



An Assessment of Application Methods of Plant Growth Promoting Rhizobacteria (PGRRs) for Their Growth Promoting Attributes in Sugarcane (*Saccharum spp. hybrid*)

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Abstract

Extensive literature has documented the impact of plant growth promoting rhizobacteria (PGPR) on various crops but studies are scant on PGPR application methods. In this study, we tested three different application methods, viz., biopriming, foliar and rhizospheric application on the growth of two sugarcane lines (CPSG-2525 and CPSG-2730). *Pseudomonas aurantiaca* (PB-St2) and *Bacillus* spp. strains (SB-1 and CRN8) were selected and their synergistic interaction was evaluated by competitive growth assay. Subsequently, sugarcane plants were inoculated with PGPRs, either individually or their consortia in pot experiment. The survival analysis of inoculated strains was carried out at various time intervals, whereas plant's morpho-chemical data were noted after 90 days of sowing. Biopriming notably enhanced the above-ground sugarcane growth, with maximum shoot length (156.1 cm) with SB-1 + CRN8 in CPSG-2525, followed by 146.4 cm in CPSG-2730 with PB-St2, compared with 113.7 and 114.13 cm respectively in control plants. Moreover, a substantial increase in shoot dry matter (11.4 g) was noted with PB-St2 in CPSG-2730 and 12.1 g in CPSG-2525 with PB-St2 + CRN8 in contrast to non-inoculated plants (5 g). Rhizospheric application of PB-St2 led to a significant increase in root length (79 cm) and dry weight (2 g) in both sugarcane lines. In terms of biochemical attributes, significantly ($p < 0.05$) higher chlorophyll content (2.98 and 3.78 mg/g), total soluble sugars (7.54 and 5.72 mg/g) and sucrose content (52.21 and 24 mg/g) were observed in biopriming in CPSG-2525 and CPSG-2730, respectively. Our findings suggest that biopriming is more convenient and effective method of applying PGPRs to sugarcane, while *Pseudomonas aurantiaca* (PB-St2) enhanced sugarcane growth, both individually and in combination with *Bacillus* results in improved plant growth and biochemical attributes.

Keywords Biopriming · Co-inoculation · Foliar application · *Pseudomonas aurantiaca* · Rhizobacteria · Sucrose content · Sugarcane

1 Introduction

In recent years, Plant Growth Promoting Rhizobacteria (PGPRs) have emerged as an effective tool to replace chemical fertilizers, thus paving a way to diminish the harmful environmental impacts of chemical fertilizers, and for improved agricultural practices (Zahir et al. 2004; Ibort et al.

2018). Their implication is encouraged in many parts of the world to improve plant growth and sustainable agricultural production. Rhizobacteria improve plant growth by colonizing root system and also play an important role to maintain soil fertility as symbionts or free living saprophytes (Vessey 2003; Goswami et al. 2016; Mukhtar et al. 2017). They directly or indirectly enable the plants to survive better under various biotic and abiotic stresses (Rodrigues et al. 2016; Sharma and Archana 2016; Gu et al. 2023). Plant root system produces exudates that act as chemoattractants for microbes, increase their growth in the rhizosphere and build a mutualistic and beneficial association with plants (Kumar et al. 2016). Roots are the interface between plants and soil for the exchange of nutrients and metabolites (Benizri et al. 2001). Therefore, manipulation of the rhizosphere with PGPRs can be one of the best ways to optimize plant growth conditions

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(Da Silva et al. 2017). Application of microbes along with synthetic fertilizers is another viable approach to minimize the excessive use of chemical fertilizers while maintaining the crop yield (Breedt et al. 2017; Li et al. 2023).

Sugarcane (*Saccharum* spp. hybrid) is one of the oldest and valuable crops in the world. It requires large amount of nutrients for growth due to its perennial nature (Chhabra et al. 2016). It is reported that sugarcane cultivation in one hectare (which usually gives around 100 tones yield) removes 140 kg Nitrogen (N), 34 kg Phosphorus (P) and 32 kg Potassium (K) from the soil (Dotaniya et al. 2016). Among macronutrients, P plays a vital role in the development of tillers, root system and significantly affect the longevity of sugarcane in field (Kingston 2013). PGPRs, on the other hand, have emerged as a sustainable and cost-effective alternate, as they have the potential to solubilize the minerals in soil, particularly phosphate, thereby resulting in decreased requirement of fertilizers but an enhanced sugarcane production (Santos et al. 2020). Various greenhouse and field studies with PGPR inoculation in sugarcane have reported enhanced root growth and improved water and mineral uptake (Moura et al. 2018), increased photosynthesis and nitrogen absorption (Silveira et al. 2018), improved biomass and yield (Rampazzo et al. 2018).

Different studies have reported the effectiveness of chemical growth promoters in sugarcane, such as humic acid (Canellas et al. 2022), 2-chloroethylphosphonic acid (Roberto et al. 2015), and a combination of Naphthaleneacetic acid with gibberellic acid (Sujatha et al. 2018). Additionally, using PGPRs like *Azospirillum brasilense* and *Bacillus subtilis*, either alone or with low-cost P_2O_5 fertilizer, have been found to be an effective fertilizer management approach for sugarcane growth and yield enhancement (Moura et al. 2018; Santos et al. 2020). Similarly, *Burkholderia* species have been observed to activate metabolic pathways in sugarcane, making more nutrients availability and enhancing physiological functions (Paungfoo-Lonhienne et al. 2016; Muthukumarasamy et al. 2017). Likewise, *Bacillus* and *Pseudomonas* genera have been assessed for both enhancing sugarcane growth and controlling phytopathogens (Li et al. 2017; Chandra et al. 2018; Rosa et al. 2020; Rossetto et al. 2021; Shair et al. 2021).

