plant growth promoting (mineral solubilization and phytohormone production). Plate assay was performed to detect the zinc, potassium and phosphate solubilization of the bacteria as per Di Simine *et al*. (1998), Parmar and Sindhu, (2013) and Pikovskaya (1948), respectively.

Indole-3-acetic acid (IAA) production was performed by the method of Bric *et al*. (1991). Quantitative assay of gibberellic acid production was carried out using the protocol of Graham and Henderson *et al*. (1961).

2.3 Plant Protecting Activities

Detection of hydrolytic enzymes *viz*., chitinase, protease and cellulase was performed by spot inoculation of bacterial culture on chitin agar plate, skim milk agar plate and CMC agar plates as per method described by Kuddus and Ahmad (2013), Patel *et al*. (2005) and Ferbiyanto *et al.* (2015), respectively. Qualitative screening of rhizospheric bacterial isolates for HCN production was performed as per method of Lorck (1948). Quantitative determination of bacterial siderophore production was performed as per method of Schwyn and Neilands (1987).

2.4 Bacterial Seed priming and its effect on germination and Early Seedling Growth of Cotton Plant

The biopriming of cotton seeds was carried out by using potent rhizospheric PGP bacteria as per method suggested by Meena *et al* (2020). For that, cotton seeds were surface sterilized (Musson *et al*.,1995) followed by inoculation in bacterial suspension (10^8 cfu ml^{-1}) with 1.0 % aqueous carboxymehtyl cellulose (CMC) as binding agent and seeds were dried under aseptic condition. The seeds treated only with CMC acted as control; while seeds without CMC and bacteria as absolute control. Each of the bioprimed cotton seeds, absolute control and control cotton seeds were aseptically transferred to half MS medium in PTC bottles (five seeds/bottle) in triplicates. Bottles were kept in a growth room at 25±2°C to 16 hrs photoperiod. The germination percentage of cotton seedling was recorded at 7^{th} day of incubation; while other morphological parameters were recorded at 15^{th} day of incubation. Five seedlings from each treatment (Bioprimed seeds, absolute control and control seeds) were selected in order to measure the morphological characters *viz*., Shoot and Root length (cm), Fresh Shoot and Root Weight (g), Dry Shoot and Root Weight (g) and Root: Shoot ratio.

2.5 Microbial and Molecular Characterization of Potent PGP Rhizobacteria

Identification of the bacteria was carried out using microbial and molecular attributes. The KB002 Hi-Assorted Biochemical test kit was used for biochemical characterization of bacteria; while gram reaction and nutrient media were used to identify the morphology and cultural characteristics of the bacteria.

Molecular identification was performed through 16S-rDNA sequence analysis. Bacterial genomic DNA was extracted, purified and was confirmed

through agarose gel electrophoresis Bacterial 16S rDNA gene was amplified using primers 357F (CCTACGGGAGGCAGCAG) and 1391R (GACGGGCGGTGWGTRCA) (Weisburg et al., 1991) and column purified amplicon was sequenced. Consensus sequence of 16S rDNA gene was generated and used to carry out BLAST with the database of NCBI Genbank. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Nei and Kumar, 2000) and evolutionary analyses were conducted in programme MEGA11 (Tamura *et al.*, 2021).

2.6 Statistical Analysis

Data analysis was done using Completely Randomized Design (CRD) in three replications. The critical difference (CD) among the variance was calculated at P≤0.05 (Panse and Sukhatme, 1961). The results are expressed as mean with standard deviation (mean±SD) or standard error (mean±SE). During the statistical analysis, percentage data has been transformed using arc sign transformation and other parameter transformed using square root transformation. The results were graphically presented using Microsoft Excel.

3. RESULTS AND DISCUSSION

The wild cotton species, *G. aridum* was known to have better biotic and abiotic stress resistance in comparison to their cultivated varieties. Further, it was speculated that the cause was due to its complex microbial community which may prove to be beneficial to plant growth and protection. In particular, the PGPR found in the rhizosphere is of great focus for this study as they play a pivotal role in enhancing plant nutrition and resistance to stress. Therefore rhizospheric soil of *G. aridum* was chosen for the present study.

3.1 Isolation and Characterization of Rhizospheric Bacteria

A total of 13 rhizospheric bacteria based on its distinct colonial characters were isolated and purified using four flame method after incubation of 24-48 hrs at room temperature. Further, it was noted that the population size of bacteria was 5.8 × 10^2 CFU $ml^{-1}g^{-1}$ of soil sample and the purified bacterial isolates were coded as NAU-RPM-1 to NAU-RPM-13. Earlier, Patel and Minocheherhomji (2020) also reported 81 diversified bacterial communities from rhizospheric soil of American cotton (rhizosphere, rhizoplane, and endorhizosphere). While, Parmar *et al*. (2024) reported isolation of 19 endophytic bacteria from the root, leaf, stem and boll samples of the desi cotton.

