ISSN: 2347-4688, Vol. 11, No.(1) 2023, pg. 337-347

Current Agriculture Research Journal

www.agriculturejournal.org

Isolation and Molecular Characterization of Plant Growth Promoting Rhizobacteria from Groundnut (*Arachis Hypogaea* L.) Rhizosphere

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Abstract

Plant growth promoting rhizobacteria (PGPR) have been extensively employed as biofertilizers to enhance the soil nutrition for several crop plants. Rhizobacteria with beneficial effects for plants could therefore be used to reduce the dependence on synthetic chemical fertilizers in conventional agriculture. Within this study, we have explored for isolation of potential PGPR for groundnut crop from agricultural fields of Saurashtra region, Gujarat. A total of forty-two isolates from rhizospheric soil with different colony characteristics were isolated. All the strains were tested for plant growth promoting (PGP) traits to observe their properties and potential for plant growth promoting of all forty-two isolates. Plant growth promoting traits such as indole acetic acid (IAA), hydrogen cyanide (HCN), ammonia production, phosphate solubilisation and gibberellins production were performed. Thirty-four isolates produced IAA in the range of 20.7–133 µg/mL, seventeen isolates were positive for ammonia production in the range of 21.4–55.5 µg/mL, twenty-six isolates produced HCN in the range of 5.65–114.3 µg/mL, 4 isolates displayed phosphate solubilisation in the range of 65.6–259.5 µg/mL, and 5 isolates were positive for gibberellins production in the range of 10.2–112.1 µg/mL. Moreover, only RGKP3 and RG12 isolates displayed positive results for all PGP traits. The potent isolate RGKP3 was further identified using 16SrRNA sequencing. The strain has close evolutionary similarities with *Priestia megaterium*. In future study, the potent PGPR will be studied to promote groundnut plant growth, enhanced crop production, and as a potential biofertilizer.

Article History
Received: 02 March 2023
Accepted: 13 May 2023

Keywords
Groundnut;
PGPR;
PGP traits (Quantitative and Qualitative);
Priestia Megaterium.

Introduction

In India, 70–75% of the population is directly or indirectly dependent on agriculture, which forms the backbone of the nation.[1] The Indian economy depends heavily on the oilseed industry because it is the world's largest producer of all major oil seeds,

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Doi: http://dx.doi.org/10.12944/CARJ.11.1.30

such as groundnut, rapeseed, mustard, sunflower, safflower, sesame, soybean, castor, and linseed.[2] Groundnut is the most important food and cash crop in India.[3] The state of Gujarat supplies approximately 40% of India's production.[4] The Solvent Extractors' Association of India reported that the groundnut oil availability of the country for 2014-15 was 2,40,000 tonnes, which was reduced by 170,000 tonnes or 41.50% from 2013 (410,000 tonnes). According to estimates, groundnut crop production has decreased globally over the past ten years.[5] The enhancement in the crop yield is usually achieved by excessive use of chemical fertilizers in the present agricultural system.[6] However, overemployment of chemical fertilizers results in severe issues, such as soil degradation, nitrogen leaching, soil compaction, and reduction in organic matter in soil.

Sustainable biological approaches for the enhancement of crop production employing rhizospheric microorganisms, especially PGPR, are gaining immense popularity worldwide. These bacteria promote plant growth and development via plant root-microbial interactions, improving nutrient availability (nitrogen, phosphorous, and potassium), controlling the levels of phytohormones (gibberellins, cytokinin), and controlling phytopathogens through the production of secondary metabolites (HCN and chitinase production). PGPR have been broadly classified into two categories-intracellular or endophytic, iPGPR and extracellular or rhizopheric, ePGPR based on their association with plant roots.[7] The ePGPR displays enhanced interaction with several plants due to their free-living ability in comparison to the iPGPR. Extracellular PGPR includes *Acetobacter*, *Azotobacter*, *Bacillus*, *Clostridium*, *Derxia*, *Enterobacter*, *Pseudomonas*, *Rhodopseudominas,*[8] which have been isolated and extensively studied on several crops including groundnut, wheat, rice, maize and soybean.[9] In the present study, we have screened for potential PGPR in the groundnut rhizosphere, which can be employed as novel bio-inoculants for enhanced production of the crop.

Materials and Method

Collection of Sample and Isolation of Rhizobacteria

Rhizospheric soil samples were collected from four different agriculture fields of Kotdapitha 21.966728, 71.204532, Virnagar 22.043104, 71.113215, District Rajkot, Kalawad 22.206375, 70.377288, District Jamnagar, and Garani 21.924582, 71.136719, District Amreli, from the Saurashtra, Gujarat. The groundnut plants were uprooted, and shoots were cut off, and roots along with the rhizosphere soil were stored aseptically in sample bags. The soil samples were stored at 4°C until further use. The samples were serially diluted in the range 10^{-3} to 10^{-8} and colonies with morphological variations were isolated.

Characterization of the Isolates for PGP Traits

All the isolates were tested for plant growth promoting traits: indole acetic acid (IAA), hydrogen cyanide (HCN), ammonia production, phosphate solubilisation, and gibberellin production. All the tests were performed in triplicate.

Indole Acetic Acid

Indole acetic acid production was performed according to the colorimetric method.[10] Briefly, isolates were transferred into 5 mL of nutrient broth (NB) containing 100 mg/mL of L-tryptophan. The tubes were incubated at 37°C for 48 h. After incubation, the broth was centrifuged at 10,000 rpm for 5 minutes. The supernatant (1 mL) was transferred into a fresh, sterile micro centrifuge tube and 2 mL of Salkowski's reagent (0.5M ferric chloride+ 35% perchloric acid) was added. The tubes were gentlymixed and incubated for 30 minutes at room temperature, and a pink coloration of the solution was observed. The color change was recorded spectrophotometrically at 530 nm. The standard curve was plotted in the range of 20–200 µg/mL.

Ammonia Production

All the isolates were analyzed for the production of ammonia.[11] The 24 h old bacterial cultures were inoculated in 10 mL peptone broth and incubated at 37°C for 48h. After incubation, 0.2 mL of freshly prepared Nessler's reagent was added to test tubes. Ammonia production was observed by change in color from yellow to brown. Furthermore, the quantitative estimation of ammonia was spectrophotometrically measured at 600 nm.[12] The standard curve was plotted in the range of 10-100 µg/mL.

Hydrogen Cyanide Production

All the isolates were screened for the production of HCN by adapting the method as described by Alstrom in 1989.[13] Briefly, 100 µL bacterial culture were streaked on nutrient agar medium containing 4.4 g/L glycine plates. Whatman filter paper no.1 was soaked in alkaline picrate solution (2% sodium carbonate in 0.5% picric acid) and placed at the top of the plates. The plates were sealed with parafilm to prevent volatilizationand incubated at 28°C for 4 days. Color changes of filter paper from yellow to light brown to reddish-brown indicated HCN production.

The HCN production by the rhizobacterial strain is determined using the picric acid method.[14] Briefly, media (NB) was supplemented with 4.4 g/Lglycine. The 3 mm strips of Whatman No.42 filter paper were sterilized and then soaked in a picrate alkaline solution. Later,filter paper strips were dried and placed in a test tube with 5 mL of inoculated bacterial culture, and the tubes were plugged with cotton to prevent volatilization. The tubes were incubated at 28±2°C for 3–5 days. After the incubation period, a color change was observed, and strips were placed in fresh tubes with 10 mL of distilled water and mixed properly with a vortex. The optical density of the samples was measured at 515 nm. The standard curve plotted with potassium cyanide in range of 10-100 µg/mL.[15]

Phosphate Solubilization Test

All isolates were screened for their qualitative ability to solubilize calcium phosphate using Pikovskaya agar.[16] Briefly, isolates were spotted on Pikovskaya agar plates and incubated at 28±2°C for 7 days. The halo zone indicated phosphate solubilization.

Quantitative Analysis of Phosphate Solubilization

The amount of phosphate released was measured by the chlorostannous reduced molybdophosphoric acid blue method. Briefly, 1 mL of bacterial culture was inoculated into 100 mL sterile Pikovskaya broth in Erlenmeyer flask and incubated at 28±2°C for 11 days with shaking at 120 rpm. The uninoculated broth was used as a control. The whole experiment was performed in triplicates. Broth (10 mL) from each sample was withdrawn on the 3rd, 5th, 7th, and 10th day for measurement of soluble phosphorous and variation in pH. The cultures were centrifuged at 10,000 rpm for 15 minutes. The supernatant (100 µL) was added in the flask containing 10 mL of chloromolybdic reagent in a shaking condition and diluted with 40 mL of distilled water. Later, 5 drops of chlorostannous acid reagent were added along the sides of the flask and mixed properly. The final volume was made up to 50 mL with distilled water.[17] The resultant blue color was measured by spectrophotometrically at 660 nm against blank. The standard curve was plotted in the range of 10–50 µg/mL.

