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Dear Authors,

We have reached a decision regarding your submission **MS no. PST 3223 / Kaneriya et al. / Amelioration of growth of maize (Zea mays L.) seedling using plant growth promoting bacteria.: Improvement of growth of maize using PGPB**

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Regards

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RESEARCH ARTICLE

Amelioration of growth of maize (*Zea mays* L.) seedling using plant growth promoting bacteria

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Abstract

This research was aimed to screen plant growth promoting bacteria (PGPB) from soil and study its effect on maize plant growth. PGPB were isolated from Saurashtra coastal region soil and cultured in Nitrogen fixing Ashby's medium to find potent of PGPBs, we conducted a thorough screening process, assessed their abilities in phosphate and zinc solubilization, siderophore production, hydrogen cyanide (HCN) release and the antifungal activity was performed against *Fusarium oxysporum*, a pathogenic fungus. These tests helped us identify bacteria with plant growth-promoting characteristics for plants. Bacterial isolates which provided better results were sequenced and sequences were submitted to NCBI. Bacterial isolates selected for application on maize in primary screening showed most treated seeds increased the seedling vigor of maize. In the latter stages of screening where bacterial consortia were developed from primary and secondary screening. In 30 days, the experiment in maize plant height, number of leaves, chlorophyll content and anatomy was analysed. All the bacterium consortiums displayed an increase in height (24.75%), number of leaves (47.77%) and total chlorophyll content (23.59%) as compared to the control maize plant. Additionally, microscopic examination of the treated plants showed improved growth, especially in the increased starch grain content in the leaves, stems and roots. Out of the eleven PGPB consortia, 3 specific PGPB consortia in this study have significantly substantiated the growth of maize plants as evidenced by the comprehensive analysis of anatomical features.

Keywords

PGPBs; phosphate solubilization; siderophore production; antifungal maize.

Introduction

In recent years, there has been a strong emphasis on taking advantage of the benefits of beneficial interactions between plants and bacteria to promote sustainable farming methods. Plant growth-promoting bacteria (PGPB) are a varied group of microorganisms that play an important role in plant growth and development. These bacteria provide major contributions by enhancing phosphate solubilization, hormone synthesis and nitrogen fixation, all of which have a direct impact on plant metabolism. Furthermore, PGPB actively promotes enhanced plant water and nutrient uptake, boosting strong root development and increasing enzymatic activity within

the plant system nutrient availability, inducing hormone production and providing resistance to diseases and environmental challenges in their host plants (1). PGPR (Plant Growth-Promoting Rhizobacteria) exhibit distinct interactions with plants, categorized into symbiotic bacteria, residing within plants and engaging in metabolite exchange and free-living rhizobacteria, which inhabit the external environment of plant cells the operational mechanisms of PGPB include direct and indirect pathways. Direct mechanisms include strategies such as biofertilization, root growth stimulation, rhizoremediation and plant stress management. The indirect process, on the other hand, involves the biological regulation imposed by rhizobacteria to promote plant growth. This is accomplished by behaviours such as antibiosis, inducing systemic resistance inside the plant and competing for both nutrients and ecological niches. Bacterial strains that have been widely studied for their roles in these processes include *Rhizobium* spp., *Azotobacter* spp., *Pseudomonas* spp., *Bacillus* spp. and *Paenibacillus* spp. have been reported for their PGP potentials (2). PGPB isolates have emerged as a promising approach to developing sustainable agriculture practices. The utilization of plant PGPB, plant growth-promoting microbes and mycorrhizal fungi enhance plant growth by generating siderophores, antioxidants, stress-resistant responses and notably, facilitating nutrient absorption from the soil (3, 4). Chemical fertilizers and pesticides should be avoided in sustainable agriculture because they impair the health of plants as well as all other living organisms such as animals and humans (5). Maize as one of the most vital cereal crops is esteemed for its rich content of macronutrients, including starch, fibre, protein and fat. Additionally, it provides essential micronutrients like the B-complex vitamins and β -carotene as well as critical minerals such as magnesium, zinc, phosphorus and copper (6). The main objective of this research was to determine how PGPB strains could effect seed germination, growth parameters of maize and yield.

Materials and Methods

Soil Sampling

Soil sampling was conducted within the Saurashtra Coastal Region of Gujarat, India, whereby samples were systematically collected. Each collected sample was assigned an accession number for accurate labelling and subsequent processing in the laboratory (Table 1).

Table 1. Details of the Collected soil samples.

Sr.	Bacterial strain no.	Latitude	Longitude	Sr.	Bacterial strain no.	Latitude	Longitude
1	SCS01	20° 43' 21.32" N	70° 55' 45.84" E	9	SCS09	22° 30' 35.5" N	070° 01' 48.5" E
2	SCS02	20° 43' 04.26" N	70° 53' 58.19" E	10	SCS10	22° 30' 36.2" N	070° 01' 52.4" E
3	SCS03	20° 45' 04.52" N	70° 54' 02.31" E	11	SCS11	22° 25' 37.4" N	069° 43' 32.4" E
4	SCS04	20° 45' 30.85" N	70° 56' 38.91" E	12	SCS12	22° 24' 29.8" N	069° 43' 16.4" E
5	SCS05	22° 13' 57.3" N	68° 58' 56.5" E	13	SCS13	22° 36' 08.7" N	070° 12' 02.8" E
6	SCS06	22° 13' 36.9" N	68° 59' 21.9" E	14	SCS14	22° 33' 04.4" N	070° 12' 08.5" E
7	SCS07	22° 13' 33.5" N	68° 59' 31.2" E	15	SCS15	22° 35' 08.7" N	070° 12' 03.8" E
8	SCS08	22° 09' 10.6" N	69° 03' 26.2" E				

Isolation and screening of bacteria

Isolation was carried out using Ashby's agar plate was used for the isolation of the bacteria, which were plated in triplicate and incubated at 30 ± 1 °C for 48 h. Ashby's agar plates were used to sub-culture and purify the bacteria colonies. Following the incubation period, the colonies of bacteria were sub-cultured and purified using Ashby's agar plates.