3.2 Plant Growth Promoting (PGP) Activities

One of the primary reasons for screening for PGP traits is to identify bacteria that can promote plant growth and increase crop yields in an environmentally sustainable manner. PGPR promote plant growth either by solubilizing and assisting nutrient acquisition or by releasing phytohormones or biocontrol agents to protect plant from various pathogens (Glick, 2012). PGP traits such as mineral solubilization (phosphate, potash, and zinc) and phytohormone production (e.g., IAA, GA_3) directly influence plant growth by improving nutrient availability and promoting root and shoot development.

The 13 pure rhizobacteria isolates were screened for their potent PGP traits under *In vitro* conditions (Fig. 1(a) and 1(b)). The plate assays for mineral solubilization revealed that NAU-RPM-4 (2.23) followed by NAU-RPM-1 (0.78) showed maximum solubilization index for potash; NAU-RPM-4 (2.53), NAU-RPM-1 (1.56), and NAU-RPM-9 (1.50) showed maximum SI for phosphate; while NAU-RPM-4 (1.56), NAU-RPM-1 (1.36) showed the highest ability of zinc solubilization followed by NAU-RPM-8 (1.22) and NAU-RPM-9 (1.11) at 72 h (Fig. 1 (a)). Recently, Zhao *et al.* (2024) also isolated and characterized potassium-solubilizing rhizobacteria (KSR) from cotton; and they reported strain the A10 (*Pseudomonas aeruginosa)* with potassium highest solubilization capacity (21.8 ppm). Shah *et al.* (2022) reported 17 thermo-tolerant phosphate solubilizing bacteria (PSB) from the 840ellulose840e of cotton. Romero-Perdomo *et al.* (2021) found that inoculating cotton plants with phosphate-solubilizing bacteria, specifically *Rhizobium* sp. B02, significantly improved plant growth, phosphorus content in shoots, and photosynthetic rate. Dhaked *et al.* (2017) reported three bacterial isolates named ZnSB-1 and ZnSB-3 as zinc solubilizers. Further, Parmar *et al.* (2024) reported solubilization index for zinc and potash of 0.58 and 0.76, respectively by potent root endophyte of desi cotton (NAU-G27E-PR1).