Application method of PGPR plays an important role to the survival efficacy of bacteria in the soil and on seeds. Among different inoculation methods of beneficial bacteria include biopriming, soil amendment, root dipping and foliar application (Podile and Kishore 2007; Mahmood et al. 2016). Microbial inoculants are applied on seeds, soil or in composting area, to increase plant metabolism, enhance the water and nutrients availability and to stimulate diverse physiological mechanisms concurrently (Tittabutr et al. 2007; Günes et al. 2014). The investigated application methods offer various scopes for promoting plant

growth, establishing a beneficial microbial community in biopriming, enhancing root growth and nutrient absorption with rhizosphere inoculation, and improving plant resistance against pathogens with foliar spray. However, labor-intensive application at field level, limited uptake with foliar application, and inconsistent results are amongst the major limitations of these studies. These challenges like competition with native soil microbes, environmental factors, and compatibility with other practices need to be addressed for successful implementation.

In sugarcane cultivation, *Pseudomonas* and *Bacillus* spp. have been utilized through various application methods including foliar spray (Rampazzo et al. 2018; Rosa et al. 2020), rhizospheric application (González et al. 2015; Kleingesinds et al. 2018; Rampazzo et al. 2018), root dipping (Chandra et al. 2018; Singh et al. 2023), and biopriming (Marcos et al. 2016; Ferreira et al. 2020). These studies have consistently shown significant improvements in sugarcane growth and yield. However, addressing challenges such as optimizing the timing of inoculation, compatibility with other chemical treatments, and specific symbiotic interactions with crops is crucial for their successful application achieving consistent and optimal outcomes in field conditions (Mahmood et al. 2016; Mujeeb et al. 2022). Therefore, a comprehensive investigation is necessary to evaluate the multiple application methods concurrently to ascertain their relative effectiveness.

Although numerous studies (Mehnaz 2011; Paungfoo-Lonhienne et al. 2016; Li et al. 2017) have reported the supportive results of PGPR application in sugarcane under controlled conditions, nevertheless their application in the field is imperative to validate their effectiveness for their practical implementation. In sugarcane farming, a major limitation is nutrients deficient soil, which fails to meet the crop's nutritional requirements and subsequently hampers its productivity. Therefore, the primary challenge in utilizing PGPRs is the identification of crop-specific rhizospheric strains with efficient mineral solubilization ability to achieve increased yield in field conditions. Thus, bridging the gap between farmers and this technology is essential for the successful implementation of PGPR technology in agriculture. Practical implementation and rural development programs for the awareness should be conducted to change farmers' mindset regarding the use of chemical fertilizers and encourage the adoption of environment friendly alternatives.

Different bacterial strains of the genera *Pseudomonas*, *Bacillus*, *Agrobacterium*, *Streptomyces* and *Burkholderia* are reported to be used as biostimulants in monocots (wheat, rice and maize) and have been progressively marketed as biofertilizer (Tahir and Sarwar 2013; Naveed et al. 2015; Chandra and Chandra 2016; Chandra et al. 2018). However, in this study, the selected strains of *P. aurantiaca* (PB-St2), *B. subtilis* (CRN8) and *B. amyloliquefaciens* (SB-1) are being

reported for the first time as co-inoculants in sugarcane. The growth-promoting capabilities of PB-St2, SB-1 and CRN8 were initially examined in an in vitro study (Idrees et al. 2024; under review elsewhere) and the sugarcane plantlets were inoculated either individually or in combination. The results of in vitro study yielded significant findings concerning various aspects of sugarcane growth, such as plant height, fresh and dry weight, number of microtillers, along with notable enhancement in root growth. These findings establish a strong foundation for the current study to further apply these strains in natural settings. Thus, we hypothesize that the inoculation of sugarcane with PB-St2, SB-1 and CRN8 using different methods should accelerate the plant growth and improve biochemical attributes. Furthermore, we envisage that co-inoculation of these strains may support an even better growth and biochemical picture compared with individual inoculation. To test our hypothesis, the present study was devised in three experimental sets with different inoculation methods of PGPRs specifically tailored for sugarcane cultivation in greenhouse conditions. Initially, the compatibility of *Pseudomonas* and *Bacillus* strains was assessed through compatibility assay, followed by their application individually and in consortia using different application methods on two sugarcane lines, i.e., CPSG-2730 and CPSG-2525. The outcome of this research could assist in selecting the most efficient application method for delivering PGPRs in sugarcane farming. Additionally, PGPRs exhibiting growth-promoting attributes and being synergistically effective can also contribute to the formulation of crop-specific biofertilizers. This approach may help reduce the reliance on chemical fertilizers to fulfill the nutrient requirements of sugarcane crop.