Screening of Phosphate solubilizing bacteria

Pikovskaya's agar plates incorporated with tri-calcium phosphate, were inoculated with the isolates to assess their ability for phosphate solubilization within an incubation period of 5 days at a controlled temperature of 30 ± 1 °C (7).

Screening of Indole acetic acid-producing bacteria

The quantification of Indole-3-acetic acid produced by each isolate was conducted employing a colorimetric method involving Salkowski reagent (8). The use of the Salkowski reagent allowed for the measurement and quantification of IAA produced by the respective isolates. Every IAA determination experiment was performed in triplicate.

Screening of Siderophore-producing bacteria

Screening for siderophore-producing bacteria was carried out by using the standard technique (9).

Screening of Hydrogen cyanide (HCN) producing bacteria

Bacteria were cultivated on an LB agar medium supplemented with glycerine. A method was employed to assess hydrogen cyanide (HCN) production: Whatman filter paper No. 1, saturated in a solution of 1% picric acid and moistened with 10% sodium bicarbonate, was affixed to the inner surface of the petri plate lid. The Petri plate was sealed with a paraffin film and then incubated at a controlled temperature of 30 ± 1 °C for a duration ranging from 24 to 48 h. The presence of a colour change from light brown to dark brown within this timeframe indicated elevated levels of hydrogen cyanide production by the bacteria (10).

Screening of Zinc solubilizing bacteria

Screening of bacterial isolates to assess their capacity for zinc solubilization by 2 insoluble zinc compounds, zinc oxide (ZnO) and zinc carbonate ($ZnCO_3$), were utilized in the experiment. Cultures of bacteria aged one day were

spot-inoculated onto an LB medium with these insoluble zinc compounds. The inoculated plates were subsequently incubated at a constant temperature of 30 ± 1 °C for a duration of 14 days. Notably, bacterial strains demonstrating the ability to solubilize zinc showcased visible clear zones surrounding their colonies on the culture medium containing insoluble zinc compounds. This phenomenon served as an indicator of their capability to solubilize zinc from these specific compounds (11).

Antagonistic activity of bacterial isolates against fungi (*Fusarium oxysporum*)

Antagonistic effects of bacterial isolates against the phytopathogenic fungus *F. oxysporum* (NCIM Accession no. 1008) were examined using Potato Dextrose Agar plate use to evaluate the antagonistic activity of bacterial isolates against the phytopathogenic fungus *in vitro*. On the PDA plates, fungal agar discs were positioned 3 cm apart from the sites of bacterial growth. Additionally, a negative control was established, comprising fungal agar discs devoid of any bacterial culture spots. Subsequently, the Petri plates were incubated for a duration of seven days at a temperature of 30 ± 1 °C. Daily observations were made over the following eight days to monitor and assess the inhibition of fungal growth, thereby evaluating the inhibitory effects of the bacterial isolates on the phytopathogenic fungus (12).

1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production by bacteria isolates

Bacterial isolates were inoculated on the minimal salts agar medium, enriched with 3 mM ACC (nitrogen source). The minimal salt agar plates were placed in an incubator set at a temperature of 30 ± 2 °C and allowed to incubate for a period of 72 h. Colonies growing on the plates are taken as ACC deaminase producers (13).

Molecular Characterization

Isolation of DNA and 16S rDNA gene amplification

The targeted gene amplification was conducted using the universal bacterial primers 1492 R (5'-GGTTACCTTGTTAC-GACTT-3') and 27 F (5'-AGAGTTTGATCCTGGCTCAG-3'). Subsequently, sequencing of the amplified gene fragments was performed utilizing a 24-capillary electrophoresis machine, specifically the 3500 Genetic Analyzer manufactured by Applied Biosystems, at the facility of GBRC. For the identification of bacterial species based on the obtained sequences, EzBioCloud's identification service was utilized. This service employs similarity-based searches against quality-controlled databases specifically curated for 16S rRNA sequences. This approach assists in determining the closest matches or similarities of the sequenced gene fragments to known bacterial species within the database, aiding in the accurate identification and classification of the bacterial strains under investigation (14). The phylogenetic tree was constructed, MEGA software version 11 (15). The sequence variations the analysed based on 16S rDNA sequences.

Preparation of inoculum and seed for pot trial

Bacterial strains selected based on their *in vitro* activity were cultured in LB broth for 24 h at 30 ± 1 °C to generate

bacterial inoculum. Maize seeds of the Kaveri-200 variety were chosen for the trials. The surface sterilization process of maize seeds involved a 2 min treatment using a 0.02% sodium hypochlorite solution. Following sterilization, the seeds underwent thorough washing with sterile distilled water to eliminate any residual sodium hypochlorite. Surface-sterilized seeds were immersed in suspensions of bacterial isolates with a concentration of 1×10^8 CFU/ml for a duration of 30 min. For the primary screening phase, each of the 3 treatments involved a triplicate of inoculated seeds placed in different conditions: sterile soil, 1% water agar, and non-sterile soil. The pots containing these seeds were then incubated at a temperature of 30 °C for 4 days in the absence of light to promote germination.