Gibberellin (GA) Production

All isolates were screened for their quantitative ability to produce phytohormone-gibberellin. Briefly, the bacterial culture was inoculated in NB media containing 1mM of L-tryptophan and incubated at 37°C for 24 h at 150 rpm condition. The culture after incubation was centrifuged at 10,000 rpm for 5 min and a cell free supernatant was collected and used for estimation of gibberellic acid.[18]

Gibberellin production was estimated with the Folin-Ciocalteu reagent.[19] Bacterial cell extract (1 mL) was added to the test tube, followed by the addition of 1 mL Folin-Ciocalteu reagent and 1mL of concentrated hydrochloric acid into the test tubes. The mixture was boiled in a water bath for 5 min and then allowed to cool at room temperature. The greenish blue color produced was recorded using a spectrophotometer at 760 nm. The standard was performed with gibberellic acid (GA3) in the range of 10–100 mg/mL.

Identification of Potent PGPR

The isolates RG12 and RGKP3 displayed all positivePGP traits. The isolate RGKP3was selected for further molecular identification.

Molecular Identification of PGPR Isolate by 16s rRNA Sequencing

DNA was isolated from the overnight culture of RGKP3. Quantification of DNA was done by evaluating on 1.0% Agarose Gel to obtain a single band of high-molecular weight DNA was observed. The fragment of gene was amplified by PCR. A single discrete PCR amplicon band was observed on a resolving agarose gel. The PCR amplicon was purified by column purification to remove contaminants. The DNA sequencing reaction of the PCR amplicon was carried out with primer27 F using the BDT v3.1 Cycle Sequencing Kit on an

ABI 3730xl Genetic Analyzer. The gene sequence was used to carry out BLAST with the database of NCBI GenBank database. Based on the maximum identity score, the first ten sequences were selected and aligned using multiple alignment software programs. The gene sequences obtained were compared with sequences available in the GenBank databases using the NCBI and BLAST at https://blast.ncbi.nlm.nih.gov.Sequencing was done by SLS Research Private Limited, Surat, Gujarat. Sequences were submitted to the NCBI GenBank database, and accession number was obtained.

Results

The rhizospheric soil samples of groundnut were collected from Rajkot (RG1-21), Jamnagar (RGK1-RGK9),Virnagar (RGV1-RGV5), and Amreli (RGKP1-RGKP9) districts of Saurashtra, Gujarat. A total forty-two rhizobacteria were isolated from rhizospheric soil (Table 1) and were analysed for their PGP traits. The PGP traits, such as IAA, ammonia, HCN, gibberellin, and phosphate solubilization augments the plant growth and development.

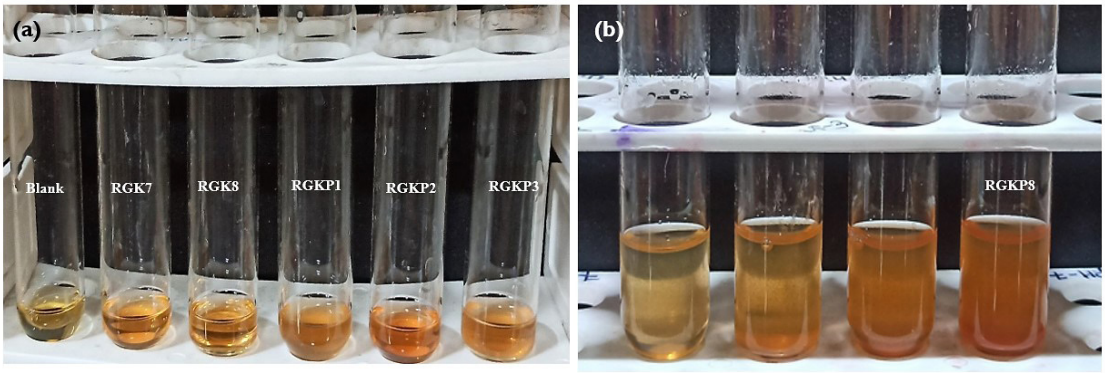

Fig 1: (a) Qualitative analysis of IAA production of thirty-three positive isolates; (b)Qualitative analysis of ammonia production of fifteen positive isolates.

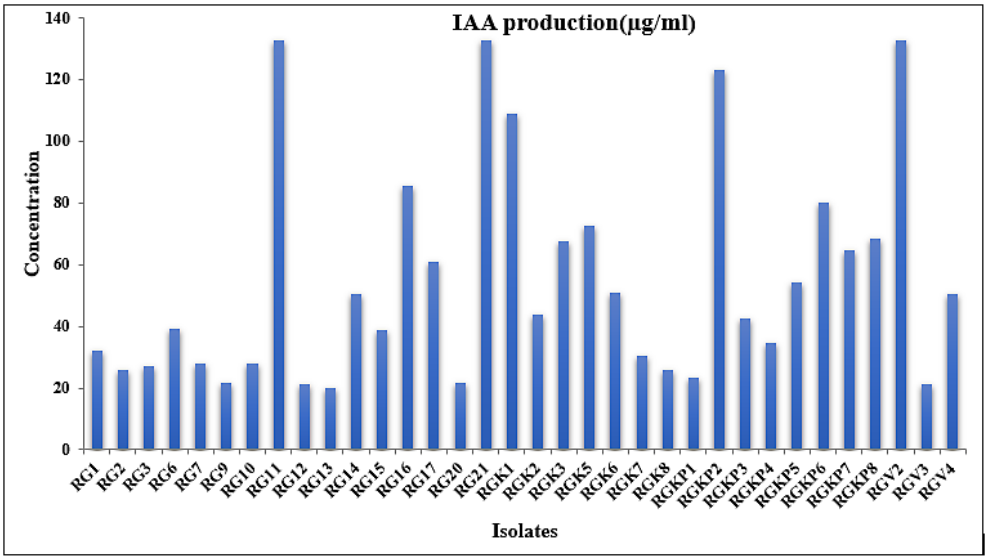

Fig 2: Quantification of IAA production of thirty-three positive isolates

IAA is a pivotal phytohormone for the division and differentiation of plant cells and tissues. Furthermore, it supports plant root elongation. Figure1(a) shows the results for IAA production with respect to

control. The quantification of IAA was done using spectrophotometric analysis (Fig.2). The results indicated that thirty-three isolates produced IAA in the range of 20.7–133 µg/mL. The isolate RG11, RG21,and RGV2 produced maximum concentration of IAA, which was 87% higher than the least IAA production by RG9. The potent PGPR KP3 produced IAA at a significantly higher levels, compared to IAA production reported in the literature.[20]

The ammonia production by the PGPR indirectly affects plant growth and development. The PGPR nitrogenous materials of peptones break down into ammonia, which is released into the soil and used by plants as their nutrient source.[21] Figure 1(b) shows the brown color formation, which depicts the production of ammonia in test tubes on addition of Nessler's reagent. The spectrophotometric analysis of the brown color produced was observed in only seventeen isolates. Figure 3 shows the maximum amount (55.5 µg/mL) of ammonia was produced by isolate RG5, while RG19 produced minimum amount of ammonia. The other isolates produced ammonia in the range of 21.4–55.5 µg/mL. Goswami *et al*. (2013) reported maximum ammonia production was 36 µg/mL which is 36% less than our findings.[22]