2 Materials and Methods

2.1 Biological Material

Three bacterial strains were used in this study, provided by Kauser Abdulla Malik School of Life Sciences, Forman Christian College (A Chartered University), Lahore. *Pseudomonas aurantiaca* (PB-St2; Accession number EU761590) and *Bacillus amyloliquefaciens* (SB-1; Accession number MF171193) were isolated from sugarcane (Mehnaz et al. 2010; Shahid et al. 2021). *P. aurantiaca* (PB-St2) is described as a PGPR acknowledged for its production of siderophore and hydrogen cyanide (HCN), and well-known for its antifungal properties (Mehnaz et al. 2010). Likewise, *B. amyloliquefaciens* (SB-1) is reported as a sugarcane endophyte renowned for plant growth promotion, attributed to its biofertilizer and biocontrol properties. Additionally, its capability to solubilize phosphorus and zinc within the rhizosphere is emphasized, alongside

its documented significant effect on wheat growth (Shahid et al. 2021), whereas *B. subtilis* (CRN8; Accession Number MZ477358; unpublished data) was isolated from corn and having PGPR attributes. Sugarcane (*Saccharum* spp. hybrids) lines CPSG-2730 and CPSG-2525 were procured from Shakarganj Sugarcane Research Institute (SSRI) Jhang, Pakistan. These lines were sown in the field of Seed Centre, University of the Punjab for further field experiments.

2.2 Competitive Growth Assay

Synergistic effect of *Bacillus* and *Pseudomonas* strains was evaluated through an in vitro competitive growth assay by following the methodology of Kumar et al. (2016) with slight modifications. The cultures of PB-St2, CRN8 and SB-1 were grown in Nutrient Broth (NB) medium separately at 28 ± 2 °C for 48 h in a shaking incubator at 150 rpm. Freshly grown cultures were centrifuged at $4500 \times g$ for 15 min and the supernatant was filtered by using 0.22 µm sterile filter. The resultant filtrate was mixed with the freshly prepared NB medium at a concentration of 10, 25 and 50% (v/v). The effect of filtered supernatant of one isolate on the growth of other bacteria was plotted by taking absorbance at 600 nm using UV–visible spectrophotometer at the intervals of 0, 24 and 48 h. The strains grown without filtrate were assigned as the control group. This experiment was replicated three times, with each group consisting of three biological replicates and significance was assessed at a 5% level of significance.

2.3 Inoculum Preparation

The inoculum was prepared from pure cultures of *Pseudomonas* and *Bacillus* strains individually in 50 ml King's Broth (KB) and Luria Bertani (LB) medium, respectively, for 36–48 h at 28 ± 2 °C and 150 rpm in a shaking incubator. After that, 20 ml of freshly grown culture was inoculated into 2 L of KB and LB media separately for each strain and grown at the same conditions for 48 h. The bacterial cells were harvested by centrifugation at $2000 \times g$ and the resultant pellet was re-suspended into the 0.85% saline while optical density was adjusted to 10^8 colony forming unit (CFU) ml⁻¹ at 600 nm. Mixture of bacterial strains was prepared by adjusting the optical density (OD) of individuals at 10^8 CFU ml⁻¹ and taking equal quantity from each of them.

2.4 Pot Experiment and Treatments

The selected rhizobacteria were utilized in the form of various treatments (either individually or in consortia) and sugarcane plants were inoculated accordingly to assess their synergistic effect. These bacterial treatments were named as T1: PB-St2, T2: SB-1, T3: CRN8, T4:

PB-St2 + SB-1, T5: PB-St2 + CRN8, T6: SB-1 + CRN8 and T7: PB-St2 + SB-1 + CRN8, whereas the control (non-inoculated) plants received water and maintained as such. The pot experiment was segregated in three experimental sets (A: biopriming, B: rhizospheric and C: foliar application) to study the methods of PGPRs application.

In biopriming (experimental set A), sugarcane was cut into mini-sets containing one viable node of each line. Five mini sets of each sugarcane line were soaked with 700 ml inoculum of individual strain (10^8 CFU ml⁻¹) along with 3% Polyvinylpyrrolidone (PVP) and incubated for 3 h. Bacterial strains in consortium were also applied in similar manner to evaluate their combined effect on plant growth. Afterward, the mini sets were dried under the shade for 30 min and then sown in the pots (12 × 11 inches) containing 5 kg sterilized soil each. Five pots for each treatment and one mini set per pot was sown for each sugarcane line. The plants were watered twice a week.

Biostimulants were applied in the rhizospheric area in experimental set B (rhizospheric application). One bud cutting of each selected sugarcane line was sown in each pot. Ten milliliters (ml) of bacterial cultures (10^8 CFU ml⁻¹) of each treatment were inoculated at the rhizospheric area at the stem base of each plant at 30th and 60th day of germination. In experimental set C, PGPR treatments were given to the plants via foliar application using a hand atomizer. For each treatment, the sugarcane leaves were thoroughly sprayed with respective bacterial suspensions until they were well soaked at 30th and 60th day of germination, while the control plants were sprayed with water only. All experimental sets were organized in a randomized block design with five replicates of each treatment. The plants were watered twice a week.

2.5 PGPR Survival Analysis

The survival of inoculated bacteria in the rhizosphere of sugarcane plants was assessed at the 30th, 60th, and 90th day after sowing using the serial dilution technique. Random soil samples were collected from the rhizosphere of each treatment in triplicate and thoroughly mixed for uniformity. One g of soil from each sample was then serially diluted in sterilized water. Aliquots of 100 µl from dilutions ranging from 10^{-3} to 10^{-5} were spread on LB agar plates and incubated at 28 ± 2 °C. After 48 h, the number of colonies that were morphologically similar to our inoculated bacterial strains was counted and expressed as CFU/g of soil. The difference in the survival of inoculants was analyzed at a 5% significance level.