Pot experiments were conducted between April 2021 and December 2022 and involved specific bacterial isolates for 8 days, 15 days and 30-day experiments. The study involved systematically computing various combinations derived from these isolates. Among the 7 bacterial isolates based on Eq.1. Bacterial strain combination is shown in (Table 2) Among these 11 bacterial consortia, finally 6 bacterial consortia were selected on the basis of maize plant to measure plant height, number of leaves and chlorophyll content in the pot experiment (16).

Number of combinations without repetition =

$${}^n C_r = \frac{ni}{(n-r)_i r_i} \dots \dots \dots (\text{Eqn. 1})$$

where ${}^n C_r$ = number of combinations, n = number of objects, r = sample size, ni = n factorial, ri = r factorial

Table 2. Bacterial Individual strains and its consortia composition.

Strains and its consortia	Combination of Bacterial strain	Notations
SCS03C1	S1	
SCS07C3	S2	
SCS12C2	S3	
SCS12C5	S4	
SCS03C1+ SCS07C3	S1 + S2=C1	Consortia C1
SCS03C1 + SCS12C2	S1+ S3=C2	Consortia C2
SCS03C1 + SCS12C5	S1 + S4=C3	Consortia C3
SCS07C3 + SCS12C2	S2 + S3=C4	Consortia C4
SCS07C3 + SCS12C5	S2 +S4=C5	Consortia C5
SCS12C2 + SCS12C5	S3 + S4=C6	Consortia C6
SCS03C1 + SCS07C3 + SCS12C2	S1+S2+S3=C7	Consortia C7
SCS03C1 + SCS07C3 + SCS12C5	S1+S2+S4=C8	Consortia C8
SCS07C3 + SCS12C2 + SCS12C5	S2 +S3 +S4=C9	Consortia C9
SCS03C1 + SCS12C2 + SCS12C5	S1 +S3+S4=C10	Consortia C10
SCS03C1 + SCS07C3 + SCS12C2 + SCS12C5	S1+S2+S3+S4=C11	Consortia C11

Microscopic analysis of plants

Control and treated maize plants parts stem, leaf and root were collected, and fixed in formalin-acetic acid-alcohol, Dehydration was performed into glass vials, with a series

of ethanol and samples were embedded in paraffin. Transverse serial sections the histological sections were contrasted, were cut with a rotary microtome measuring 12 µm to 14 µm thick to visualize the tissue structures, histological sections underwent staining procedures utilizing 1% toluidine blue dissolved in 1% aqueous borax solution as well as safranin O/fast green. These stained sections were examined and captured under varying magnifications utilizing a DME research microscope manufactured by Leica, Germany.

Statistical analysis

The primary and secondary screening results were statistically analysed using two-way ANOVA and Bonferroni post-tests. In addition, Dunnett's Multiple Comparison Test and a one-way ANOVA were used at the final screening stage to assess the efficacy of treated and control plants. The results are shown as means with standard error of the mean (SEM). The statistical analysis and graph plotting were carried out using GraphPad Prism 9 software, which enabled a thorough display and understanding of the experimental results.

Results and discussion

The current investigation focused on the isolation and screening of (PGPB) from the coastal region of Saurashtra. A total of 7 bacterial strains were screened and characterized based on their plant growth-promoting activities, as outlined in (Table 3). Out of 7 Bacterial strains, all bacterial strains are capable of PS (phosphate solubilization), IAA (indole acetic acid) and Siderophore production. Out of 7 bacterial isolates 4 bacterial strains SCS03C1, SCS07C3, SCS12C1 and SCS12C2, exhibited the production

of hydrogen cyanide (HCN), except for strains SCS12C3 and SCS06C1. The bacterial strains were testing for the antifungal activity against *F. oxysporum*. Among these 7 bacterial strains 4 bacterial strain SCS03C1, SCS07C3, SCS12C2 and SCS12C5 demonstrated antifungal activity were able to inhibit the growth of *F. oxysporum* species. Additionally, the solubilization of zinc was facilitated by all bacterial strain except SCS06C1. 1-aminocyclopropane-1-carboxylate, a precursor of ethylene produced by SCS03C1, SCS07C3, SCS12C2, SCS12C3 and SCS12C5 bacterial strain.

The investigation meticulously outlined the distinctive traits and functionalities of the bacterial strains isolated from the Saurashtra coastal region. This comprehensive analysis provided insights into their diverse capacities and constraints concerning plant growth promotion. Based on the plant growth-promoting activity (Table 3), a total of 7 bacterial strains were screened and characterized for Phylogenetic analysis of 16S rRNA-based identification of bacterial isolates through EzTaxon server is given in (Table 4). The molecular characterization of isolated bacteria aids in the comprehension of phenotypes and heterogeneity. Phylogenetic analysis revealed the relationship between the isolates (Fig. 1). A thorough investigation of each bacteria the initial screening for bacteria that support plant growth, A variety of bacterial isolates, including SCS03C1, SCS07C3, SCS06C1, SCS12C1, SCS12C2, SCS12C3 and SCS12C5, were used to treat maize seeds. Three distinct growth media water agar, sterile soil and soil were used to promote growth. The seed vigor index was calculated. A formula was used to calculate the seed vigor index, which was determined as the seed length and germination %.

Table 3. Characterization of bacterial isolates for plant growth-promoting activities.