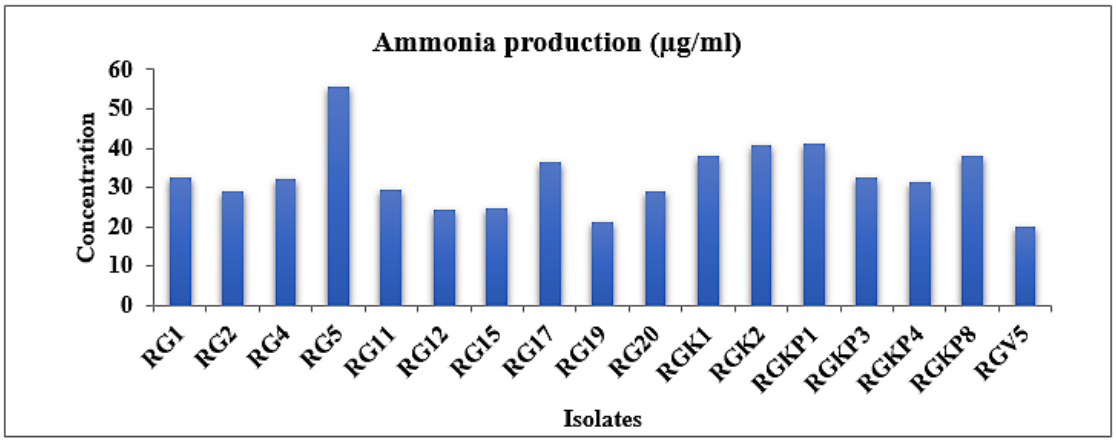

Fig 3: Quantification of ammonia production of fifteen positive isolate

The HCN production is associated with bioremediation and as a bio control for growth enhancement and antagonistic activities. The qualitative estimation of HCN was confirmed by the change in coloration of filter paper soaked in sodium picrate solution from yellow to orange-brown. Twenty-six isolates produced HCN and showed orange to reddish brown coloration of solution (Fig. 5a) and total sixteen isolates were not able to produce HCN. Jadav *et al*. (2020) isolated only four HCN producing bacteria from the *Limonium stocksii* rhizosphere that supported our HCN trait finding.[23]

Fig 4: Qualitative analysis of HCN production of thirty-three positive isolates

Phosphorous is the second-key nutrient after nitrogen for plant growth.[24] The results indicated that out of forty-two isolates, only 4 isolates demonstrated the capacity to solubilize phosphorous from an insoluble phosphate source present in the media.

Table 1: Plant growth promotional properties of PGPR isolates

S. No.	Name of the Isolate	Indole acetic acid Production	Ammonia Production	Hydrogen cyanide	Phosphate solubi lization Production	Gibberellins
1.	RG1	+	+	-	-	-
2.	RG2	+	+	+	-	-
3.	RG3	+	-	-	-	-
4.	RG4	-	+	-	-	-
5.	RG5	-	+	+	-	-
6.	RG6	+	-	+	-	+
7.	RG7	+	-	-	-	-
8.	RG8	-	-	+	-	-
9.	RG9	+	-	+	-	-
10.	RG10	+	-	+	-	-
11.	RG11	+	+	+	-	-
12.	RG12	+	+	+	+	+
13.	RG13	+	-	-	-	-
14.	RG14	+	-	-	-	-
15.	RG15	+	+	+	-	-
16.	RG16	+	-	-	-	-
17.	RG17	+	+	+	-	-
18.	RG18	-	-	-	-	-
19.	RG19	-	+	+	-	-
20.	RG20	+	+	+	-	-
21.	RG21	+	-	+	-	-
22.	RGK1	+	+	+	-	-
23.	RGK2	+	+	-	-	-
24.	RGK3	+	-	-	-	-
25.	RGK4	-	-	-	-	-
26.	RGK5	+	-	+	-	-
27.	RGK6	+	-	-	-	-
28.	RGK7	+	-	-	+	+
29.	RGK8	+	-	+	-	-
30.	RGKP1	+	+	-	-	-
31.	RGKP2	+	-	+	-	-
32.	RGKP3	+	+	+	+	+
33.	RGKP4	+	+	+	-	-
34.	RGKP5	+	-	+	-	-
35.	RGKP6	+	-	+	-	-
36.	RGKP7	+	-	+	-	-
37.	RGKP8	+	+	+	-	-
38.	RGV1	-	-	+	-	-
39.	RGV2	+	-	-	-	-
40.	RGV3	+	-	+	-	+
41.	RGV4	+	-	-	-	-
42.	RGV5	-	+	+	+	-

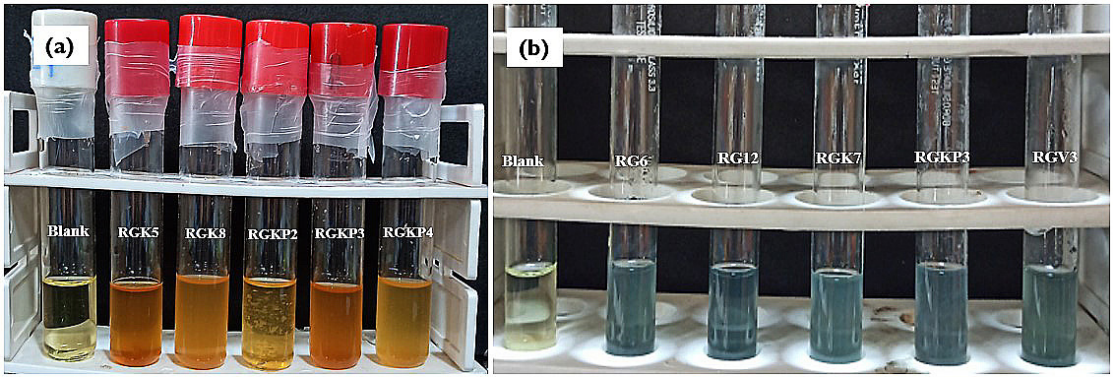

Fig 5: (a) Quantitative analysis of HCN production of isolates with compared to control (b) Quantitative analysis of Gibberellins (GA) production with compared to control.

Furthermore, 4 positive isolates were studied for quantitative estimation of phosphorous using the colorimetric method. Phosphate solubilizing isolates shows blue color compared to yellow colored control on addition of chlorostannous reagent on the 5th day of the assay, the solubilization concentration of RGK7 was recorded to be maximum. The 4 isolates had potential to solubilize phosphate from Pikovskaya's media in range of 65.6–259.5 µg/mL. On the 3rd and 5th days, phosphate is solubilized in a range of 65.6–108.5 µg/mL and on the 7th day, the isolate K7 had 259.5 µg/mL phosphate solubilization. After the 7thdays, the amount of free phosphate gradually decreases during phosphate solubilization by isolates.[25] The standard curve of TCP (tri-calcium phosphate) was plotted in the range of 50–500 µg/mL. Figure 7b shows that the RGKP3 had maximum solubilization after 10 days. Tahir *et al.*(2013) supported our findings reporting that *Azospirillumstrain* WS-1 solubilized 218.1 µg/mL phosphate, which is 16% less than our findings.[26]

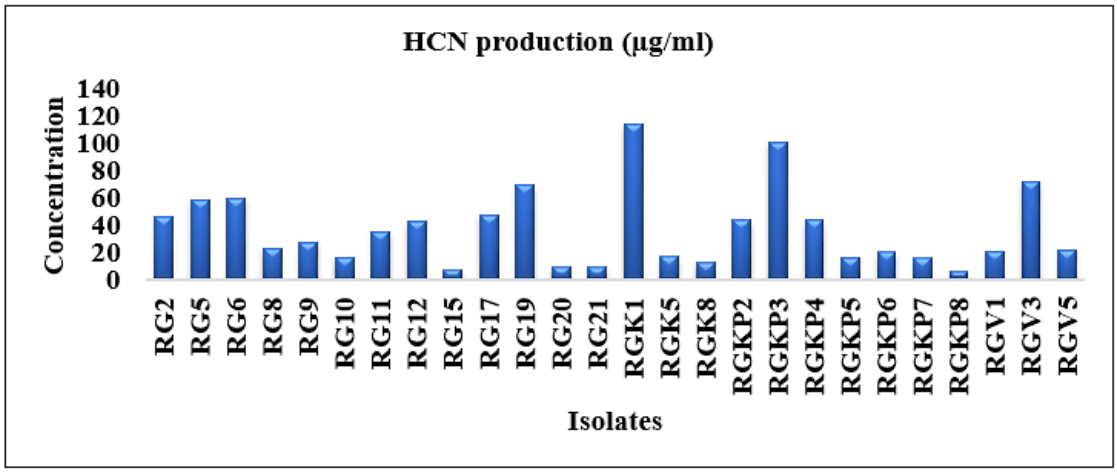

Fig 6: Quantification of HCN production of twenty-six positive isolates

Gibberellins are plant regulators and play a major role in germination and elongation of the stem.[27] Recent studies hypothesise that bacteria have developed an independent biosynthetic pathway for the production of gibberellins.[28] Only five isolates produced gibberellin in the range of 10.2–112.4 µg/mL. The isolate K7 produced the highest amount of gibberellin in the range of 112.4 µg/mL, while

isolate V3 was found to produce the lowest amount of GA (10.2 µg/mL). Youssef *et al*. (2010) also found the gibberellin production in the range of 18.75–49.95 µg/mL, which is 43.35% less than our findings.[29]