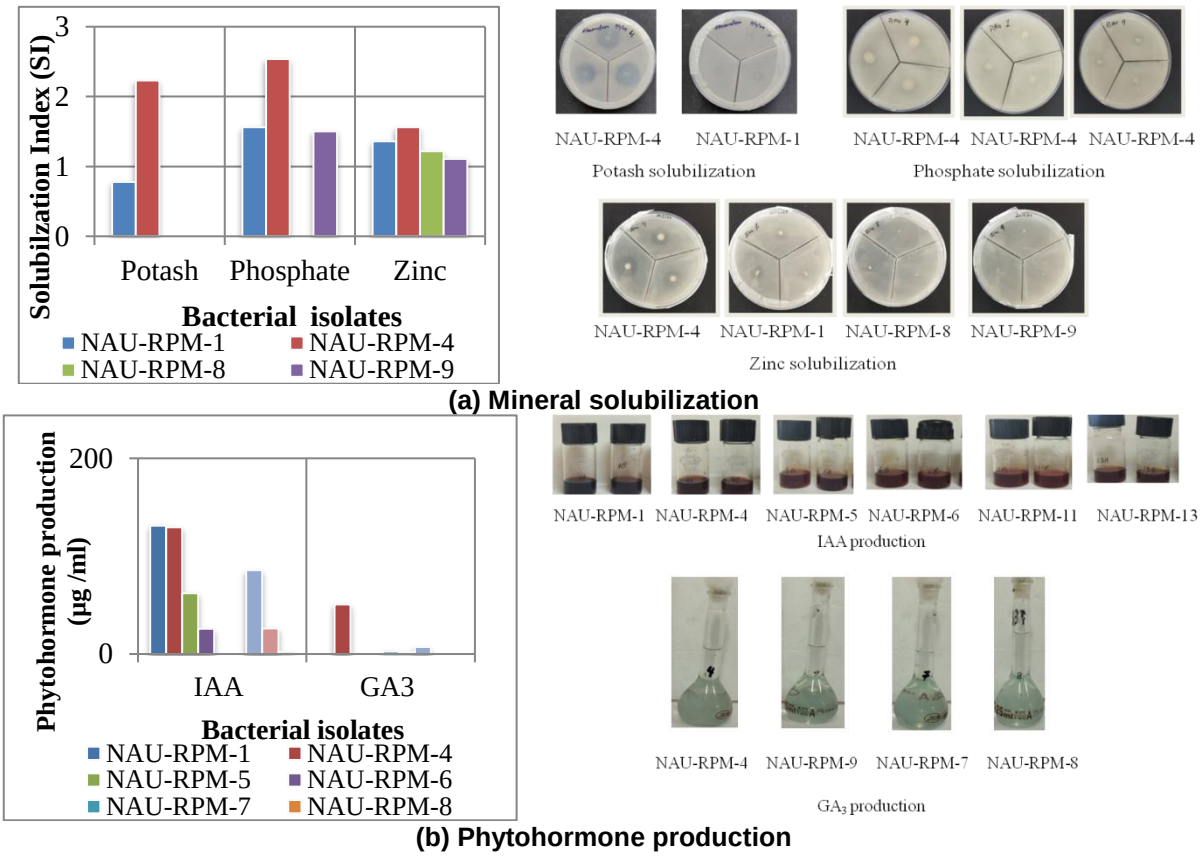


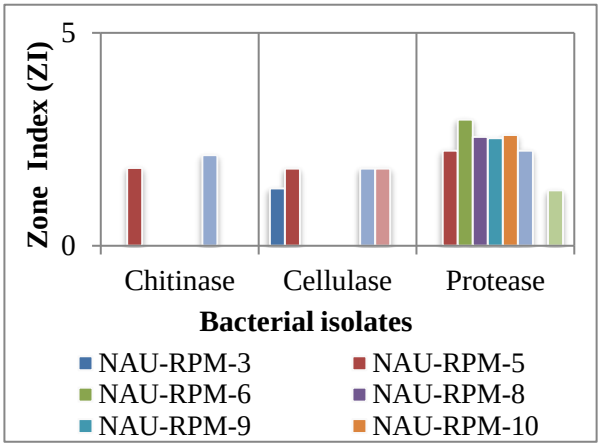
Fig. 1. Plant growth promoting activities of rhizospheric bacterial isolates of *G. aridum*

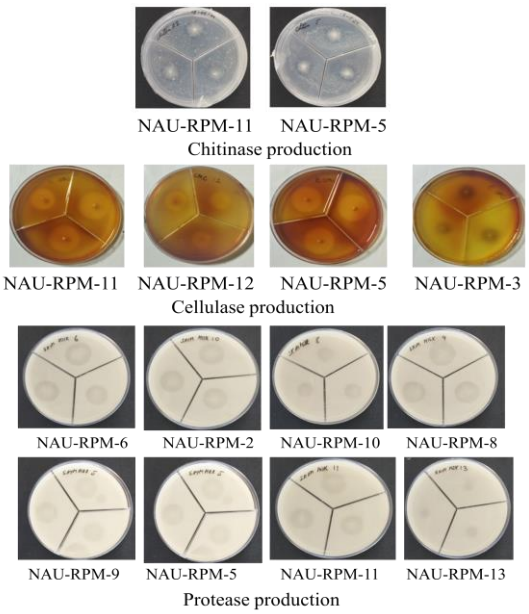
Indole-3-Acetic Acid (IAA) is one of the most common and essential naturally occurring plant hormone, classified as auxin that know to promotes cell elongation, root initiation, fruit development, and facilitates nutrient uptake. Gibbberellic acid (GA) is also a plant hormone belonging to the gibberellin group and it regulates cell elongation and division, leading to increased shoot length, leaf expansion, and root development. Thus, identification of IAA and GA producing strains and optimization to maximize IAA and GA production could promote sustainable agriculture. Phytohormone production *viz.,* IAA and gibberellic acid revealed that seven isolates *viz.,* NAU-RPM-1 (131.00 µg ml^{-1}), NAU-RPM-4 (129.51 µg ml^{-1}), NAU-RPM-9 (85.54 µg ml^{-1}), NAU-RPM-5 (62.25 µg ml^{-1}), NAU-RPM-11 (26.12 µg ml^{-1}), NAU-RPM-6 (25.70 µg ml^{-1}) and NAU-RPM-13 (2.24 µg ml^{-1}) produced IAA. Whereas, four isolates *viz*., NAU-RPM-4 (50.73 µg ml^{-1}), NAU-RPM-9 (7.06 µg ml^{-1}), NAU-RPM-7 (2.65 µg ml^{-1}) and NAU-RPM-8 (2.10 µg ml^{-1}) recorded GA_3 production (Fig. 1 (b)). Similar range of IAA production (0.070 – 0.317 ug ml^{-1}) and GA_3 production (0.001- 0.364 µg ml^{-1}) was reported by Patel and Desai (2015) among 67 bacteria of 841ellulose841e of cotton plant (*Gossypium hirsutum*), Shaikh *et al.* (2017) reported seven cotton endophytes (EB3, EB13, EB8, EB11, EB15, EB2 and EB17) as IAA producers in the range of 15-28 µg ml^{-1} at 72 h. Meena *et al.* (2023) characterized plant growth promoting microbial isolates from rice 841ellulose841e (2 Actinomycetes and 3 fungal strains) and phyllosphere (4 bacterial stains, 2 Actinomycetes and 2 fungal strains) and evaluated PGP traits. They reported highest IAA (Indole Acetic Acid) production (23.75µg ml-1) by RRS5 (Rice Rhizospheric Sample 5). Recently, Parmar *et al*. (2024) reported IAA production (19.97 ± 0.14 µg ml^{-1}) and gibberellic acid production (33.03 ± 0.60 µg ml^{-1}) in desi cotton root endophyte.