PGP Trait	Bacterial strains						
	<i>Acinetobacter pittii</i> SCS03C1	<i>Pseudomonas extremorientalis</i> SCS07C3	<i>Bacillus licheniformis</i> SCS12C1	<i>Bacillus haynesii</i> SCS12C2	<i>Bacillus vallismortis</i> SCS12C3	<i>Priestia aryabhatai</i> SCS12C5	<i>Bacillus pumilus</i> SCS06C1
PS	+	+	+	+	+	+	+
IAA	+	+	+	+	+	+	+
Siderophore	+	+	+	+	+	+	+
HCN	+	+	+	+	-	+	-
Anti-fungal Activity	+	+	-	+	-	+	-
Zinc Solubilization	+	+	+	+	+	+	-
ACC Deaminase	+	+	-	+	+	+	-

+ indicate positive activity, - indicate no activity. **HCN**= Hydrogen cyanide production. **IAA**=Indole Acetic Acid production. **PS**= Phosphate solubilization.

Table 4. 16S rRNA-based identification of bacterial isolates through EzTaxon server.

Genebank	Accession No.	Bacterial Strain	Top-hit taxon	Top-hit strain	Similarity (%)
ON344839.1		<i>Pseudomonas extremorientalis</i> (SCS07C3)	<i>Pseudomonas extremorientalis</i>	KMM	99.9
ON146311.1		<i>Priestia aryabhatai</i> (SCS12C5)	<i>Priestia aryabhatai</i>	B8W22	100
ON556603.1		<i>Bacillus vallismortis</i> (SCS12C3)	<i>Bacillus nakamura</i>	NRRL B-41091	99.19
ON545808.1		<i>Bacillus pumilus</i> (SCS06C1)	<i>Bacillus pumilus</i>	ATCC 7061	99.72
ON533626.1		<i>Bacillus licheniformis</i> (SCS12C1)	<i>Bacillus licheniformis</i>	ATCC 14580	99.89
ON514165.1		<i>Bacillus haynesii</i> (SCS12C2)	<i>Bacillus licheniformis</i>	ATCC 14580	99.79
ON350852.1		<i>Acinetobacter pittii</i> (SCS03C1)	<i>Acinetobacter pittii</i>	CIP 70.29	100

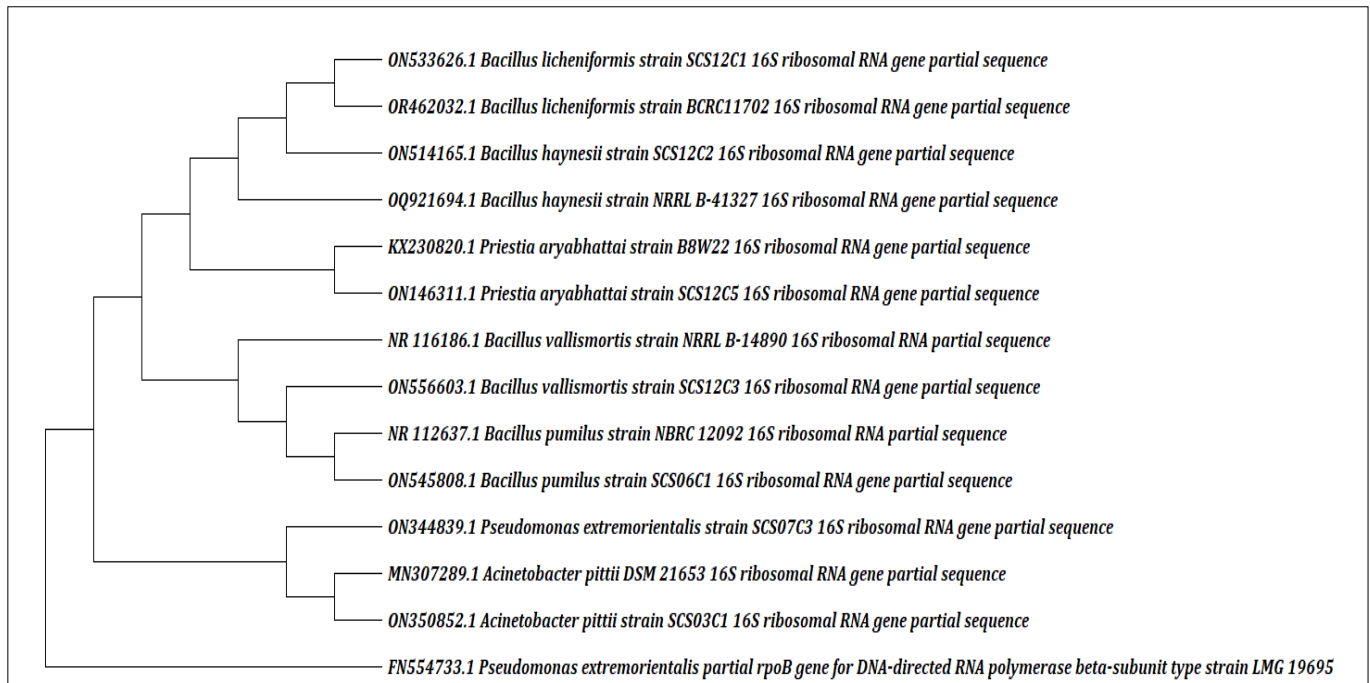


Fig. 1. The evolutionary history was inferred using the Neighbour-Joining method (38). The optimal tree is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method (39) and are in the units of the number of base substitutions per site. This analysis involved 14 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1599 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

Effect of Plant growth promoting bacteria isolates on Maize

Primary screening

Primary Screening in this study, after 8 days to evaluate the treatments results. Four bacterial isolates out of the 7 studied displayed positive plant growth-promoting activity, according to the primary screening results. Particularly, as compared to the control, SCS07C3, which showed as s (24.56%), SCS03C1 showed a significant (23.10%), SCS12C1 a (4.85%), SCS12C2 a significant improvement with a 22.11% increase in seed vigor. Additionally, SCS12C5 and SCS06C1 both demonstrated significant increase in seed vigor index as compared to the control (19.77%) and (14.74%) respectively.