2.6 Morpho-Chemical Analysis of Sugarcane Plants

Sugarcane plants were harvested 90 days after plantation. For morphological data analysis, the mean of five replicates from each group was calculated. The shoot and root length was measured from the base of the plant to the apex of leaf + 1 using a suitable flexible measuring tape. Both fresh and dry masses were determined using an analytical balance (Shimadzu, Japan). To obtain plant dry weight, the plant tissues were dried in a dry heat oven (Binder, Germany) at 65°C until a constant dry mass was achieved. Fresh leaves from each experimental group were collected in triplicates and utilized for biochemical analysis. Total chlorophyll content was measured following the method outlined by Porra et al. (1989). Estimation of Total soluble sugars (TSS) in fresh sugarcane leaves was carried out by the method of Dubois et al. (1956) and sucrose content by using the protocol of Van Handel (1986). Optical density (OD) of the plant extract was measured at the specified wavelengths as outlined in their respective protocol using a spectrophotometer (UV-VIS4000, O.R.I, Germany). Separate standard curves of glucose and sucrose were prepared to calculate the TSS and sucrose in sugarcane leaves.

2.7 Statistical Analysis

The experiment was conducted in a Randomized Complete Block Design (RCBD) with 7 bacterial treatments, either individually or their consortia along with one control (non-inoculated) in five replicates of each. Mean values and standard error were also computed. The analysis of variance (ANOVA) was performed for morpho-chemical parameters with computer software program IBM SPSS (version 23.0). The significant difference among treatment means were compared at probability ($p < 0.05$) by Duncan's Multiple Range test (DMRT). The correlation among observed growth and biochemical parameters was confirmed by Pearson's correlation plot with the use of statistical software Origin 2023. Principle Component Analysis (PCA) was performed to evaluate the interaction of selected sugarcane lines with different PGPR treatments, their application methods and measured plant physio-chemical parameters.

3 Results

3.1 Competitive Growth of PGPRs

Initially, the competitive growth assay was performed by co-culturing of PB-St2, SB-1 and CRN8 in NB, to check their in vitro growth patterns. The variance among growth means was statistically analyzed and compared at 5% level of significance. Pairwise growth pattern indicated no antagonistic

interaction among them (Fig. 1). Absorbance at 600 nm revealed a non-significant ($p > 0.05$) difference in growth of bacterial strains compared with control. Although, negative

interactions may also occur depending on the strains and their metabolites, *Pseudomonas* and *Bacillus* strains in this study have shown no negative interaction in co-culture assay.

