Evaluation of Plant Growth Promoting Traits of Silicate Solubilizing *Bacillus Tequilensis* SKSSB09 in *Zea mays* L.

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ABSTRACT

Background: *Zea mays* L., the world's third-largest crop, faces productivity challenges due to abiotic and biotic stresses. With synthetic fertilizers posing adverse effects, microbial inoculants offer a sustainable alternative. This study examines the role of silicate-solubilizing bacteria (SSB) in improving soil fertility and plant nutrition, focusing on its synergy with NPK microbial inoculants and synthetic fertilizers.

Methods: Microbial cultures from Biofac Inputs Pvt. Ltd., Hyderabad, were tested in a randomized block design (RBD) with seven treatments and four replications. Plant growth parameters and pigment content were analyzed.

Result: The T6 treatment (NPK+microbial consortia+SSB) showed significant improvements in shoot dry weight (89.6±1.372 g), shoot length (219.95±3.96 cm), root dry weight (17.05±0.676 g), root length (61.55±1.17 cm), yield (17.0±0.196 g), chlorophyll (0.925±0.038 mg/g), carotenoids (0.974±0.037 mg/g) and NPK content (1.94%, 18.19%, 15.1%). The microbial consortia and SSB enhanced soil nutrient availability and plant health while demonstrating compatibility with synthetic fertilizers.

Key words: *Bacillus tequilensis*, Carotenoids, Chlorophyll, Silicate solubilizing bacteria.

INTRODUCTION

Maize (*Zea mays* L.) is a pivotal and extensively cultivated crop globally. In developed nations, it is primarily used as animal feed and raw material for agricultural processing industries.

In developing countries, it provides food, assuring nutritional security for humans and livestock (Prasanna *et al.,* 2021; Lelago *et al.,* 2025). Maize tends to show resilience to various adverse weather and agroecological conditions (Ssemugenze *et al.,* 2024; Bhaumik *et al.,* 2025). With its exceptional significance, maize is widely acclaimed as the crown of cereals. Maize is a complete source of nutrients for starch, calcium, fibre, essential minerals and vitamins (Adinarayana *et al.,* 2024; Rouf Shah *et al.,* 2016).

In recent decades, due to adverse abiotic conditions *viz.* drought, water logging, salinity and heavy metal toxicity, the world has noticed a drastic decline in productivity, threatening the security of food. Among all the abiotic stress, drought created devastating losses leading to sharp decline in the productivity (Sukumar *et al.,* 2023). Silicon is one of the major minerals on the earth which occupies 31% of the soil and absorbed by plants in the form of monosilicic acid. Silicon has determined benefits in securing plants from lethal injuries of biological and environmental stresses (Karuganti *et al.,* 2023).

The silicate solubilizing bacteria group is responsible for transforming insoluble silicates into soluble silica, enhancing soil fertility and plant nutrition. These silicate solubilizing bacteria secrete the silicase enzyme and organic acids responsible for silicate breakdown into soluble silica (Cruz *et al.,* 2022; Jain, 2025). In this scenario, silicate solubilizing bacteria was much studied due to its ability to enhance drought alle*via*tion in *Zea mays* L. and other crops. These silicate solubilizing bacteria has the ability to secrete organic acids, ACC deaminase and plant growth-promoting molecules which induce systemic acquired resistance to the plants in fighting against biotic and abiotic stresses.

A major auxin family hormone, indole acetic acid (IAA) is produced by plant beneficial microbial inoculants. This IAA regulates the wide range of plant bodily functions including germination, cell division, vegetative growth, secondary metabolite biosynthesis and stress response, highlighting its importance in plant development and adaptation. Some of the silicate solubilizing bacteria like *Bacillus tequilensis* has multipotential abilities *viz.* solubilizing the complex silicates, secretion of IAA and

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showing antagonistic activity against plant pathogens (Sukumar *et al.,* 2023; Karuganti *et al.,* 2023).

Globally, plant beneficial microbial inoculants gained significant importance due to their plant growth promoting attributes and the ability to induce resistance in plants. Although individual microbial inoculants are widely available in the global market, their utility is limited as they typically offer only a single benefit. Microbial consortia-based formulations gained importance due to their multiple plant growth promoting abilities (Lugtenberg and Kamilova, 2009).