These outcomes clearly validate the treatment's efficacy in boosting seed vigor. However, it's important to note that SCS12C3 showed decrease in seed vigor index by 3.30% compared to the control. This negative alteration implies that the treatment might not have been

appropriate or advantageous for this particular maize variety. This finding gives the way for further investigation into these promising strains and their potential applications in secondary screening experiments.

Secondary screening

In this study Seed growth experiments were carried out using water agar, sterile soil and regular soil, with the seed vigor index calculated after a 15-day period (Fig. 2). In this phase of the study, seed vigor served as the primary criterion, with values of seed vigour index exceeding 700 being the threshold for selection in the secondary screening. Among the bacterial isolates that were investigated, the results were SCS03C1 exhibited (34.88%), SCS07C3 showed (45.86%), SCS12C2 showed (40.18%) and SCS12C5 exhibited a (38.37%) increase in the seed vigor index compared to the control. These findings further underscore the effectiveness of the treatment in enhancing seed vigor.

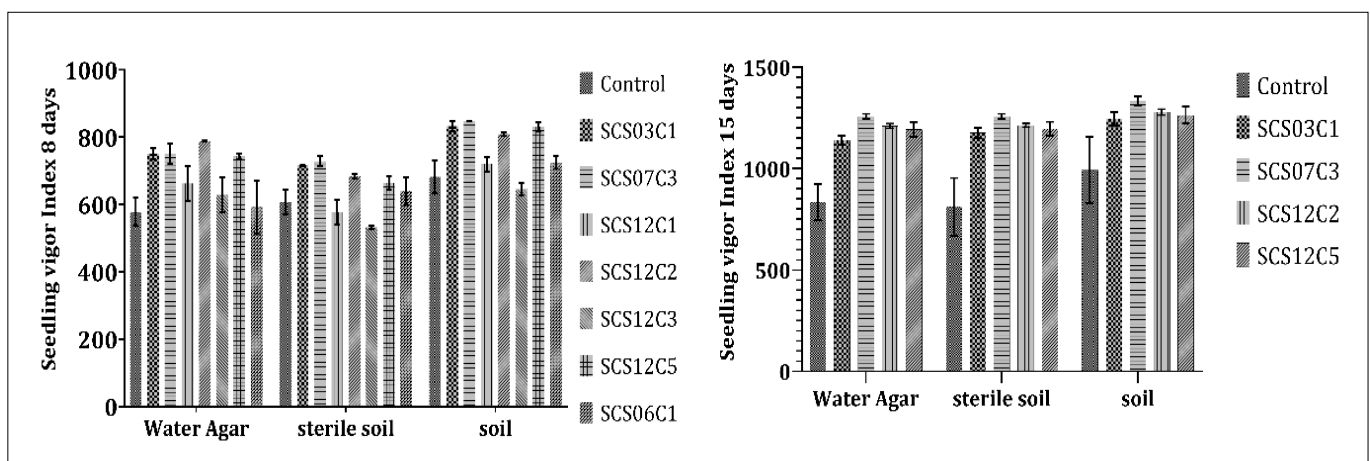


Fig. 2. Shows the Seedling vigor of the 8-day and 15-day germination periods, Bars represents mean values and standard errors and marked with * indicate statistically significant differences using Bonferroni post-tests (*- $p < 0.05$; **- $p < 0.01$; ***- $p < 0.001$; ****- $p < 0.0001$). $n = 3$.

Tertiary screening

In this study, bacterial consortia used (Fig. 3) for seed germination and growth for 8 days experiment. The highest percentage of increase was observed in consortium 5 (48.61%). The other consortia also displayed positive changes in length, ranging from (19.95%) to (39.00%). The treatments seem to have had a beneficial impact on the growth of these maize varieties, with consortium 5 and consortium 6 showing the most significant improvements.

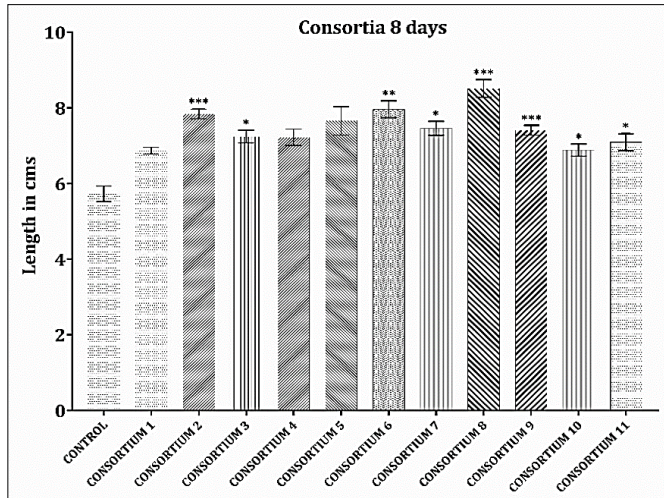


Fig. 3. Shows the Seedling vigor of the 8-days germination periods. Bars represents mean values and standard errors and marked with * indicate statistically significant differences using Bonferroni post-tests (*- $p < 0.05$; **- $p < 0.01$; ***- $p < 0.001$; ****- $p < 0.0001$). $n = 12$.