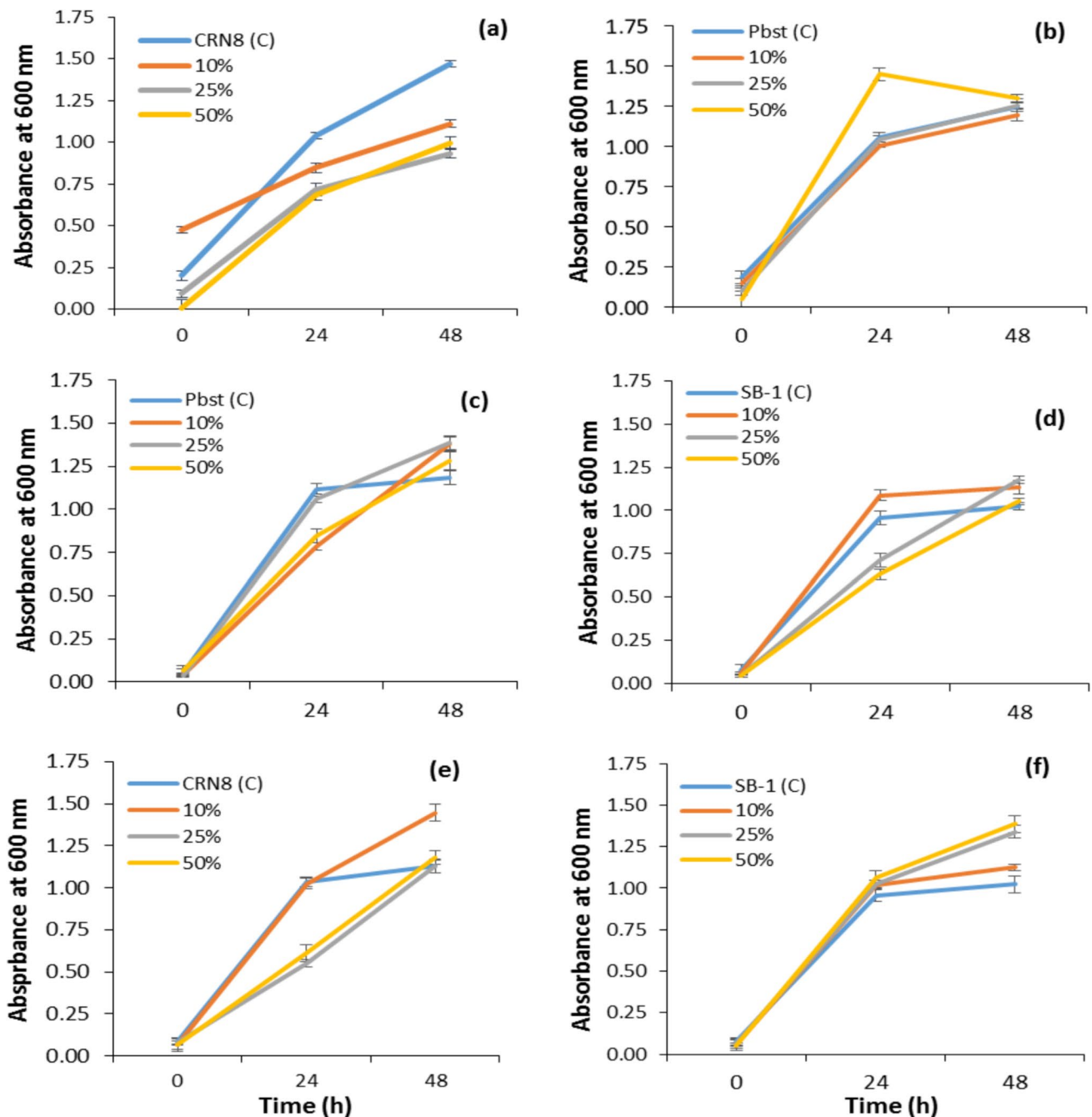


Fig. 1 Competitive growth of PGPRs **(a)** Filtered supernatant of *P. aurantiaca* (10, 25 and 50%) in Nutrient Broth (NB) to check *B. subtilis* (CRN8) growth at different time intervals. **b** Filtered supernatant of *B. subtilis* (CRN8; 10, 25 and 50%) in NB to check *P. aurantiaca* (Pbst-St2) growth at different time intervals. The data presented in graphs are an average of three replicates. **c** Filtered supernatant of *B. amyloliquefaciens* (SB-1; 10, 25 and 50%) in NB to check *P. aurantiaca* (Pbst-St2) growth at different time intervals. **d** Filtered superna-

tant of *P. aurantiaca* (10, 25 and 50%) in NB to check *B. amyloliquefaciens* (SB-1) growth at different time interval. The data presented in graphs is the average of three replicates. **e** Filtered supernatant of *B. subtilis* CRN8 (10, 25 and 50%) in NB to check *B. amyloliquefaciens* (SB-1) growth at different time intervals. **f** Filtered supernatant of *B. amyloliquefaciens* (10, 25 and 50%) in NB to check *B. subtilis* (CRN8) growth at different time interval. The data presented in graphs is the average of three replicates

These results, therefore, endorsed the application of these PGPRs in consortia on sugarcane plants to study their possible role in enhancement of various growth attributes.

3.2 PGPR Survival Analysis

Population density (log CFU/g soil) of inoculated *Pseudomonas* PB-St2 and *Bacillus* strains CRN8 and SB-1, was determined at 30th, 60th and 90th day of inoculation in the rhizosphere of sugarcane plants, individually and in combination. Variation in bacterial population ($p=0.05$) was observed with increase in time (Fig. 2). Bacterial density declined by almost half after 30 days and more than 50% survival of strains was recorded till the 30th day. However, after 60–90 days, a slight reduction in bacterial cell count was observed. PB-St2 showed a maximum ($p<0.05$) survival rate (2.8×10^8 CFU/g soil) on the 90th day followed by SB-1 (2.2×10^8 CFU/g soil). Among different combinations, PB-St2 + SB-1 showed the highest ($p<0.05$) population density (2.6×10^8 CFU/g soil).

3.3 Effect of PGPRs Application Methods on Selected Morphological Parameters

The results of bio-primed sugarcane lines exhibited a significant ($p<0.05$) increase (37%) in shoot length with T6 (SB-1 + CRN8) in CPSG-2525 and 28% with T1 (PB-St2) for CPSG-2730 over control (Fig. 3a). Maximum increase (59 and 57%) in root length for CPSG-2525 and CPSG-2730 was observed with the bacterial consortia PB-St2 + SB-1 (T4) and PB-St2 + CRN8 (T5) respectively than either the individually primed or the control plants (Fig. 3b). Highest value for shoot dry weight (143%) in CPSG-2525 was noted with T5 (PB-St2 + CRN8), whereas T1 (PB-St2) resulted in 128% increase for CPSG-2730 (Fig. 3c). A 78% increase