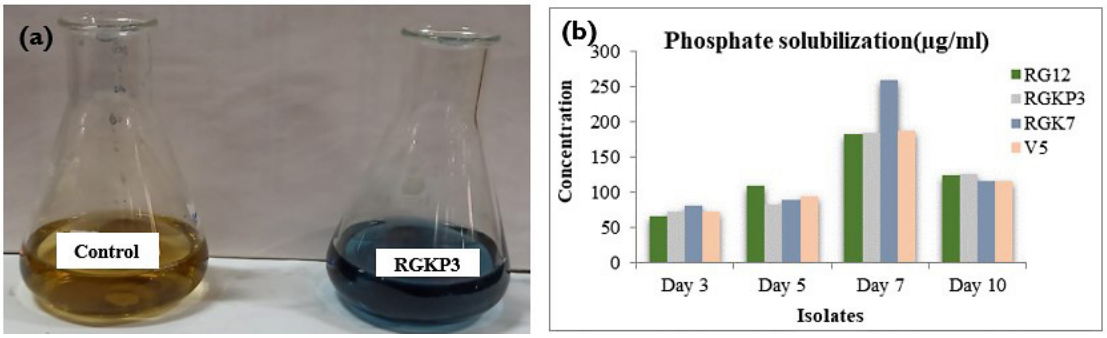

Fig 7: (a)Quantitative analysis of phosphate solubilization control with positive result of isolate RGKP3 (b)Quantification of phosphate solubilization of 4 isolates.

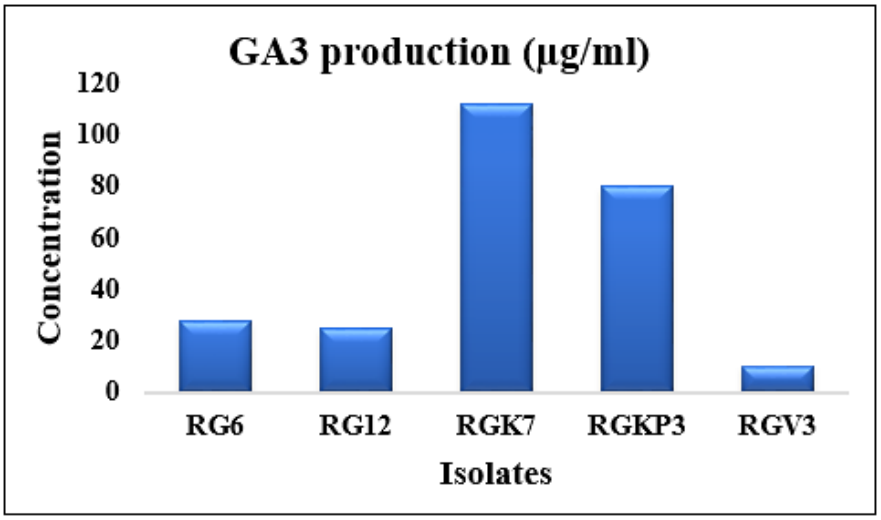

Fig 8: Quantification of Gibberellins (GA) production of six positive isolates

The most promising rhizobacteria isolate has multiple PGP traits that are positive. Gram staining revealed that potent PGPR is a gram-positive bacterium. The isolates were identified by 16S rRNA partial sequencing. The 16S rRNA sequence of RGKP3 and PGPR has been placed in GenBank with the accession number OP528743. Figure 9 displays the phylogenetic analysis of the identified PGPR RGKP3 isolate.

Moreover, using Genbank data, the KP3 PGPR isolate presented close homology with *Priestia megaterium.*

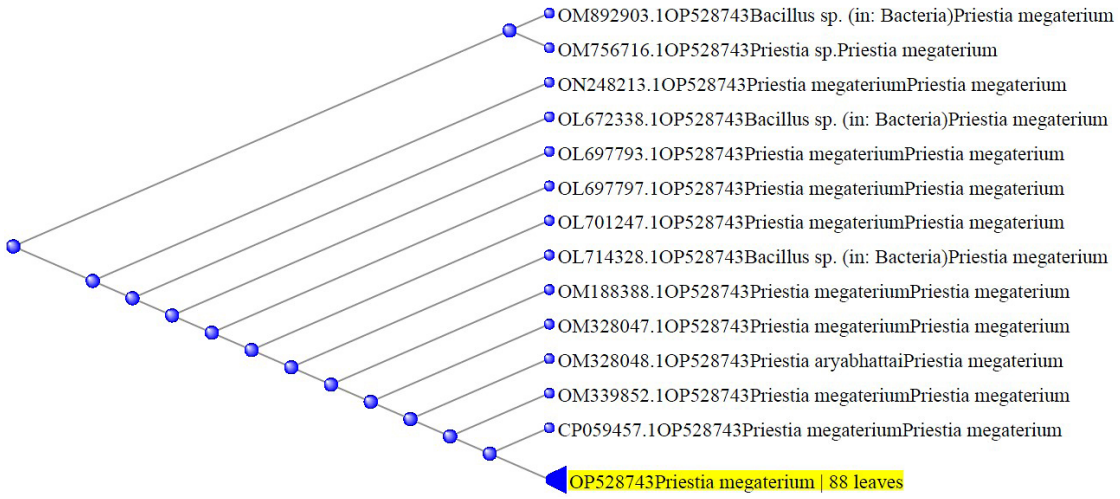

Fig 9: Phylogenetic tree showing the evolutionary relationship between RGKP3, a PGPR isolate and reference strain from GenBank database.

Conclusion

In the present study, a total of forty-two isolates were obtained from the rhizospheric region of the groundnut crop. Qualitative and quantitative analysis for PGP traits found only two isolates with positive results for all multiple PGP traits. A potent RGKP3 strain was identified by 16S rRNA sequencing. The investigation suggests the potent PGPR must be studied further for its plant growth-promoting ability.

Acknowledgement

The authors would like to acknowledge the department of biotechnology and microbiology at Atmiya University in Rajkot, Gujarat, for providing research facilities and technical assistance.

Funding

We are thankful to Government of Gujarat for providing financial assistance under its 'SHODH SCHEME'

Conflict of Interest

There are no conflict of interest.

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Chapter 12

Revitalization of PGPR through integrating nanotechnology for sustainable development in agriculture

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1 Introduction

The world population will be approximately 9.6 billion by 2050 as stated by UN report 2013 [1]. The need for enhanced yield of food production with reduced harmful aftereffects on the soil is a challenge for sustainable agriculture. Furthermore, the crops need to be made tolerant to abiotic and biotic factors including salt, drought, disease-causing organisms, and heavy metals. The mentioned desirable properties can be made possible by the use of rhizospheric organisms in soil. The potent organisms present in the soil, which augment plant growth rate without contaminating the environment, are called plant growth-promoting rhizobacteria (PGPR) [2].

The favorable microorganisms colonizing around the rhizoplane, microhabitats, and root endosphere provide plant growth-promoting activities [3,4]. The carbon compounds secreted by the plants into the soil lead to high microbial populations, i.e., approximately a thousand times higher in the rhizospheric soil relative to the bulk soil [4–6]. The plant secretes numerous signal compounds, which attract specific species and regulate their biochemical and genetic activities [7–9]. Thus, the microbial community present in the rhizosphere varies from the bulk soil on account of different root exudates [10]. The PGPR are approximately up to 5% of total rhizospheric bacteria [11,12]. They affect plant growth by direct and indirect mechanisms (Fig. 1). The direct mechanisms include increasing the quantity and absorption of nutrients present in the soil to plants through providing phytohormones (cytokinin, abscisic acid, gibberellins, auxins, and ethylene) [13,14], biological nitrogen fixation, solubilizing nutrients (K, P, Zn) to plant available form, siderophore production [5,15,16].

Plant-Microbe Interaction—Recent Advances in Molecular and Biochemical Approaches
https://doi.org/10.1016/B978-0-323-91876-3.00009-9

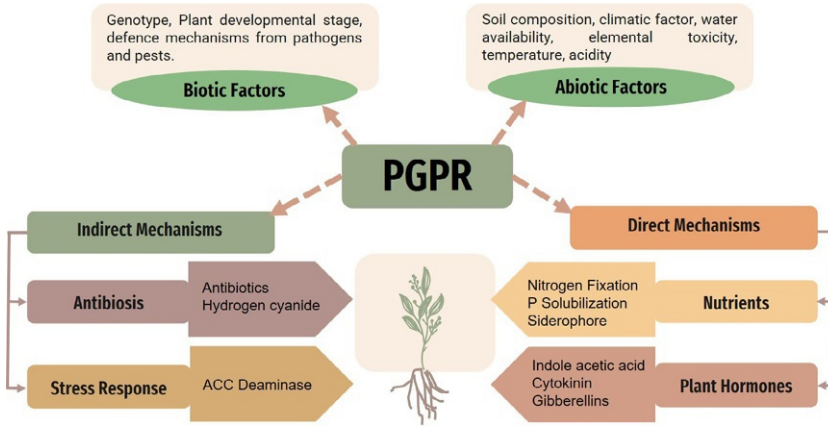

FIG. 1 Schematic representation of plant growth by PGPR.