3.3 Plant Protection Activities

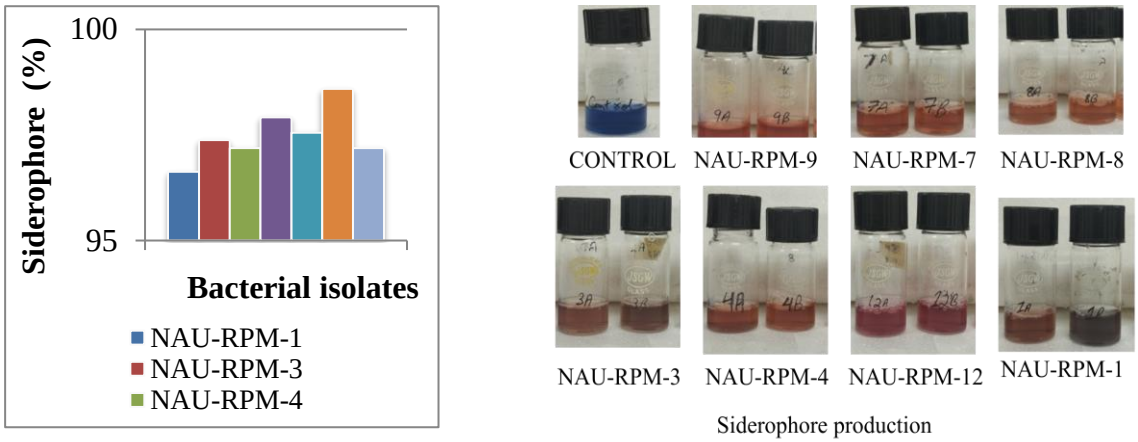
PGPR can also promote plant growth through the suppression of pathogen growth by production of siderophores, antibiotics, HCN and cell wall degrading enzymes like chitinase, protease, and 841ellulose (Soltani *et al.*, 2010). Many bacterial species like *B. subtilis*, *B. cereus*, *B. subtilis, B.*

thuringiensis, and *S. marcescens etc*. are shown to synthesize such enzymes that can affect the cell wall integrity of the pathogens and inhibit those (Panicker and Sayyed, 2022).