This research focuses on the plant growth-promoting effects of silicate solubilizing *Bacillus tequilensis* SKSSB09 in *Zea mays* L. The study aims to test plant growth promoting efficiency of *Bacillus tequilensis* individually and in combination with the *Paenibacillus azotofixans* BFPA09 (nitrogen fixing bacteria), *Bacillus megaterium* KLBM01 (phosphate solubilizing bacteria), *Bacillus mucilaginous* BFBM06 (potash solubilizing bacteria) and synthetic fertilizers.

MATERIALS AND METHODS

Sourcing of microbial cultures

The microbial cultures used in this study were sourced from Biofac Inputs Private Limited in Hyderabad and included *Bacillus tequilensis* SKSSB09 (silicate solubilizing), *Paenibacillus azotofixans* BFPA09 (nitrogen-fixing), *Bacillus megaterium* KLBM01 (phosphate solubilizing) and *Bacillus mucilaginous* BFBM06 (potash solubilizing). These cultures were revived, subcultured on soybean casein digest agar and stored at 4°C for long-term use.

Formulation of the microbial consortium

For each bacterial culture, 100 mL of soybean casein digest broth was autoclaved and cooled. A loopful of each bacterial culture was inoculated and incubated at 120 rpm for 24 to 48 h on an orbital shaker. Microbial samples were collected and centrifuged using the REMI C-24 BL. The cell pellet was used to determine the total microbial count using the spread plate technique, expressed as CFU/g. 1 g of each cell biomass was suspended in 1 L of double distilled water and mixed homogeneously using the magnetic stirrer (REMI- 5MLH) for 15 min. The microbial cells were encapsulated with 1% of glycerol to increase the *via*bility of the cells. The total microbial count of the formulated microbial consortium was as assessed on N-free malate agar with composition of malic acid (5 g/L), yeast extract (0.1 g/L), potassium hydroxide (4 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4$ (0.1 g/L), NaCl (0.02 g/L), $FeSO_4$ (0.05 g/L), $MnSO_4$ (0.01 g/L), Na_2MoO_4 (0.002 g/L), bromothymol blue (0.5% alcoholic solution) of 2 mL/L, adjusted to pH 7.0. Pikovask's agar (composition: glucose 10 g/L, aluminium sulphate 0.5 g/L, NaCl 0.3 g/L, KCl 0.3 g/L, $MgSO_4$ 0.3 g/L, $FeSO_4$ 0.03 g/L, $MnSO_4$ 0.03 g/L, tricalcium phosphate 3 g/L and pH adjusted to 6.8) and Aleksandrov's agar (composition: $MgSO_4$ 0.5 g/L, $CaCO_3$ 0.1 g/L, dextrose 5 g/L, $FeCl_3$ 0.005 g/L, Ca_3PO_4 2 g/L and agar 20 g/L) to check the compatibility and presented as CFU/ml (Raju *et al.,* 2020).

In-planta experimental design for studying the plant growth attributes

Maize seeds (Rasi RMH 3499) were surface sterilized with 0.1% $HgCl_2$ and rinsed with sterile water. The seeds were then sown in seedling trays with double-sterilized coco peat and after 10 days, transplanted into 8 Kg pots with a sterilized pot mix (40% garden soil, 20% coco peat, 20% vermicompost and 20% vermiculite). Treatments were applied based on the experimental design, with four replications per treatment (Table 1). Irrigation was daily and data were collected twice per week. The trial continued until cob harvesting, recording shoot dry weight, shoot length, root dry weight, root length and yield. Dry weights were determined by drying plant parts at 80°C for 24 h and one-factor ANOVA was used for data analysis (Raju *et al.,* 2020; Karuganti *et al.,* 2023).