Further, PGPR exhibit indirect mechanisms including abiotic and biotic stress tolerance [17,18], suppression of plant pathogens [7,16,19], and secretion of various biocontrol specialists such as Volatile Organic Compounds (VOCs). The proclaimed group of PGPR includes bacteria belonging to genera *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Gluconacetobacter*, *Klebsiella*, *Mesorhizobium*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, and *Thiobacillus* [5,17,20].

2 Optimal PGPR

A rhizobacterial strain is viewed as an evident PGPR when it exhibits plant development advancing qualities and can upgrade plant development on inoculation. An optimal PGPR follows the indispensable criteria:

(1) It needs to be profoundly rhizosphere-capable and eco-accommodating.
(2) On inoculation, it should colonize the plant in critical number.
(3) It needs to have the option to advance plant development.
(4) It should display a wide range of activity.
(5) It should be viable with different microscopic organisms in the rhizosphere.
(6) It ought to be tolerable toward physicochemical variables like oxidants, temperature, parching, and radiation.

3 Role of PGPR in enhancement of plant growth

The plant growth is enhanced by direct and indirect mechanisms exhibited by PGPR. Plant development is highly affected by an assortment of stresses which

can be grouped/categorized into two types—biotic and abiotic. Biotic stress alludes to the plant pathogens and pests, for example, such as fungi, viruses, bacteria, nematodes, insects, while abiotic stress focuses on drought, salinity, concentration of various heavy metals in soils, nutrient deficiency, temperature, and so on [2,16,21,22]. PGPR colonization profoundly improves the stress tolerance in plants and enables enhancement of its growth.

4 PGPR and plant hormones

Phytohormones play an important role in plant growth regulation. They function as molecular signals in response to environmental factors, which may otherwise restrict plant growth or become fatal if uncontrolled [23]. Numerous rhizospheric bacteria are known to secrete hormones and boost the growth of plants, stimulate agricultural production, and alter the stress response. Numerous microorganisms have the competence to produce growth regulators such as indoleacetic acid (IAA), gibberellic acid (GA), cytokinin, and ethylene.

According to Spaepen and Vanderleyden, IAA plays a crucial role in plant growth and its development including primary root elongation, enhancement of root surface area and length [24]. Auxin plays an important role in the beneficial plant-PGPR interaction. PGPR strains producing IAA such as *Azospirillum brasilense* Sp245, *Aeromonas punctata* PNS-1, and *Serratia marcescens* 90–166 stimulate growth and activate morphological changes in *Arabidopsis thaliana* [24].

The process of seed germination, flowering, fruit development, leaf and stem growth involves the hormone gibberellin (GA), a type of phytohormones, which also plays a pivotal role in shoot elongation. Gibberellin-producing PGPR *Enterococcus faecium* LKE12 and *Leifsonia soli* SE134 trigger shoot growth in mutated rice plants deficient in gibberellin synthesis [25]. The gibberellin-producing PGPR strains of *Promicromonospora* sp. SE188 and *Bacillus amyloliquefaciens* RWL-1 result in an augmented amount of gibberellins in the plant.

Cytokinin enhances plant vascular differentiation, cell division, vascular cambium sensitivity and increases root hairs proliferation, but inhibits primary root elongation [26]. Various PGPR strains are synthesizing cytokinin which enhances shoot growth and fruit formation of plants [27,28]. *Bacillus megaterium* UMCV1 was reported to stimulate the growth of lateral roots in *Arabidopsis thaliana*, and the cytokinin receptor genes AHK2 and RPN12 are involved in the mechanism of this stimulation. Cytokinin-producing PGPR strain *Pseudomonas fluorescens* stimulated main roots growth and repressed lateral roots formation in *Brassica napus* [29]. Bacterial cytokinins also have the feature to exhibit plant resistance to biotic and abiotic stresses. For instance, PGPR *Pseudomonas fluorescens* G20-18 synthesizes cytokinin, which improves the resistance of *Arabidopsis thaliana* plants to infection with *Pseudomonas syringae*.

Another hormone is ethylene, which controls many processes including the germination of seed, shoot and root growth, abscission of leaves and fruit ripening. Furthermore, excessive amounts of ethylene result in defoliation, premature senescence, and root and stem growth retardation. This eventually leads to restricted plant growth and development. Several abiotic and biotic stresses such as flood, heavy metals, pathogens lead to synthesis of 1-aminocyclopropane-1-carboxylate (ACC), a precursor of ethylene. The ethylene then causes reduction in root elongation and nitrogen fixation causing premature senescence.

PGPR degrade ACC and assist the growth of the root system. Glick has explained that PGPR producing ACC deaminase and IAA facilitate the growth of plants to a greater extent. Ahmad evidenced that *Pseudomonas* and *Rhizobium* ACC-deaminase-producing strains are able to augment the quality, growth, physiology of mung beans under saline environments.

5 Nutrient availability for plant growth

Various PGPR assist in fixing nitrogen into organic form that can be utilized by the plants. Several collections of soil and root-associated nitrogen-fixing microorganisms have been reported in the literature such as *Azotobacter vinelandii*, *Azospirillum brasilense*, *Acetobacter diazotrophicus*, *Achromobacter insolitus*, *Burkholderia tropica*, *Burkholderia xenovorans*, *Burkholderia silvatlantica*, *Burkholderia caballeronis*, *Bradyrhizobium japonicum*, *Delftia tsuruhatensis*, *Enterobacter sacchari*, *Bacillus megaterium*, *Gluconacetobacter diazotrophicus*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Pseudomonas koreensis*, and *Pseudomonas entomophila*, which colonize different crops and enhances plant growth directly or indirectly. Their activity, however, is influenced by soil type, soil condition, and crop species [2,10,11].

Numerous PGPR are also reported to have the ability to solubilize phosphate and increase the phosphate ions availability and accessibility to the plants. *Kocuria turfanensis* strain 2M4 PGPR is a phosphate solubilizer, a siderophore producer, and an IAA producer. Kumar et al. [30] have reported that the employment of *Bacillus megaterium*, *Arthrobacter chlorophenolicus*, and *Enterobacter* resulted in a twofold increase in wheat grain yield in greenhouse experiments [30]. PGPR with a phosphate solubilizing capacity including *Bacillus megaterium* [31], *Pseudomonas*, *Delftia* sp., *Azotobacter*, *Xanthomonas* and *Rhodococcus*, *Arthrobacter*, *Serratia*, *Phyllobacterium*, *Chryseobacterium*, and *Gordonia* increased phosphate availability in soil by approximately 30% [32,33]. Furthermore, phosphate deficiency was reported to reduced crop yield by 5%–15% [34]. Phosphate-deficient plants show symptoms such as dark, dull, and reddish colored leaves, necrosis in old leaf tips, and a smaller size of new leaves [35,36]. Employment of phosphate solubilizing bacteria can prove to be highly cost-effective and lead to enhancement of plant growth and development.

Another macronutrient in plant growth is potassium. Inoculation of seeds and seedlings of different plants with potassium solubilizing bacteria (KSB) displays significant enhancement in germination percentage, seedling vigor, plant growth, yield, and K uptake by plants under greenhouse and field conditions.

6 Enzymes by PGPR

The two main hydrolytic enzymes produced by PGPR are chitinase and glucanase. The major components of the fungal cell wall are chitin and beta-glucan; hence, PGPR producing chitinases and beta-glucanases would inhibit fungal growth. *Pseudomonas fluorescens* LPK2 and *Sinorhizobium fredii* KCC5 produce chitinase and beta-glucanases and dictate the fusarium wilt produced by *Fusariumudum*. *Pseudomonas* spp. a PGPR that inhibits *Phytophthora capsici* and *Rhizoctonia solani*, two of the most destructive crop pathogens in the world.