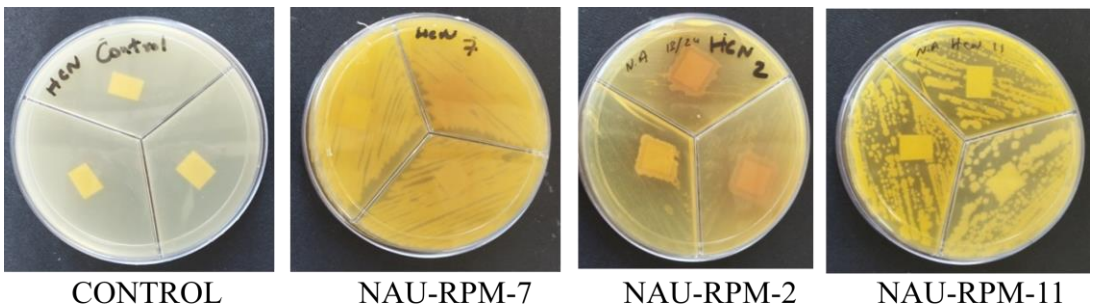




(a) Hydrolytic enzyme production



(b) Siderophore production



(c) HCN production

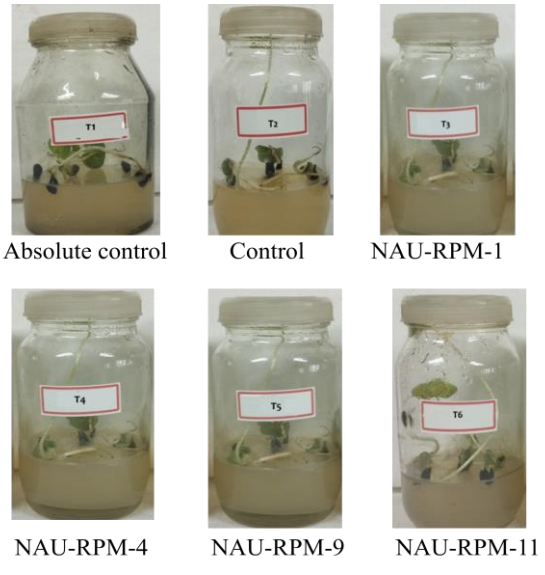
Fig. 2. Plant protection activities of rhizospheric bacterial isolates of *G. aridum*

The plant protection traits like production of hydrolytic enzymes (chitinase, cellulase and protease) and biomolecules (HCN and siderophore) production were studied [ig.2 (a-c). Two isolates NAU-RPM-11 (2.13) and NAU-RPM-5 (1.83) were chitinase producers; four isolates *viz.,* NAU-RPM-11 (1.81), NAU-RPM-12 (1.81), NAU-RPM-5 (1.81), and NAU-RPM-3 (1.35) were potent cellulase producers; and eight isolates were protease producers *viz.*, NAU-RPM-6 (2.96), NAU-RPM-2 (2.63), NAU-RPM-10 (2.60), NAU-RPM-8 (2.56) NAU-RPM-9 (2.53), NAU-RPM-5 (2.23), NAU-RPM-11 (2.23) and NAU-RPM-13 (1.30) (Fig. 2(a)). Further, three isolates showed HCN production NAU-RPM-2 (orange), NAU-RPM-7 (orange) and NAU-RPM-11 (light orange) and seven showed siderophore production NAU-RPM-9 (98.56 %), NAU-RPM-7 (97.92 %), NAU-RPM-8 (97.55 %), NAU-RPM-3 (97.38 %), NAU-RPM-4 (97.19 %), NAU-RPM-12 (97.19 %) and NAU-RPM-1 (96.63 %) (Fig. 2(b)). Recently, Parmar *et al*. (2024) reported zone index for chitinase (0.51), protease (0.39) and siderophohre production by root endophytic bacteria from desi cotton variety, G.27. Afrin *et al.* (2024) characterized protease-producing bacterial strains and they reported seven bacterial strains (S1, S2, S3, S4, S5, S6, and S7) for protease activity using skimmed milk agar and gelatin hydrolysis methods. HCN and siderophore production of cotton flora was reported by Shahid *et al.* (2017).

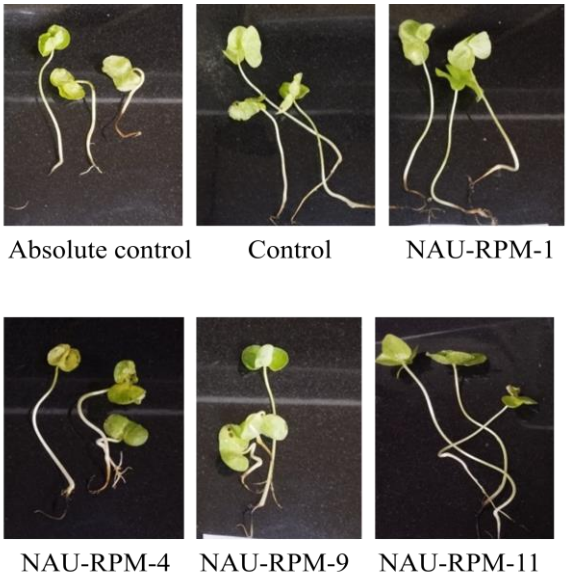
Based on the data generated on 10 parameters comprising plant growth promoting and plant protection across 13 rhizobacterial strains, six parameters each found statistically significant in NAU-RPM-4 (potash, phosphate and zinc solubilization; IAA and GA_3 production; and siderophore production), NAU-RPM-9 (phosphate and zinc solubilization; IAA and GA_3 production; protease activity and siderophore production); and NAU-RPM-11 (IAA and GA_3 production; Chitinase, cellulase and protease activity ; and HCN production), while five statistically significant parameters recorded in NAU-RPM-1 (potash, phosphate and zinc solubilization; IAA production; and siderophore production). Therefore, these four rhizospheric bacteria of wild cotton species were used for seed priming study.