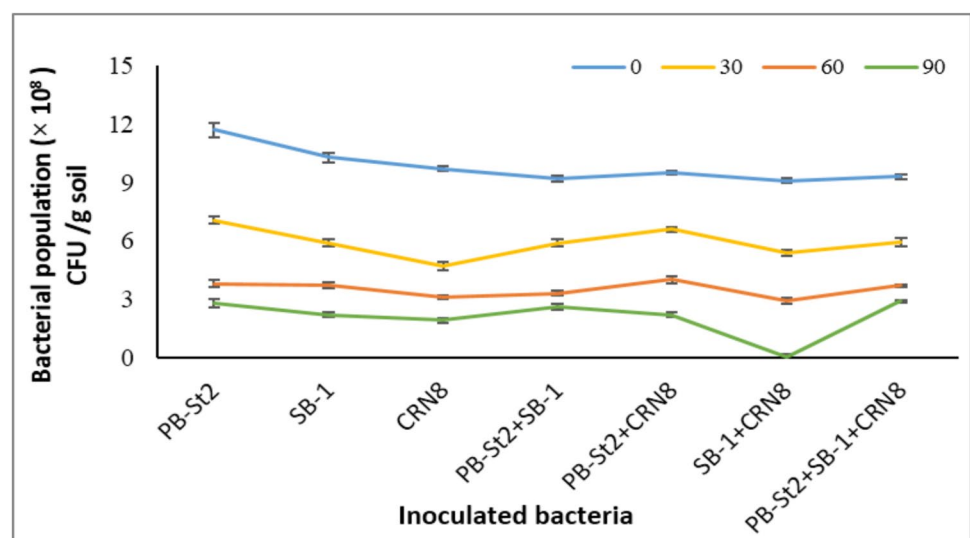
in root dry weight was observed in CPSG-2730 with T6 (SB-1 + CRN8) inoculation while it was increased up to 77% in CPSG-2525 with T5 (PB-St2 + CRN8). However, other treatments showed statistically non-significant ($p>0.05$) results as compared to the non-inoculated control (Fig. 3d).

Foliar application of T1 (PB-St2) resulted in a significant ($p<0.05$) increase of shoot (11 and 27%) and root length (56 and 44%) for CPSG-2730 and CPSG-2525, respectively (Fig. 4). Additionally, the application of T1 (PB-St2) also led to the highest value for root dry weight, with an increase of 128% in CPSG-2730 and 52% in CPSG-2525. However, T7 (PB-St2 + SB-1 + CRN8) inoculation yielded the maximum shoot dry weight (111 and 89%) in CPSG-2525 and CPSG-2730 respectively as compared to the non-inoculated control plants.

The data regarding the rhizospheric application of PGPRs are presented in Fig. 5. The results indicate that rhizospheric application of T1 (PB-St2) has a positive impact on root growth in both sugarcane lines. A significant increase ($p<0.05$) in root length (152%), and root dry weight (156 and 113%) was observed for CPSG-2730 and CPSG-2525 correspondingly. However, the consortium of SB-1 + CRN8 (T6) exhibited the promising results for shoot growth in CPSG-2525 with highest increase (28%) in shoot length and shoot dry weight (63%). However, other treatments did not show a significant difference ($p<0.05$) for shoot growth as compared to the control. In CPSG-2730, a 7% increase in shoot length was observed with T1 (PB-St2), while 14% increase in shoot dry weight was obtained with T2 (SB-1).

The results of the present study indicate that plant growth was significantly ($p<0.05$) enhanced when they were inoculated with selected growth-promoting bacterial strains as compared to non-inoculated control group. Among different treatments, the individual *P. aurantiaca* (PB-St2) inoculation had a pronounced (positive) effect on all growth parameters.

Fig. 2 Population of *Pseudomonas* and *Bacillus* strains ($\times 10^8$ CFU/g soil) in the rhizosphere of pot growing sugarcane plants at different time intervals (0, 30, 60 and 90th day)