7 Abiotic stress tolerance in plants

Abiotic stress plays a major role in reducing agricultural production. The strength of abiotic stresses changes on the basis of the type of plant factors and type of soils [37]. Sarma and Saikia reported that the *Pseudomonas aeruginosa* strain enhanced the growth of *Vigna radiata* (mung beans) during drought conditions [38]. The stomata of the leaf balance the water content in the leaves and also water uptake by the roots. Ahmad et al. and Naveed et al. reported that the stomatal conductance of leaves in plants inoculated with PGPR was higher than that in plants without PGPR under drought conditions. PGPR increase water use efficiency of plants. Marulanda et al. reported that the *Bacillus megatertum* strain augments the absorption of water by roots under saline conditions. A similar behavior was exhibited by *Pantoea agglomerans* when observed with maize roots. Gabriela et al. used *Azospirillum* for lettuce growth under salinity stress [39]. The results showed that inoculation with *Azospirillum* sp. augments the quality of lettuce and the storage life of lettuce under salt stress, which further increases the yield.

8 Macronutrients and micronutrients

Plants require various minerals throughout their life cycle. Carbon, hydrogen, and oxygen are derived by plants from air; however, thirteen elements are made available to plants from soil. Based on their requirement by plants, they are classified into micro- and macronutrients. Microorganisms play a significant role in enhancing nutrient availability to plant roots by solubilizing minerals. This section enlists various essential macro- and micronutrients, their role and responsibility in plant growth and development [40].

8.1 Potassium

Potassium is involved in numerous biochemical and physiological systems of plants. Potassium is not included in any chemical structure of plants; however, its role in plant development has been widely studied and reported in the literature [41–44]. Potassium is essential for activation of several enzymes (~60 enzymes) involved in the growth and development of plants. Potassium neutralizes various ions in the plant system and hence assists in the maintenance of the pH (7–8), which is crucial for the enzymatic reactions. Potassium is vital for the opening and closing of stomata, which regulate the nutrient transport, photosynthesis, and cooling of plants. Furthermore, potassium aids in the uptake of water by the plant roots by developing a gradient of osmotic pressure with its accumulation. Potassium is also reported to be responsible for the transport of sugars, synthesis of starch and proteins, transportation of water and nutrients in the plant system. It also helps in the enhancement of crop quality and extends the shelf life of fruits and vegetables [44–47].

8.2 Phosphorus

The abundance of phosphorus is essential for plants as it is a key component in several cellular processes such as synthesis of biomolecules (nucleic acids-DNA, RNA), sugar phosphates (intermediates of various metabolic pathways), and energy-rich compounds (adenosine/cytidine/guanosine/uridine-triphosphate and other phosphorylated compounds). Furthermore, phosphorus energizes photosynthesis and respiration making it indispensable for plant survival. Phosphorus is accountable for the maintenance of cell membrane (phospholipids), germination of seeds, formation of roots (morphology, clusters, and architecture), increment in shoot and root length, flowering, and seed formation in plants [35,36,48] (Fig. 2).

8.3 Calcium

Calcium is an important element that regulates growth and development in plants [49]. It has been vividly reported as the second messenger in animal cells; however, its role has been determined to be essential and indispensable in plant cells. It is a crucial component in determining the structural rigidity of the cell wall and maintains selective permeability of the membrane. It has also been reported to promote root hair growth in various plants. The calcium uptake by plants has been reported to protect them against heavy metal toxicity and several pathogenic microorganisms (yeast, bacteria, etc.). Moreover, the role of calcium has been extended to several developmental processes such as pollen tube elongation, cell division, seed germination, apoptosis, stomatal closure, and auxin responses [48–51].

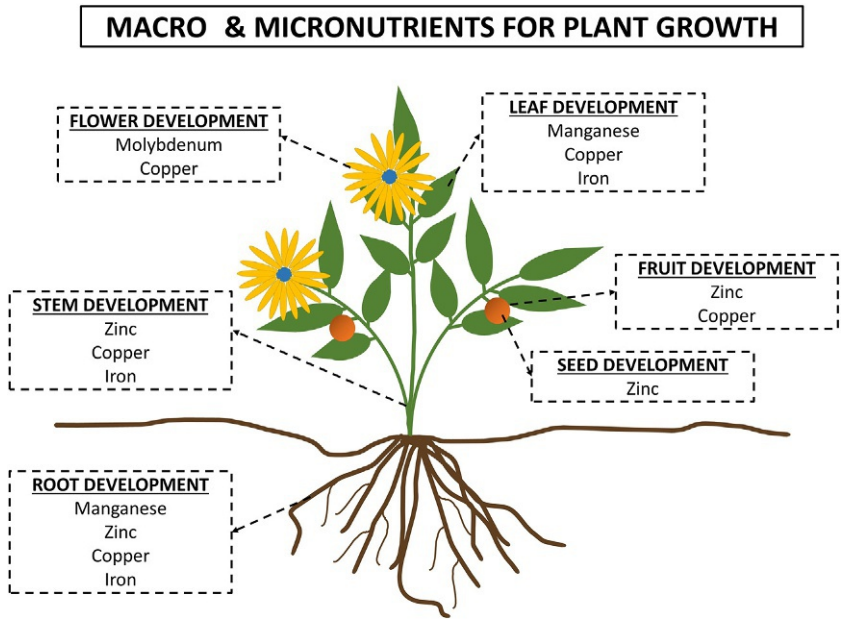

FIG. 2 The role and function of various macronutrients and micronutrients in plant growth and development.

8.4 Magnesium

Magnesium performs various biological functions in plant and animal systems by being a dissociable cofactor in enzymes that activate the phosphorylation process. In plant systems, magnesium is the central atom in the tetrapyrrole ring of chlorophyll *a* and *b* present in the leaf chloroplast. Hence, its concentration affects photophosphorylation and the phosphorylation reactions in chloroplast. The magnesium in plant leaves has been directly and indirectly associated with protein synthesis. Furthermore, it has been reported to be critical for maintaining the stability of ribosomal subunits in the plant cells. Magnesium is also required for activation of several metabolic pathways such as lipid metabolism and carbohydrate metabolism. It has been reported that magnesium ions improve the produce and quality of crops.

8.5 Iron

Iron is involved in the synthesis of chlorophyll and maintenance of chloroplast. The concentration of iron and chlorophyll has been reported to be interrelated in green plants. Furthermore, in plant systems, it plays a vital role in several biological processes such as photosynthesis and respiration (energy yielding electron transfer reactions), nitrogen fixation, hormone production, and nutrient

uptake mechanisms. Moreover, iron plays a major role in major metabolic processes as it is a constituent of several electron carriers and enzymes. Sufficient amounts of iron result in improved nutritional quality and better yield.

8.6 Zinc

Zinc is a constituent of several enzymes and is required as a cofactor for enzymes such as peroxidases, oxidases, etc. Zinc has also been associated with the regulation of the nitrogen metabolism (utilization of nitrogen in seed formation), multiplication of cells, and photosynthesis in plants [52,53]. In various metabolic pathways, such as starch, carbohydrates, hormones (indoleacetic acid and auxin) and proteins, zinc plays a significant role by aiding the activity of the necessary enzymes. Zinc has also been linked to the maintenance of membrane integrity, formation and turgidity in the leaves in most plants. Furthermore, zinc has also been potent in reducing heavy metal accumulation in plants [54,55].

8.7 Manganese

The manganese in plant cells acts as a cofactor and is beneficial in controlling the conformation of various metalloproteins such as superoxide dismutase, oxalate oxidase, etc. It activates several enzymes, such as phosphokinase and phosphotransferase, by bridging adenosine triphosphate (ATP) with the enzyme complex. There are various metabolic processes which are dependent on divalent manganese such as glycosylation and ROS scavenging. Furthermore, divalent manganese ion itself acts as an antioxidant and supports in the reduction of oxidative damage in plants. Manganese also plays a crucial role in water splitting, chlorophyll production, lignin biosynthesis, and photosynthesis.

8.8 Copper

Copper has been extensively studied for its role in several physiological processes in plants including photosynthesis, electron transport, respiration, metabolism of cell wall, hormones, carbohydrates and nitrogen, and oxidative stress response. At cellular levels, it has been identified to be essential for transcription and protein trafficking, phosphorylation and iron mobilization in plant system. It plays an important role in activation of enzymes such as superoxide dismutase, several oxidases (amino, ascorbate, polyphenol, and mitochondrial cytochrome *c* oxidase), and laccase. It has been reported to impart disease resistance to several plants, improve the fertility of flowering plants and improve fruit formation.