Quaternary screening

In this study, length was taken into account as a criterion, and average length values above 7.3 cm were chosen of maize plant from tertiary screening. All the bacterial consortia displayed an increase in the height of the maize after 15 days of treatment (Fig. 4). The highest % of increase in height (69.41%) and number of leaves (34.16%) was observed in consortium 5. The other consortia also displayed positive changes in length of the maize. The treatments seem to have had a beneficial impact on the

growth of these maize varieties, with consortium 5, consortium 6 and consortium 9 showing the most significant improvements.

Quinary screening

In this study, height and number of leaves were taken into account as a criterion, from quaternary screening and consortia were selected were chosen for Quinary screening. In this screening height of the plant, number of leaves, chlorophyll content of leaves and anatomy of plant were analysed after 30 days (Fig. 5). No of selected bacterial consortia displayed an increase in the height, no of leaves and chlorophyll content of the maize as compared with the control after 30 days of treatment. Consortium 9 displayed highest percentage increase in height (38.90%), number of leaves (60.18%) and total chlorophyll content (32.52%) as compared to control maize plant.

Anatomical features of maize leaf, stem and root

Anatomical analysis of the maize plant was done of the control and consortium treated maize plants 30-day period. When plants were treated with consortia it was observed that the veins in the leaf get bigger, more starch accumulates and the bundles that carry nutrients grow larger (Fig. 6). The substantial starch accumulation serves as a clear indicator of the plant's remarkable proficiency in harnessing carbon resources and producing photo assimilates, which significantly contribute to optimizing plant architecture (17). This vital process holds immense significance for the plant's overall well-being and survival, as evidenced by the current findings in the studied plant species (18). When the control maize plant was compared with the consortia-9 treated maize, differences in their anatomy, particularly in the quantity of sclerenchyma tissue surrounding the vascular bundles. Sclerenchyma tissue offered additional structural support to the stem (Fig. 7).

A plant's root comprised 3 main components the outer layer, referred to as the epidermis, the middle layer,

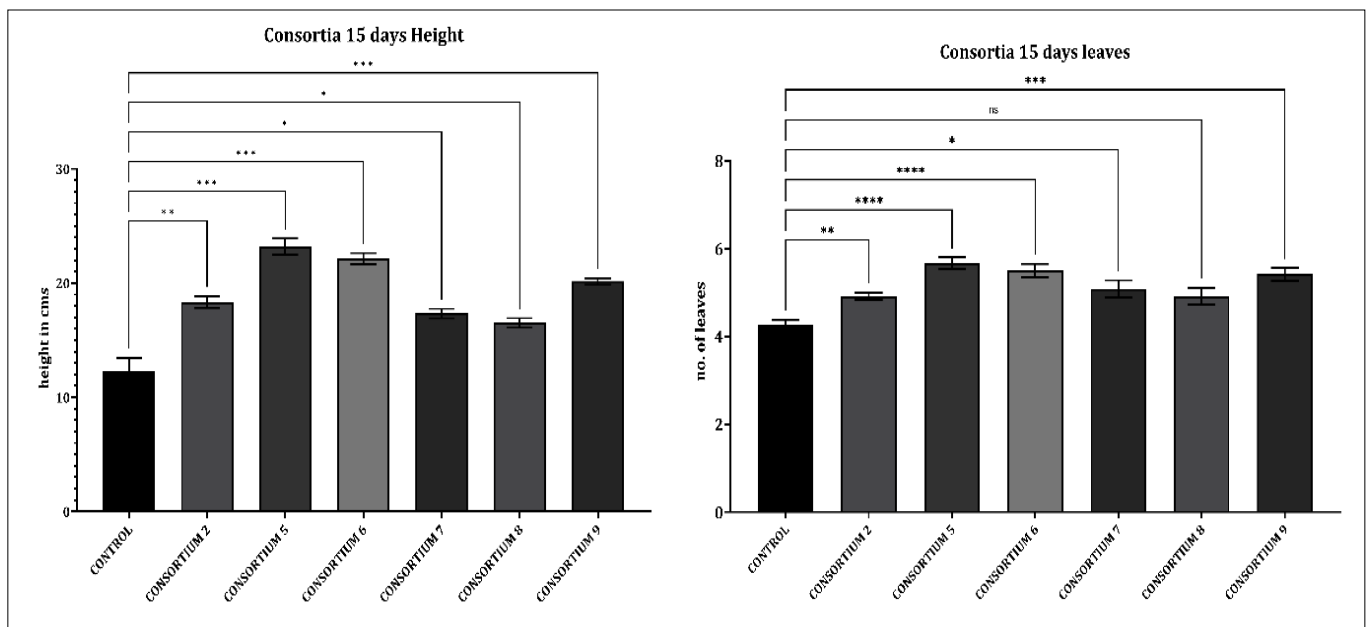


Fig. 4. Height of maize plants and the Number of leaves on maize plants after a 15-day period. The samples were run in triplicate, averaged, and the error bars represent standard error. Bonferroni post-tests (*- $p < 0.05$; **- $p < 0.01$; ***- $p < 0.001$; ****- $p < 0.0001$). $n = 12$.