Estimation of N, P, K in leaves

Older leaves from the base of plants were collected before harvesting (130 days) and dried in a hot air oven at 63°C for 48 h to ensure complete desiccation. Total nitrogen content was determined using the Kjeldahl apparatus. For phosphorus (P) and potassium (K) analysis, the samples were incinerated at 400°C for 2 h, then treated with magnesium nitrate and vaporized. After moistening with 1N nitric acid and drying, the ash was dissolved in 0.1N HNO_3 and aliquots were analysed for P and K using a Perkin-Elmer flame photometer (Bennet *et al.,* 1962).

Estimation of pigments

Pigment extraction was performed on 1 g fresh leaf samples collected before harvesting (130 days), cut into 0.5 cm pieces and immersed in 10 mL of 80% acetone, then incubated at 4°C for 24 h. Chlorophyll a, chlorophyll b and carotenoid contents were measured spectrophotometrically at 663, 645 and 480 nm using a UV Visible spectrophotometer 117 TS (Saleem *et al.,* 2021).

Chlorophylla (mg/g) =

(12.7 × Absorbance at 663 - 2.69 × Absorbance at 645] × (Volume of extract in mL/1000) × Fresh weight of the sample

Chlorophyll b (mg/g) =

(22.9 × Absorbance at 645- 4.68 × Absorbance at 663] × (Volume of extract in mL/1000) × Fresh weight of the sample

Carotenoid (μg/g) =

$$\frac{\text{Absorbance value of the carotenoid extract}}{\text{Maximum extinction coefficient}} \times 100$$

RESULTS AND DISCUSSION

India has experienced a notable increase in maize production, marked by a 35% rise in cultivated area and a

48% improvement in yield. However, productivity faces challenges from abiotic and biotic stresses, worsened by climate change, particularly drought (Yadav *et al.,* 2016; Song *et al.,* 2020). Microbial inoculants, such as *Bacillus tequilensis*, are essential for sustainable agriculture as they enhance nutrient availability and plant growth. *Bacillus tequilensis* helps alle*via*te drought stress by solubilizing silicates and producing indole-3-acetic acid (IAA).

This study evaluates *Bacillus tequilensis* SKSSB09 alongside other microbial inoculants and synthetic fertilizers, utilizing cultures sourced from Biofac Inputs Private Limited in Hyderabad. A pot trial with seven treatments was conducted to assess the effectiveness of different plant growth-promoting rhizobacteria (PGPR) strains and synthetic fertilizers (Table 1).

Enhancement of shoot length and dry weight in maize

The combined application of NPK, SSB (*Bacillus tequilensis*, 1.0×10^8 CFU/mL at 10 mL/L) and microbial consortia (*Paenibacillus azotofixans*, *Bacillus mucilaginosus* and *Bacillus megaterium*, 1.0×10^8 CFU/mL at 10 mL/L) in T6 treatment significantly enhanced maize shoot length and dry weight. The values recorded before harvesting in T6 recorded the highest shoot length (219.95±3.96 cm), followed by T5 (205.13±2.28 cm), compared to the control (T7, 118.33±1.91 cm) (Fig 1). This improvement was attributed to phytohormone production, including indole acetic acid (IAA), atmospheric nitrogen fixation and phosphorus and potassium solubilization by the PGPR.

T6 had the highest shoot dry weight at 89.6±1.37 g, followed by T5 at 84.5 ± 0.98 g, compared to the control at 54.08±1.41 g (Fig 2). This increase in dry weight was linked to greater shoot length and enhanced nutrient availability, phytohormone synthesis and stress tolerance due to plant growth-promoting rhizobacteria (PGPR).

The PGPR consortia showed greater plant growth-promoting abilities compared to individual treatments. This finding aligns with previous studies that reported improved plant growth from microbial inoculants, such as *Azotobacter, Pseudomonas, Azospirillum* and *Serratia spp.* (Gholami *et al.,* 2008; Ali *et al.,* 2017; Akther *et al.,* 2024; Ghosh *et al.,* 2018). Additionally, these results support earlier reports indicating that microbial inoculants, including *Sinorhizobium spp.,* can enhance growth while reducing the need for chemical fertilizers without compromising yield (Zahir *et al.,* 2006).