3.4 *In vitro* Study of Effect of Bacterial Seed Priming on Germination and Early Seedling Growth of Cotton Plant

Seed priming is a very simple and powerful technique to enhance the seed performance as well as plant growth and development that supports sustainable agriculture by reducing dependency on chemical fertilizers offering a cost-effective and eco-friendly solution for improving cotton productivity.

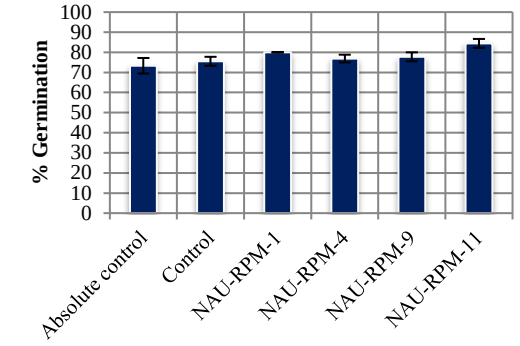


(a) Bioprimed cotton seeds in half MS medium in PTC bottles

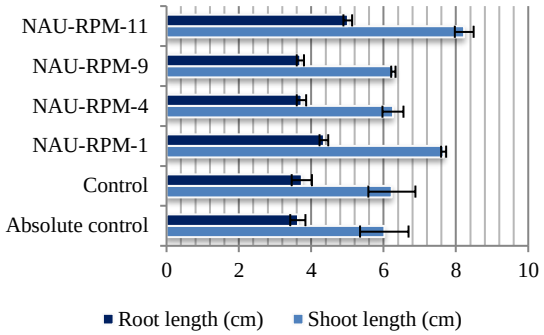


(b) Bioprimed Cotton seedling at 15 days

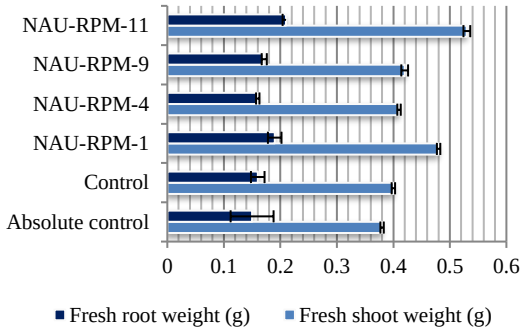
Fig. 3. Biopriming of *G. hirsutum* seeds with potent bacterial isolates *in vitro*



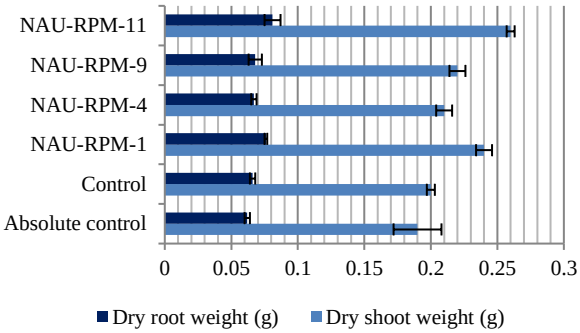
(a) Percent Germination



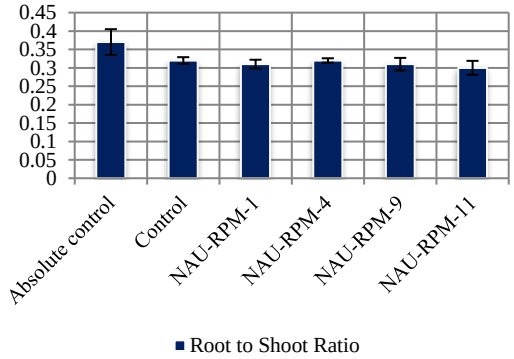
(b) Root and Shoot length (cm)



(c) Fresh root and shoot weight (g)



(d) Dry root and shoot weight (g)



(e) Root to Shoot ratio

Fig. 4. Seed biopriming effect on cotton seedling growth

The study of seed biopriming of cultivated cotton (G. Cot. 40) with the potential four rhizobacterial isolates (Fig. 3a and 3b) revealed that the isolate NAU-RPM-1 significantly improved the growth parameters such as germination percent, shoot length, root length, fresh and dry weight of shoot and root and root-shoot ratio to an extent of 9.09 %, 27.24 %, 19.83 %, 26.31 %, 26.31%, 26.66 %, 22.58 % and 16.21 %, respectively in comparison to absolute control. Data are shown in Fig. 4 (a-e).