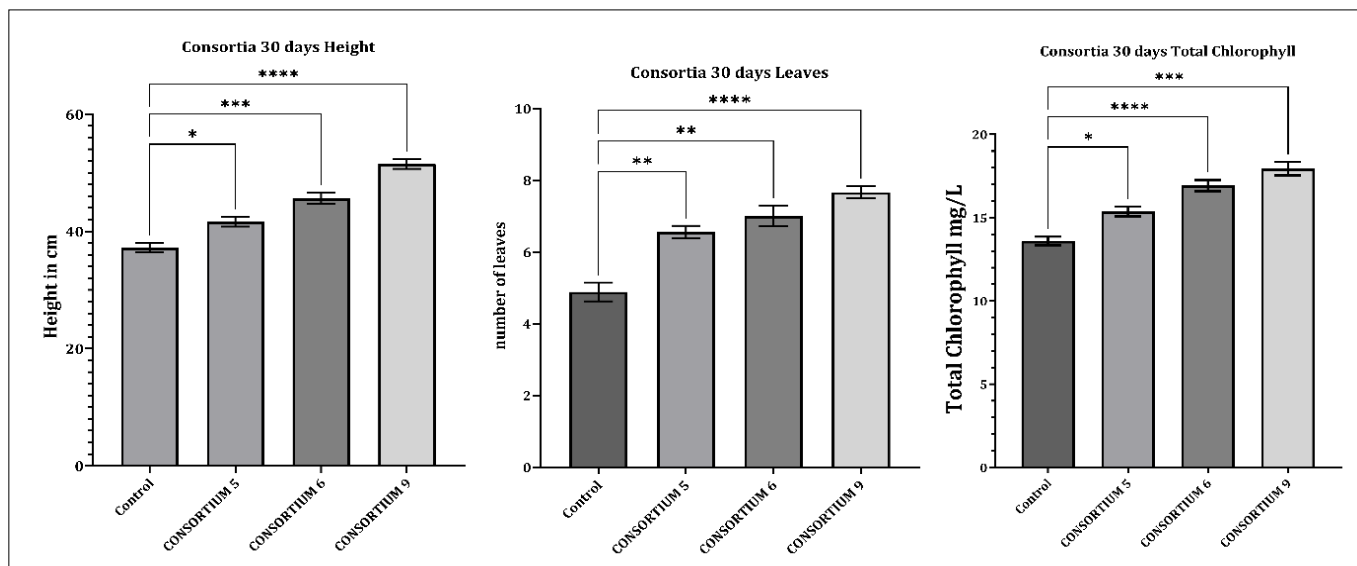


Fig. 5. Height of maize plants, the Number of leaves on maize plants, and the Total chlorophyll content in maize plants after a 30-day growth period. Bars represents mean values and standard errors and marked with * indicate statistically significant differences using Bonferroni post-tests (*- $p<0.05$;**- $p<0.01$;***- $p<0.001$;****- $p<0.0001$). $n=9$.

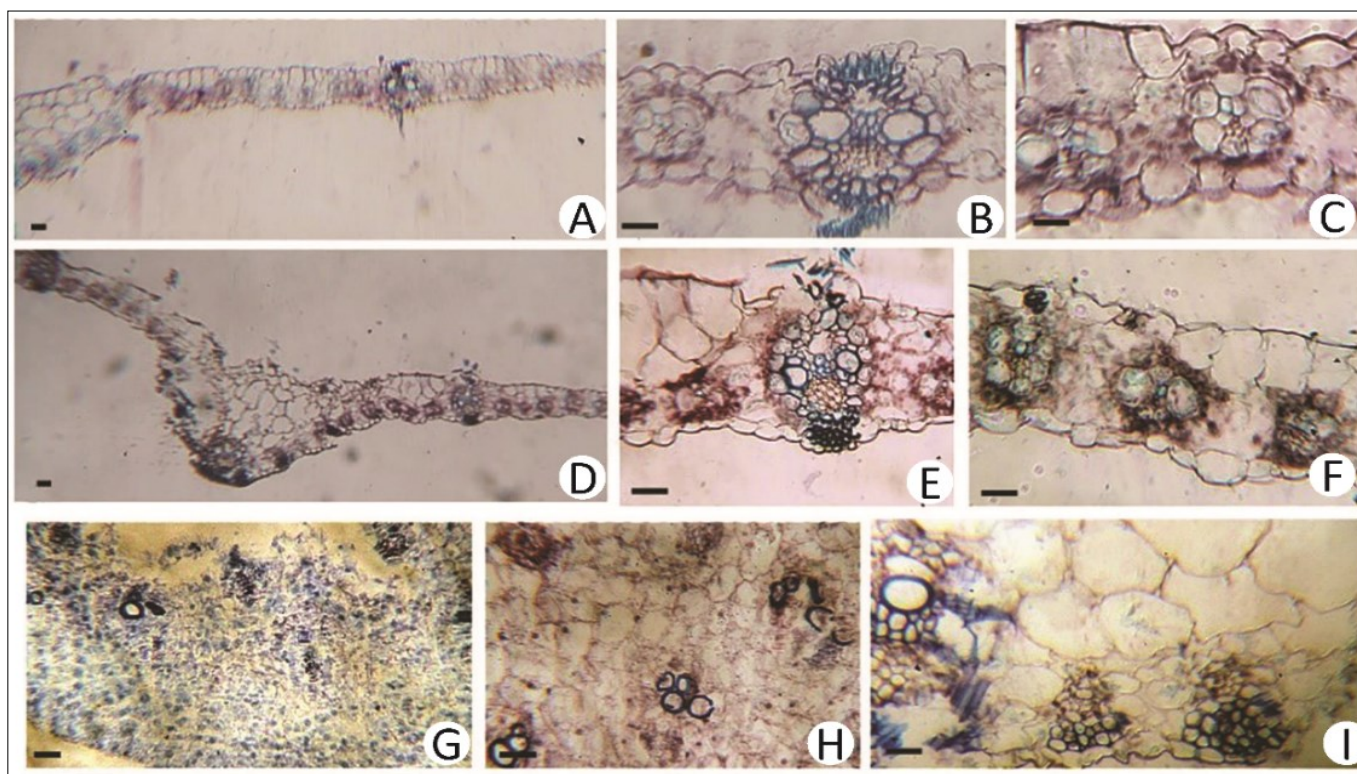


Fig. 6. Anatomical Characteristics feature of maize leaf and stem. **A-C:** Transverse section of control leaf plant. **D-F:** Transverse section of treated (Consortium-9Treated) Plant leaf. **D,E:** Shows 1 Vascular bundle in lamina region. **C,F:** Shows 2^oVascular bundle in lamina region. (Bar size Fig. **A & D,** = 125 μm . **B, C, E, F, H, I,** =12.5 μm).