Moreover, silicate-solubilizing bacteria enhanced stress tolerance, including salinity resistance, by improving photosynthetic efficiency (Kubi *et al.,* 2021). This research underscores the potential of microbial inoculants in sustainable agriculture to boost crop productivity while decreasing dependence on chemical fertilizers.

Effect of PGPR on root length and dry weight in maize

The T6 treatment, which combines NPK, SSB (*Bacillus tequilensis*) and microbial consortia (including *Paenibacillus azotofixans, Bacillus mucilaginosus* and *Bacillus megaterium*), showed the highest root proliferation at 61.55±1.17 cm. This result is significantly better than the control group (T7), which measured 37.4 cm (Fig 3). These findings align with previous research that demonstrated enhanced root lengths with Bacillus species (56.46 cm) and *Pseudomonas* species (29.33 cm) (Imran *et al.,* 2020; Zahir *et al.,* 2006).

The increase in root length is due to phytohormones that stimulate cell elongation and division in root tissues, enhancing growth and nutrient uptake (Ahemad *et al.,* 2014; Hayat *et al.,* 2010). PGPR (Plant Growth-Promoting Rhizobacteria) likely improves nutrient cycling, soil structure and microbial communities, promoting root development (Bashan and de-Bashan, 2010).

In terms of root dry weight, treatment T6 showed the highest increase (17.05±0.68 g), followed by T5 (16.48±0.58 g), compared to the control (11.8±0.47 g) (Fig 4). This aligns with studies using microbial inoculants like *Pseudomonas putida* and Acinetobacter *spp.,* which also enhanced root biomass (Kumar *et al.,* 2023). The increased dry weight in T6 is linked to better nutrient acquisition and rhizosphere conditions improved by PGPR (Glick *et al.,* 2014; Berg *et al.,* 2009). The microbial consortia likely optimized soil health, contributing to stronger root growth (Lambers *et al.,* 2009).

Effect of PGPR on grain weight in maize

T6 exhibited the highest average grain weight of 17.0±0.196 g, followed by T5 at 16.875±0.312 g, while the control (T7) showed a significantly lower weight of 8.725±0.582 g (Fig 5). The increase in grain weight is attributed to the combined effects of *Bacillus tequilensis*

Table 1: Comprehensive treatment details for *in-planta* studies.

Treatment	Description
T1	NPK mixture (Urea, DAP and MOP) at 2 g each per 8 kg of potting mix per pot.
T2	Silicate solubilizing bacterial consortium (1.0×10^8 CFU/mL) at 10 mL per 8 kg of potting mix per pot.
T3	Application of *Paenibacillus azotofixans*, *Bacillus mucilaginosus* and *Bacillus megaterium* (1.0×10^8 CFU/mL each) at 10 mL per 8 kg of potting mix per pot.
T4	NPK mixture (T1) and microbial consortium (T3) at 10 mL per 8 kg of potting mix per pot.
T5	NPK mixture (T1) and silicate-solubilizing bacterial consortium (T2) at 10 mL per 8 kg of potting mix per pot.
T6	NPK mixture (T1), silicate-solubilizing bacterial consortium (T2) and microbial consortium (T3), each at 1.0×10^8 CFU/mL, with 10 mL per 8 kg of potting mix per pot.
T7	No additional treatments applied.

and PGPR on nutrient uptake and stress tolerance (Ojuederie and Babalola, 2023).

Bacillus tequilensis enhances silicon availability, improving cell wall strength, drought tolerance and photosynthetic efficiency, all contributing to increased grain weight (Ali *et al.,* 2017). PGPR further boosts growth by enhancing nutrient uptake and producing phytohormones, promoting better grain filling. The dual inoculation of *Bacillus tequilensis* and PGPR enhances nutrient absorption and stress resilience, leading to increased grain weight, as supported by previous studies (Akhter *et al.,* 2024; Babaji *et al.,* 2014).

Enhancement of nutrient uptake

Analysis of NPK content in pot trials revealed that T6 had the highest nitrogen (1.94%), phosphorus (18.19%) and potash (15.1%) content among all treatments, while the control recorded significantly lower levels (0.86% N, 8.1% P, 7.2% K) (Fig 6).