9 Nanotechnology and PGPR

Nanotechnology is the study and design of materials (with at least one dimension between 1 and 100 nm) and their exploitation in various applications across the environment, agriculture, biomedical, textile, medicine, engineering, etc. [56,57]. The advent of nanotechnology has promised to improve the agricultural sector and has gained immense popularity in the past few decades. Metal and its oxide nanoparticles have gained considerable consideration by researchers due to the high surface to volume ratio and hence enhanced reactivity. Furthermore, nanomaterials can improve the nutrient uptake and utilization by plants over other conventional methods. Moreover, several nanoparticles have been reported in the literature, which can extensively aid plants in their metabolism and improve physicochemical parameters such as root, shoot, dry weight, wet weight, leaf area, etc. Nanoparticles (NPs) augment plant metabolism through their physicochemical properties and hence enhance crop yield and supply nutrients to the soil [58]. Several research groups are exploring the cumulative effect of various nanomaterials with PGPR for crop improvement and higher yield (Table 1).

Nanomaterials are of various types including metal nanoparticles, organic, carbon nanoparticles, and semiconductor nanoparticles [58,89]. The silver [90], titanium, zinc oxide [73], silica [83], calcium, boron [91], gold [67], and zeolite [75] nanoparticles are reported to exhibit plant growth-promoting effects. The plant growth-promoting rhizobacteria (Bacillus sp.) and silver nanoparticles are utilized on *Zea mays* and were reported to show increase in root, shoot growth and inhibit fungal infections too [92]. Timmusk et al. [93] reported that the utilization of Nanotitania (TNs) provides an effectual method for PGPR to stably attach with plant roots and facilitates PGPR for reproducible field applications.

9.1 Silver nanoparticles

Silver nanoparticles with PGPR have been elaborately studied with various plant systems and are being accepted in the agricultural sector. In addition to being highly reactive, these nanoparticles possess antimicrobial and antipest activities. The silver nanoparticles (Ag NPs) in combination with PGPR have been reported to be more effective to increase plant growth; however, their toxicity and underlying risks are still under consideration. Siddiqi and Husen [90] reported the significant impact of silver nanoparticles on fenugreek seedlings. The plant displayed improved physicochemical parameters such as increase in shoot and root length, leaf number, phytochemicals, and diosgenin [90]. Khan and Bano [94] employed three PGPR strains (*Pseudomonas* sp., *Pseudomonas fluorescence*, and *Bacillus cereus*) with silver nanoparticles and evaluated their cumulative effect on maize seeds [94]. The treated plants had enhanced root area and length and growth hormones, such as ABA, IAA, GA, and proline production [94]. Furthermore, Vishwakarma et al. [95] reported that the treatments

TABLE 1 Effect of various nanoparticles on plant with PGPR.

S. no.	Nanoparticles/ nanomaterials	Plant (common and scientific name)	PGPR	Results	References
1.	Molybdenum NPs	Wheat (*Triticum*)	*Bacillus* sp. strain ZH16	Increase in morphological characteristics, nutrients availability and balance of ions in the plants	[59]
2.	Silicon dioxide NPs	Wheat (*Triticum*)	*Azospirillumlipoferum* and *Azospirillum brasilense*, *Bacillus* sp.	Improvement in physicochemical parameters, and yield; improved relative water content, nutrients uptake, antioxidant enzymes—such as catalase, superoxide dismutase and peroxidase increased their upregulation	[60]
3.	Silicon nanoparticles NPs	Lemon balm (*Melissa officinalis* L.)	(*Pseudomonas fluorescens* and *Pseudomonas putida*)	Increment in free radical scavenging activities of plant extracts	[61]
4.	Magnesium oxide NPs	Radish (*Raphanus sativus* L.)	–	Increment secondary metabolite production, total phenolic and dry biomass	[62]
5.	Silver NPs	–	*Azotobacter vinelandii*	Silver nanoparticles display size dependent (10 and 50 nm) effect on plant; inhibited the growth of bacteria and induced cell apoptosis, effective against nitrogenase activity and ROS detection	[63]
6.	Silver NPs	–	*Nitrosomonas europaea* ATCC19718	Restricts the biosynthesis of protein, gene expression, and production of energy	[64]
7	Iron NPs	–	*Paracoccus* sp.	Excess amount of iron leads to oxidative damage to cells; iron (II) adhered to cell membranes and changed bionitrification of the microorganism	[65]

8.	Silver nanoparticles and iron oxide nanoparticles	–	Soil microbial activity	Silver NPs reduced soil microbial metabolic activity, nitrification ability and count of the microorganism Iron oxide nanoparticles promotes microbial metabolic activity, nitrification and positively influence on C and N cycle	[66]
9.	Gold nanoparticles	Cow pea (*Vigna unguiculata* L.)	*Pseudomonas monteilii*	Increased growth and IAA production	[67]
10.	Zero valent iron nanoparticles	White willow (*Salix alba* L.)	*P. fluorescens*	Dose-dependent effect of iron nanoparticles; at low concentration root length and leaf area per plant improved; at higher concentration it reduced plant growth and induced stress	[68]
11.	Magnesium oxide NPs	Radish (*R. sativus* L.)	–	Displayed enhanced plant growth, production of secondary metabolites, free radical scavenging activity, and phytoaccumulation of lead	[69]
12.	Zero valent iron nanoparticles	White clover (*Trifolium repens*)	PGPR	Increases photosynthesis, plant growth and phytoremediation performance	[70]
13.	Silver nanoparticles	Wheat (*Triticum*)	*Burkholderia* sp., *Bacillus cereus*, *Bacillus* spp.	Improved sugar production and its translocation to the grains, biocontrol potential against yellow rust	[71]
14.	Graphite and silica nanoparticles	Potato (*Solanum tuberosum*)	*Lysinibacillus* sp., *B. subtilis*, and *P. fluorescens*	Isolated strain reduced the wilt disease caused by *Ralstonia solanacearum*	[72]
15.	Titanium dioxide NPs	Beans (*Phaseolus vulgaris* L.)	*Bacillus subtilis* Vru1	Improved the vegetative growth parameters of plant and metabolites production such as indole-3-acetic acid	[73]

Continued

TABLE 1 Effect of various nanoparticles on plant with PGPR—cont'd

S. no.	Nanoparticles/ nanomaterials	Plant (common and scientific name)	PGPR	Results	References
16.	Gold nanoparticles		*P. fluorescens*, *B. subtilis*, *P. gii*, and *P. putida*	NPs displayed no significant with *P. putida*; significant increase was observed in the case of *P. fluorescens*, and *B. subtilis*, *Paenibacillus elgii* and displayed a potential to be used as a nanobiofertilizer	[74]
17.	Nanozeolite	Maize (*Zea mays*)	*Bacillus* spp.	Improved growth parameters and crop productivity	[75]
18.	Silver nanoparticles	Onion seedlings (*Allium cepa*)	*Bacillus pumilus* and *Pseudomonas moraviensis*	Increased the sugar and proline contents; enhanced protein content of bulb, decrement in leaf flavonoids and increase in the bulb flavonoid contents	[76]
19.	Molybdenum (Mo) nanoparticles	Chickpea (*Cicer arietinum* L.)	*B. subtilis*	Improved the physiological status of the plant, increasing structural diversity of the microbial community of the rhizosphere through changes in the activity of root exudates	[77]
20.	Iron oxide NPs	(*Brassica napus* L.)		Enhanced growth by reducing ROS damage and improved oxidative defense system	[78]
21.	Iron oxide nanoparticles	Thale cress (*Arabidopsis thaliana*)		Inhibitory effects on development	[79]
22.	Iron nanoparticles	Cow pea (*V. unguiculata* L.)		Increased seedling growth	[80]

23.	Silicon dioxide nanoparticles	Perennial ryegrass (*Lolium perenne*)		Improved mineral nutritional value and other quality indexes	[81]
24.	Silicon dioxide nanoparticles	Tomato (*Solanum lycopersicum*)		Enhances seed germination	[82]
25.	Silicon dioxide nanoparticles	Maize (*Z. mays*)	*Azotobacter, Bacillus megaterium, B. brevis,* and *P. fluorescens*	Nanoparticles had no toxic effects on microorganisms	[83]
26.	Zinc oxide nanoparticles	Sorghum		Reduced the negative influences on drought stress	[84]
27.	Zinc oxide nanoparticles	*B. napus*		Displayed concentration dependent effect on plant; at lower concentration, enhanced plant growth, while at higher concentration toxicity was observed	[85]
28.	Zinc oxide nanoparticles	*A. cepa* L.		Seed germination was observed to be concentration dependent; at higher concentration of NPs germination rate decreased while at lower concentration seed germination rate increased	[86]
29.	Calcium phosphate nanoparticles	Strawberry		Nano-CaPNPs at 15 ppm improved quality and storability of fruits and gave good appearance with the lowest values of weight loss, and zero decay percentage	[87]
30.	Calcium phosphate nanoparticles	Rice		NPs reduced the amount of fertilizer requirement for the crops thus reducing the fertilizer wastage	[88]

of *Brassica juncea* seedlings with silver nanoparticles and *Bacillus thuringeinsis* KVS25 were observed to significantly reduce the stress in the plant seedlings.