Similarly, seed bioprimed with isolate NAU-RPM-11 also recorded significantly improved growth parameters such as germination percent, shoot length, root length, fresh and dry weight of shoot and root and root-shoot ratio to an extent of 15.15 %, 36.00 %, 38.01 %, 39.47 %, 36.84 %, 40.00 %, 30.64 % and 18.91 %, respectively as compared to absolute control. Data regarding the biopriming with NAU-RPM-4 and NAU-RPM-9 also improved the early seedling growth parameters but it was found on par with the absolute control. Earlier, Nehra *et al.* (2016) reported significantly improved shoot (hypocotyl)

length (75 %), root length (84 %), fresh root weight (129%) and dry root weight (135%) in cotton seeds bioprimed with the PGPR *Brevibacillus brevis* than control. Ragadevi *et al*. (2021) also reported maximum shoot length (12 cm) and root length (6 cm) with an increase of 24.4 % and 42.8 % respectively, in cotton seeds treated with PGPR *Azospirillum* sp7 strain.

Higher shoot and root length, higher fresh/dry weight of root and shoot in bioprimed cotton plants indicates the positive impact of PGPR in developing the better root system that can support the overall plant resilience and growth. Further, Ma *et al*. (2024) was also of opinion that reduced root-shoot ratio enhances photosynthetic activity, boll formation and biomass restoration, ultimately leading to better cotton yield. Similar trend was also reported in the present study that establishes the importance of PGPR (NAU-RPM-1 and NAU-RPM-11) in biomass accumulation. Therefore, NAU-RPM-1 and NAU-RPM-11 were further characterized at microbiological and molecular level for precise identification.

3.5 Microbial and Molecular Characterization of Potent PGP Rhizobacteria

3.5.1 Microbial characterization

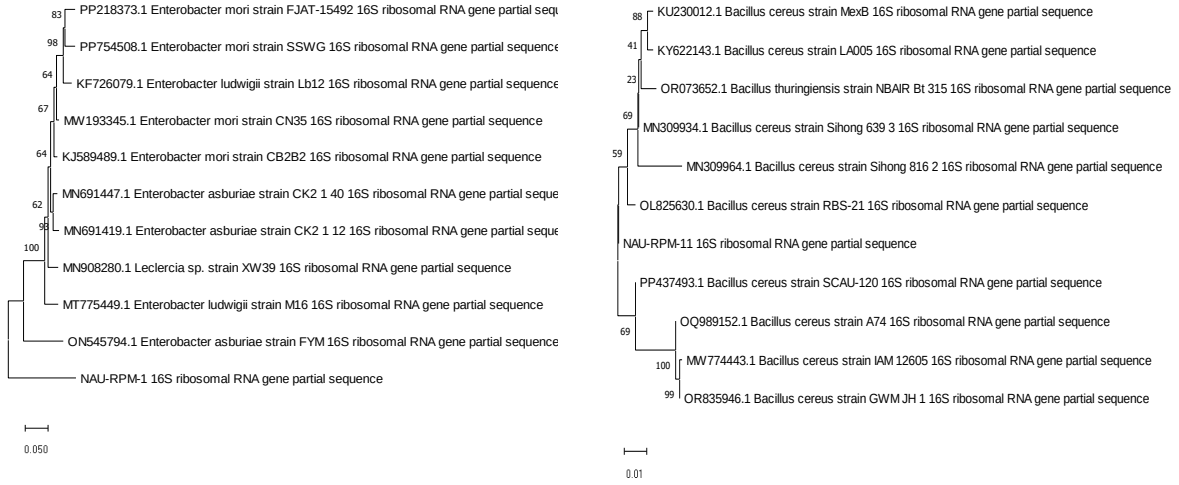
Microbiological characterization revealed that NAU-RPM-1 was gram negative, short rod shaped forming single colonies; while NAU-RPM-11 was gram positive long rods occurred in short chain. Further, cultural characterization revealed that NAU-RPM-1 was small, round, smooth, transparent, glistening and non-pigmented colonies; while NAU-RPM-11 was large, round, rough, opaque and off white color colonies on nutrient agar plates. Arunachalam and Gayathri (2010) also reported the variation for cultural characterization of endophytic bacteria. Shaikh *et al.* (2017) also characterized 19 bacterial leaf endophytes of *G. hirsusutm* and reported ten as gram positive and nine as gram negative rods.