The increased NPK content in T6 is attributed to the synergistic action of PGPR and SSB. PGPR fixes atmospheric nitrogen, while SSB solubilizes silicate minerals, improving soil fertility. This combined effect enhances mineralization in the rhizosphere, increasing nitrogen availability and promoting chlorophyll production and growth. In general, during the harvesting period, plant nitrogen requirement will be less and the same results were reciprocated in the trial conducted.

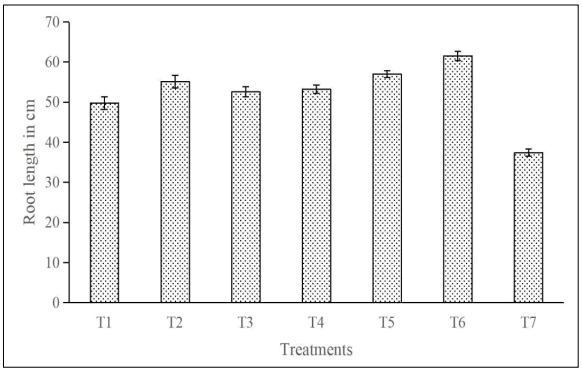


Fig 3: Root length enhancement in maize post PGPR treatment.

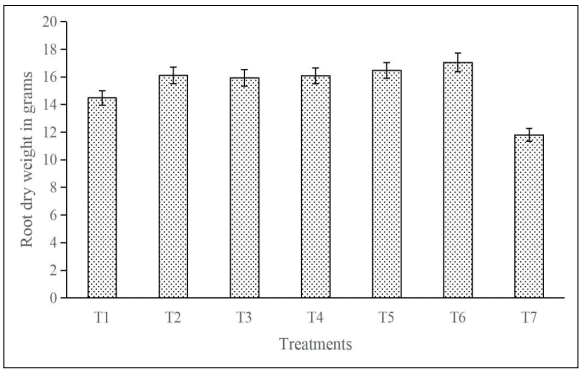


Fig 4: Root dry weight in maize plants post treating with PGPR.

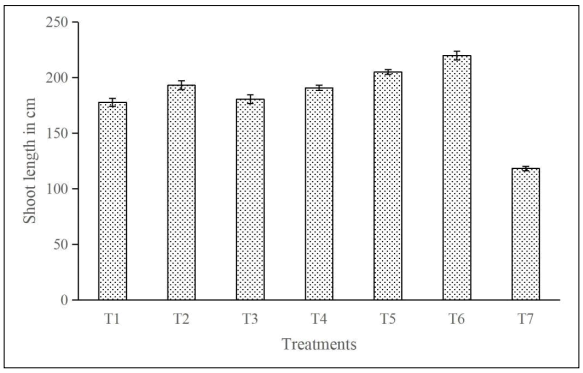


Fig 1: Shoot length enhancement after PGPR treatment in maize.

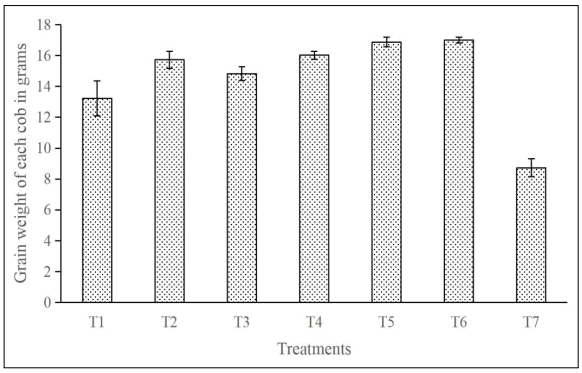


Fig 5: Total grain weight in maize plants treated with PGPR.

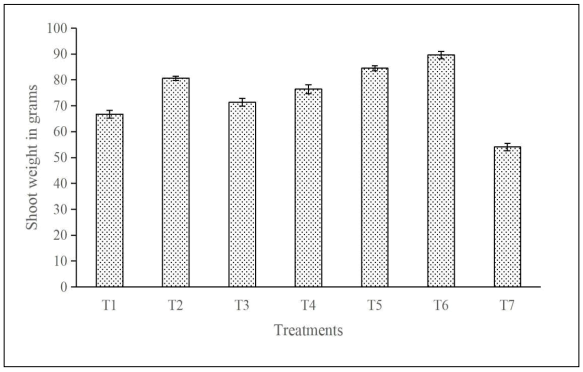


Fig 2: Shoot dry weight enhancement post PGPR treatment in maize.