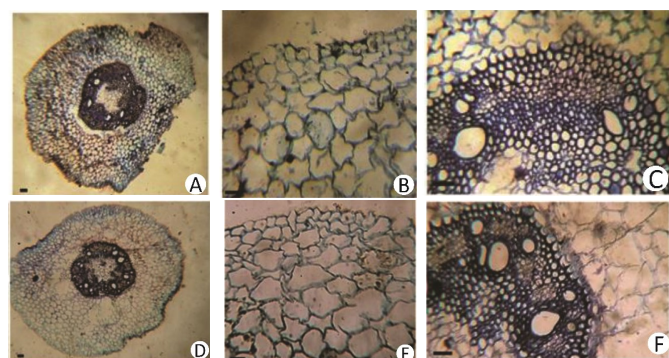


Fig. 7. Anatomical Characteristics features of maize root. **A, B, C:** Transverse section of controlled plant root. Plant root. **D,E,F:** Transverse section of consortium-9 treated Plant root. **A,D:** show different layers of shoot. **B,E:** Shows Epidermal and cortical region. **C,F:** Shows Vascular region. (**A, D** = 125 μm . **B,C,E,F**=12.5 μm).

cortex; and the inner vascular region (Fig. 7). When consortia-9 treated maize plant, it was observed that it retained similar anatomical features compared to the untreated plant. However, there was an increase in the number of metaxylem and protoxylem cells (19). This increase in these cells enhanced the root's capacity to transport water, resulting in improved hydraulic conductivity. Xylem vessels play an essential role in facilitating the predominant axial movement of water and essential nutrients across the root system (20).

The important screening criteria for the selection of plant growth-promoting bacteria reported positive impact on in several plant. *Acinetobacter pittii* (SCS03C1) was

reported to have been isolated from the maize rhizosphere and for growth promotion in paddy plants (21, 22). In present study it was found that *A. pittii* was test positive in all *in vitro* testing. Not only *in vitro*, but this bacterium also showed positive results in all plant trials. The bacterial isolate was a part of a consortia among the selected. *B. pumilus* (SCS06C1) is the well-studied PGPB. Several studies support its capability and it was reported that *B. pumilus* has an excellent PGP property (23). This bacterium has the capability of high production of gibberellins, a plant growth hormone responsible for overall plant growth. *B. pumilus* was also reported for the production of chitinase and antifungal activity (24, 25).

P. extremorientalis (SCS07C3) has been well described as PGPB. Its PGP activity has been studied in a variety of plants, including, common bean, cucumber, Jute mallow and many more (26, 27). In this study, its excellent plant growth promoting activity was noticed. Out of 3 final selected consortiums, *P. extremorientalis* is a member of 2, which showed its high plant growth promoting properties against maize plants. *B. licheniformis* (SCS12C1) as a plant growth promoter of plants (28, 29). *B. haynesii* (SCS12C2) PGP activity against rice was recently reported from holy basil plants (30). *B. vallismortis* (SCS12C3) PGP characteristics (IAA production and phosphate solubilisation) were reported (31). The ability of *B. vallismortis* to enhance the growth of maize was examined in our research. Findings suggest *B. vallismortis* has poor outcomes as compared to other isolates. *P. aryabhatai* (SCS12C5) is well studied for its plant growth against a variety of plants and crops, including wheat, soyabean and tomato (32, 33). In this investigation, it was discovered that *P. aryabhatai* is an excellent growth promoter for maize plants. It's a member of concluding consortiums 9.

Consortium 5, Consortium 6 and Consortium 9 displayed better results each in the case of height, number of leaves and Chlorophyll of the plant. These 3 consortiums clearly showed the improvement in growth of the maize plant as indicated by use of study anatomical features. These consortiums hold a promising potential in the improvement in the growth of the maize in field. It was reported that the growth parameters, including leaf, root and stem lengths, plant height and leaf count, demonstrated a significant increase in microbial consortia treatments compared to single-inoculant treatments (34). There was a report, significantly improved plant activities by adding of bacteria such as *Azospirillum* and *Azotobacter* to the soil (35). PGPB helped significantly by elevation of chlorophyll content with an increase ranging from 30% to 45% in leaves (36). Simultaneous application of bacterial consortia resulted in a substantial 90% increase in germination %, protein content in maize plants as compared to control plant was reported as earlier (37).

Conclusion

This study emphasizes the crucial role of plant growth-promoting bacteria (PGPB) in improving maize growth. Based on our studies, the use of PGPB is positively

correlated with enhanced plant height, nutrient uptake and improvement in anatomical features. A potential approach toward sustainable agriculture that lessens dependency on chemical inputs and lessens environmental effects is to use PGPB as biofertilizers. The production of maize crops can be improved by selective microbial treatments made possible by the identification of particular PGPB strains with superior features. To understand molecular pathways and investigate potential synergies with sustainable agriculture methods, more research is required. In the changing global context, using PGPB-based farming practices holds great promise for attaining environmental sustainability and food security.

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Authors contributions

All the authors shared the equal contribution. All authors read and approved the final manuscript.

Compliance with ethical standards

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