Biochemically, isolate NAU-RPM-1 NAU-RPM-11 both showed positive results for urease, citrate utilization, esculin hydrolysis, arabinose, xylose, and glucose; while both isolates showed negative results for lysine utilization, ornithine utilization, phenylalanine deamination, H_2S production, Voges Proskauer's, methyl red, indole, raffinose, trehalose, lactose and oxidase. Further, NAU-RPM-1 showed positive results for ONPG, nitrate reduction, malonate utilization, rhamnose and saccharose, while negative results for, adonitol, cellobiose, melibiose. On the contrary NAU-RPM-11 showed positive results for adonitol and melibiose, while negative results for ONPG, nitrate reduction, malonate utilization, rhamnose, mellobiose, and saccharose. Recently, Parmar *et al.* (2024) also microbiologically characterized endophyte of desi cotton NAU-G27E-PR1 as gram positive rods with short chains and large, irregular, entire, smooth, opaque and white colonies on nutrient agar; while biochemically the endophyte recorded positive for nitrate reduction, methyl red, Vogus-Proskaurer's (VP), oxidase, escluline, saccharose, glucose, xylose, maltose, dextrose, galactose, sucrose, insulin and sorbitol tests. They reported NAU-G27E-PR1 belongs to genus *Bacillus* based on microbial characters.

3.5.2 Molecular characterization

The 16S rDNA sequencing was employed to assess the molecular identity of potential PGPR. The 16S rDNA bacterial genes of NAU-RPM-1 and NAU-RPM-11 were successfully amplified using PCR with universal primers for species identification. The sequences obtained were then searched for homology using BLAST in NCBI GenBank. Further, homology analysis indicated that the sequence for 16S rDNA gene of NAU-RPM-1 and NAU-RPM-11 showed a high sequence similarity to the genus *Enterobacter mori* (81 %) and *Bacillus cereus* (100 %), respectively. Finally, the phylogenetic tree constructed using neighbour-joining method in MEGA 11 software for NAU-RPM-1 and NAU-RPM-11 **(**PQ410308**)** (Fig. 5 (a) and (b)). Although the BLAST analysis of NAU-RPM-1 showed a 82.01 % sequence similarity to *Enterobacter* species suggesting that the isolate is related to the identified species but it may represent a distinct or less characterized strain or species. However, its plant growth-promoting traits and biochemical properties may contribute to distinct mechanisms for plant-microbe interactions.

Majority of the biochemical tests of NAU-RPM-1 and NAU-RPM-11 were same as *Enterobacter mori* and *Bacillus cereus*, respectively. Thus, isolates NAU-RPM-1 and NAU-RPJ-11 identified as *Enterobacter mori* NAU-RPM-1 and *Bacillus cereus* NAU-RPM-11. The study conducted by Ibrahim *et al.* (2020) also revealed that *Bacillus* was the most prevalent genus, constituting 92 % of the bacterial isolates in the *Bt* cotton



(a) **(b)**

Fig. 5. Phylogenetic tree based on 16S rDNA sequence of a) NAU-RPM-1(*Enterobacter mori*) and b) NAU-RPM-11 (*Bacillus cereus*)

rhizosphere and 88 % in the non-*Bt* cotton rhizosphere, with *Bacillus cereus* being the most common species. Recently, Parmar *et al.* (2024) reported root endophyte of desi cotton, NAU-G27E-PR1 as *Bacillus halotolerans* based on 16S rRNA gene sequence, which showed 99.93 % similarity.

4. CONCLUSION

The present study results implicated that rhizospheric bacteria isolated from wild cotton species exhibited significant plant growth promoting (potash, phosphorus and zinc solubilization: IAA and GA_3 production) and plant protection (chitinase, cellulase, protease, siderophore and HCN production) activities. Further, seed biopriming with identified two potent rhizobacteria *Enterobacter mori* NAU-RPM-1 and *Bacillus cereus* NAU-RPM-11 showed promising improvement in early seedling growth parameters like germination percent, shoot length, root length, fresh and dry weight of shoot and root and root-shoot ratio in cultivated cotton. Thus, it can be concluded that the seed biopriming with potential rhizobacteria identified improved the early seedling growth but still comprehensive understanding and studies are required to explore it in agriculture practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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