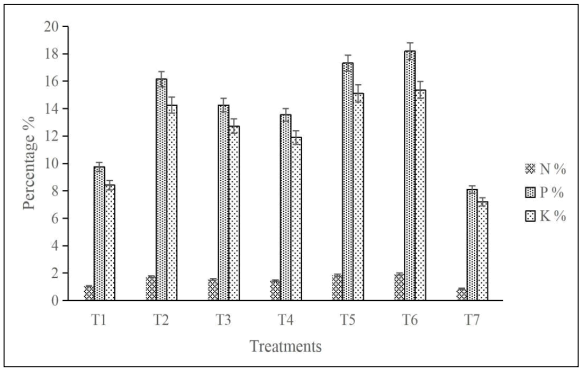


Fig 6: Impact of PGPR on nutrient uptake in maize.

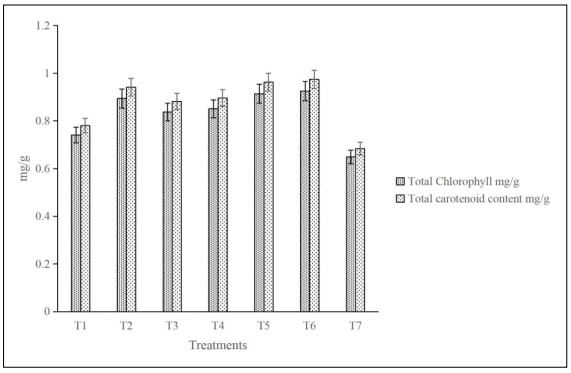


Fig 7: Chlorophyll and carotenoid content of maize after PGPR treatment.

Bacillus tequilensis, by producing organic acids and phosphatase enzymes, solubilizes phosphorus, silica and enhances root development. The higher silica availability improves drought tolerance, nutrient transport, enzyme activities and stress resilience in maize (Solomon *et al.,* 2024; Kiran *et al.,* 2022).

Effect of PGPR on chlorophyll and carotenoid content

The values recorded before the harvesting showed highest chlorophyll content in T6 (0.925±0.038 mg/g), with T5 and T6 also demonstrating superior carotenoid content (0.974±0.037 and 0.974±0.041, respectively). In contrast, the control treatment had lower chlorophyll (0.649±0.027 mg/g) and carotenoid (0.683±0.029 mg/g) levels (Fig 7). These results align with prior studies reporting similar increases in chlorophyll and carotenoid content (Ali *et al.,* 2017).

The enhanced photosynthetic pigments in this study are attributed to PGPR and *Bacillus tequilensis*, which improve chlorophyll content in maize leaves, thereby enhancing photosynthesis. The silicate-solubilizing bacteria and microbial consortia help to stabilize chlorophyll, reducing degradation and improving photosynthetic efficiency. Additionally, the increased carotenoid content, including lutein and zeaxanthin, boosts antioxidant activity, protecting against oxidative stress and enhancing overall plant health (Katagiri *et al.,* 2002; Ali *et al.,* 2017).

CONCLUSION

The results obtained revealed the plant growth-promoting abilities of the silicate solubilizing *Bacillus tequilensis* SKSSB09. The particular microbial strain also can induce drought mitigation in *Zea mays* L. as per the previous research reports. The *Bacillus tequilensis* strain showed synergism with the other microbial inoculants and synthetic fertilizers. Encouraging plant growth promotion results were obtained revealing the ability of the *Bacillus tequilensis* and microbial consortium in the nutrient uptake ability and plant growth. Further research focuses on field trials to evaluate the long-term benefits and scalability of these treatments in various agroecosystems.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Not applicable.

Conflict of interest

The authors have no conflict of interest.

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