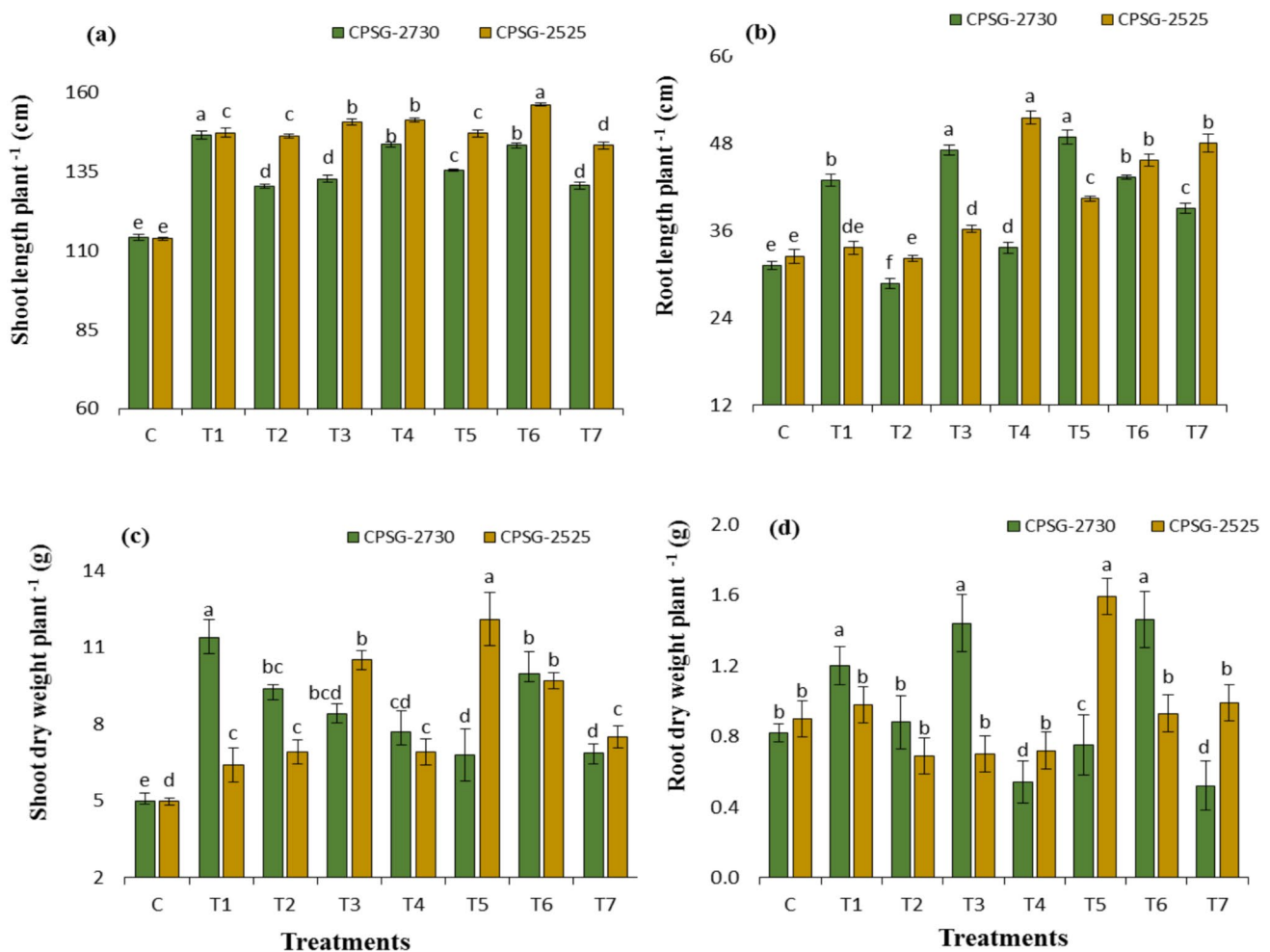


Fig. 3 Effect of biopriming with Plant growth promoting rhizobacteria on (a) shoot length; (b) root length; (c) shoot dry weight and (d) root dry weight of sugarcane lines CPSG-2730 and CPSG-2525. The value of each treatment is mean ($\pm$ S.E.) of five replicates. Different small letters indicate the significant difference among treatments of

both sugarcane lines separately according to DMRT at 5% level of significance. Control (C) means non-inoculated plants. T1: PB-St2, T2: SB-1, T3: CRN8, T4: PB-St2+SB-1, T5: PB-St2+CRN8, T6: SB-1+CRN8 and T7: PB-St2+SB-1+CRN8

However, other treatments resulted in variable growth response in both sugarcane lines. Interestingly, CPSG-2525 exhibited better foliar growth than CPSG-2730, while the root growth was significantly ($p < 0.05$) improved in CPSG-2730 with PGPRs application in comparison with the control. Biopriming was found to be most effective method for promoting foliar growth, whereas rhizospheric application of PGPRs significantly ($p < 0.05$) enhanced root growth in both sugarcane lines.

3.4 Effect of PGPRs Inoculation on Selected Biochemical Attributes of Two Sugarcane Lines

The results regarding biochemical attributes of sugarcane lines with PGPRs priming are presented in Fig. 6a, b, and c. In case of CPSG-2730, co-inoculated with PB-St2+CRN8 (T5) supported a substantial increase in total chlorophyll

content (1.09 to 3.78 mg/g) whereas in CPSG-2525, a significant increase (2.98 mg/g) for chlorophyll content was observed with T1 (PB-St2) inoculation than control (1.5 mg/g). Total soluble sugars (TSS) were found to be improved significantly ($p < 0.05$) from 3.15 to 7.54 mg/g with T1 (PB-St2) in line CPSG-2525 while in CPSG-2730, T2 (SB-1) inoculation yielded the highest value (5.72 mg/g) than control (3.03 mg/g). T1 (PB-St2) priming of the line CPSG-2525 led to a significant increase (52.21 mg/g) in sucrose concentration than non-inoculated control plants (17.22 mg/g). In CPSG-2730, both T1 (PB-St2) and T7 (PB-St2+SB-1+CRN8) showed a similar response, with a non-significantly ($p > 0.05$) different value (24 mg/g) for sucrose content.

Significant ($p < 0.05$) difference was observed in biochemical parameters of sugarcane plants with foliar application of PGPRs as compared to non-inoculated control.