The efficacy of PGPR strains *Bacillus pumilus* and *Pseudomonas moraviensis* with silver nanoparticles on onion bulb weight under salt stress displayed an increase in the sugar content of bulb, root proliferation, and bulb growth [76]. Furthermore, *Pseudomonas moraviensis* with Ag NPs was more effective under saline conditions and had elevated bulb phenolic content (stress related compounds). Bano and Habib in 2020 reported the supplementation of AgNPs with *Bacillus cereus* for enhanced antifungal activity in wheat plants. The cumulative effect of *Bacillus cereus* with AgNPs and salicylic acid effectively reduced the yellow rust in plants [71].

9.2 Zinc oxide nanoparticles

Zinc is a vital micronutrient in the plant cells for the synthesis of tryptophan, which is the precursor of indoleacetic acid, a phytohormone responsible for physiological and biochemical functions [52,53,55]. The effect of zinc oxide nanoparticles (ZnO NPs) on the plants depends on their size, concentration, and the plant species. Recently, the foliar application of ZnO NPs (10 mg/L) led to a higher biomass and photosynthetic rate in the crops. ZnO NPs slightly increased the dry and fresh weight of biomass at a lower concentration. It has been stated that the high concentration of ZnO NPs inhibited root growth. Furthermore, it is reported to have a significant role in the inhibition of chlorophyll biosynthesis, leading to the reduction in photosynthesis efficiency [96].

Dimkpa et al. [84] demonstrated that soil amended with ZnO-NPs mitigated the negative influences of drought stress (40% of field moisture capacity) in sorghum plants [84]. Canola (*Brassica napus*) showed improvement in plant growth with ZnO NPs at 10 mg/L, while a higher concentration (1000 mg/L) resulted in toxic effects [85]. Rahmani et al. [85] reported that on application of ZnO NPs, the seed germination enhanced at lower concentrations, while at higher concentrations of ZnO NPs the germination was limited in onion (*Allium cepa* L.).

9.3 Silicon oxide nanoparticles

Employment of silicon nanoparticles (SiO_2 NPs) has been reported to improve the growth performance of plants and attenuate the adverse effects of abiotic stresses and reduces toxicity. Nano-Si at lower concentrations of 1- or 2-mM improved the germination rate of the plants under drought stress. Nanoparticles of silica influenced seed germination, root elongation, and biomass of plants. Silica NPs (10 nm) at 200 mg/kg induced the cucumber plants to alleviate water deficit and soil salinity due to the effect of high silicon and potassium in regulating transpiration and maintaining ion homeostasis.

Under severe drought conditions, SiO_2 NPs at 1 mM improved the mineral nutritional value and other quality indexes in perennial ryegrass [81]. It was reported that the lower concentration of SiO_2 NPs enhances the seed germination of tomato. Nano and bulk SiO_2 particles were nontoxic to PGPRs at very high concentrations (up to 1000 mg/L) in *Bacillus megaterium*, *Bacillus brevis*, *Pseudomonas fluorescens*, and *Azotobacter vinelandii* with various plants [83].

9.4 Iron oxide nanoparticles

Iron nanoparticles have advantageous properties for plant growth. They have inhibitory effects on the development of phytotoxicity. They increase nutrient uptake and transportation. The use of zerovalent iron with PGPR might be suggested as a feasible and environmentally friendly technique to enhance the phytoremediation of heavy metals in contaminated soils. Iron chelates and PGPR had a positive and significant effect on the growth, yield, and physiological characteristics of plants. They also increase the seedling growth. Furthermore, They have the capacity to improve yield, yield components, and oil percentage. They increased photosynthesis and decreased oxidative stress and reduced reactive oxygen species damage in plants.

Palmqvist et al. [78] reported that iron oxide nanoparticles enhanced the growth and agronomic traits by reducing ROS damage and improving the oxidative defense system in *Brassica napus* L. Yang et al. concluded that the impact of iron oxide nanoparticles on *Arabidopsis thaliana* it has inhibitory effects on development. Rahimi et al. [80] showed that iron nanoparticles increased the seedling growth traits in *Vigna unguiculata* (L.) [80].

9.5 Other nanomaterials

The macronutrients and micronutrients have a crucial role in the growth and development of plants. Calcium is a major essential plant element. Synthesized calcium nanoparticles can be exploited for the formulation of new nanogrowth promoters and nanofertilizers in agriculture. Employment of calcium in nanoformulations can potentially reduce the quantity of fertilizers that are applied to the crops. The decreased use of fertilizers can directly and indirectly aid in the reduction of pollution of the environment due to agricultural malpractices [88]. The foliar application of nanofertilizers gives rise to a significant increase in the concentration of various amino acids, increased germination and growth rate of the plant. Furthermore, it has been reported that calcium nanoparticles affect plant height, branch number per plant, pod number per plant, seed number per pod, seed weight (g), and seed yield in several plants.

The foliar spray of calcium phosphate nanoparticles (CaPNPs) in strawberry plants improved the quality and storability of fruits. Furthermore, the appearance of the berries was better and the lowest values of weight loss and zero decay percentage were reported [87]. Calcium Borate nanoparticles

(CaB_4O_7 NPs) as nanofertilizers were reported to promote shoot and root biomass production by ~twofold compared to untreated plants [91].

Manganese (Mn) is a micronutrient required for growth regulation of the plants. It plays a vital role in photosynthesis, enhances the activity of the electron transport chain in photosynthesis, and reduces oxidative stress [97,98]. Manganese nanoparticles (MnNPs) can be employed as a manganese micronutrient fertilizer or plant growth enhancer. Manganese nanoparticles were biocompatible toward soil microorganisms. MnNP can be employed as a suitable alternative for salts employed in agriculture for the supplementation of manganese in soil and crop management. MnNPs are considered to be an essential constituent of the catalytic center which is responsible for water oxidation at photosystem (PS II) [99]. MnNPs transport electrons to the thylakoid bound electron transport chain (ETC), which produces reducing power and ATP for carbon dioxide assimilation [100].

10 Conclusions

The present chapter indicates the benefits of PGPR, such as biofertilization, biocontrol, and bioremediation, that have a favorable impact on crop productivity. The employment of PGPR (*Bacillus*, *Pseudomonas*, *Azospirillum*, etc.) has significantly improved physicochemical parameters in economically chief crops such as rice, maize, tomatoes, wheat, sugarcane, etc. The formulation prepared of PGPR is a promising alternative to chemical fertilizers which can be employed in sustainable agriculture. The advent of nanotechnology and its inclusion in the agricultural sector has a potential to improve the current biofertilizers and has captivated the interest of various researchers. The interaction of nanomaterials with PGPR can promote and enhance the performance of rhizobacteria and thus has immense potential to be exploited as an environmently friendly fertilizer for the crops. The amalgamation of nanotechnology and PGPR can be employed as a budget- and eco-friendly sustainable alternative to chemical fertilizers for the growth and development of plants.

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Dear Author,

Congratulations on the acceptance of article for publication in **Journal of Polymer and Composites.** This letter serves as our formal act **"Stimulation of Plant Growth Promoting Rhizobacteria with ZnO Nanoparticles to improve growth and development of Groundnut plants (Arachis hypogaea L.)"** acceptance of your paper. We affirm that your paper has met the Journal's Peer-Reviewed publication criteria.

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Corresponding Author Name: Dr. Ragini Raghav

Co-Authors: Ms. Gunja Vasant, Mr. Jahal Dangar and Dr. Shweta Bhatt

Manuscript Title: Stimulation of Plant Growth Promoting Rhizobacteria with ZnO Nanoparticles to improve growth and development of Groundnut plants (Arachis hypogaea L.)

Article received date: 2 May 2024

Article accepted date: 16 July 2024

Indexing: Chemical Abstracts Service (CAS), Cite Factor, Genomics, Google Scholar, Index Copernicus, Journal TOCs, Scientific Literature (SCILIT), Web of Sciences

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Manuscript Title: Seed priming with zinc oxide nanoparticles and plant growth-promoting rhizobacteria to enhance the seedling development of Fenugreek

Article received date: 02 May 2024

Article accepted date: 09 September 2024

Indexing: Chemical Abstracts Service (CAS), Cite Factor, Genomics, Google Scholar, Index Copernicus, Journal TOCs, Scientific Literature (SCILIT), Web of Sciences

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