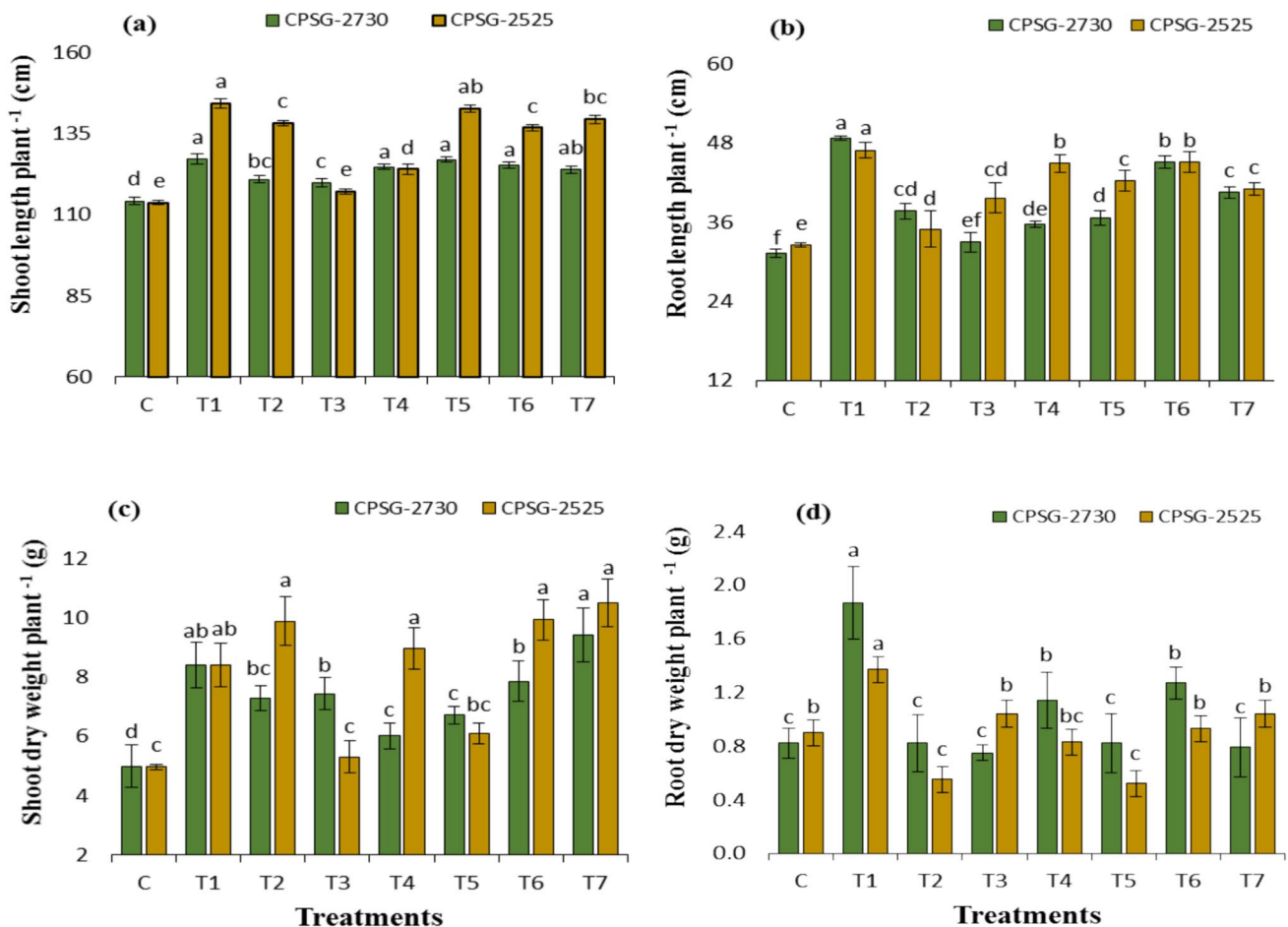


Fig. 4 Effect of foliar application of Plant growth promoting rhizobacteria on (a) shoot length; b root length; c shoot dry weight and (d) root dry weight of sugarcane lines CPSG-2730 and CPSG-2525. Different small letters indicate the significant difference among

treatments of both sugarcane lines separately according to DMRT at 5% level of significance. Control (C) means non-inoculated plants. T1: PB-St2, T2: SB-1, T3: CRN8, T4: PB-St2+SB-1, T5: PB-St2+CRN8, T6: SB-1+CRN8 and T7: PB-St2+SB-1+CRN8

Among provided treatments, T1 (PB-St2) was identified as the most effective inoculant for both sugarcane lines (Fig. 6d, e, and f). In CPSG-2730, maximum total chlorophyll (1.58 mg/g) was recorded along with total soluble sugars (5.67 mg/g) and sucrose content (24.69 mg/g). Similarly, for CPSG-2525, highest values for chlorophyll content (3.15 mg/g), total soluble sugars (6.33 mg/g) and sucrose content (34.38 mg/g) were observed with foliar inoculation of T1 (PB-St2), as compared to control plants.

In rhizospheric application, T1 (PB-St2) was the most effective PGPR for all the noted biochemical parameters in both sugarcane lines. In CPSG-2525, maximum values for chlorophyll content (2.41 mg/g), total soluble sugars (6.89 mg/g) and sucrose content (25.91 mg/g) in fresh leaves were noted than the non-inoculated control plants. As for the sugarcane plants of line CPSG-2730, chlorophyll content increased by 1.8 mg/g when compared to the control (1.09 mg/g), while it was slightly reduced

(0.98 mg/g) with T7 (PB-St2+SB-1+CRN8) inoculation. Similarly, highest concentration of TSS (5.78 mg/g) and sucrose content (20.03 mg/g) was observed in plants with T1 (PB-St2) inoculation as compared to the control (Fig. 6g, h, and i).

From the above, it may be concluded that biopriming is probably the most suitable inoculation method which has resulted in noteworthy increase in biochemical attributes. Among the selected sugarcane lines, CPSG-2525 performed better with all the PGPR treatments as compared to CPSG-2730 and non-inoculated control group. Among individually inoculated strains, *P. aurantiaca* (PB-St2) was noticed as the promising strain with significant increase ($p < 0.05$) in chlorophyll, TSS and sucrose content. Moreover, among consortia, T7 (PB-St2+SB-1+CRN8) in CPSG-2525 and T4 (PB-St2+SB-1) in CPSG-2730 exhibited the noteworthy results over control.

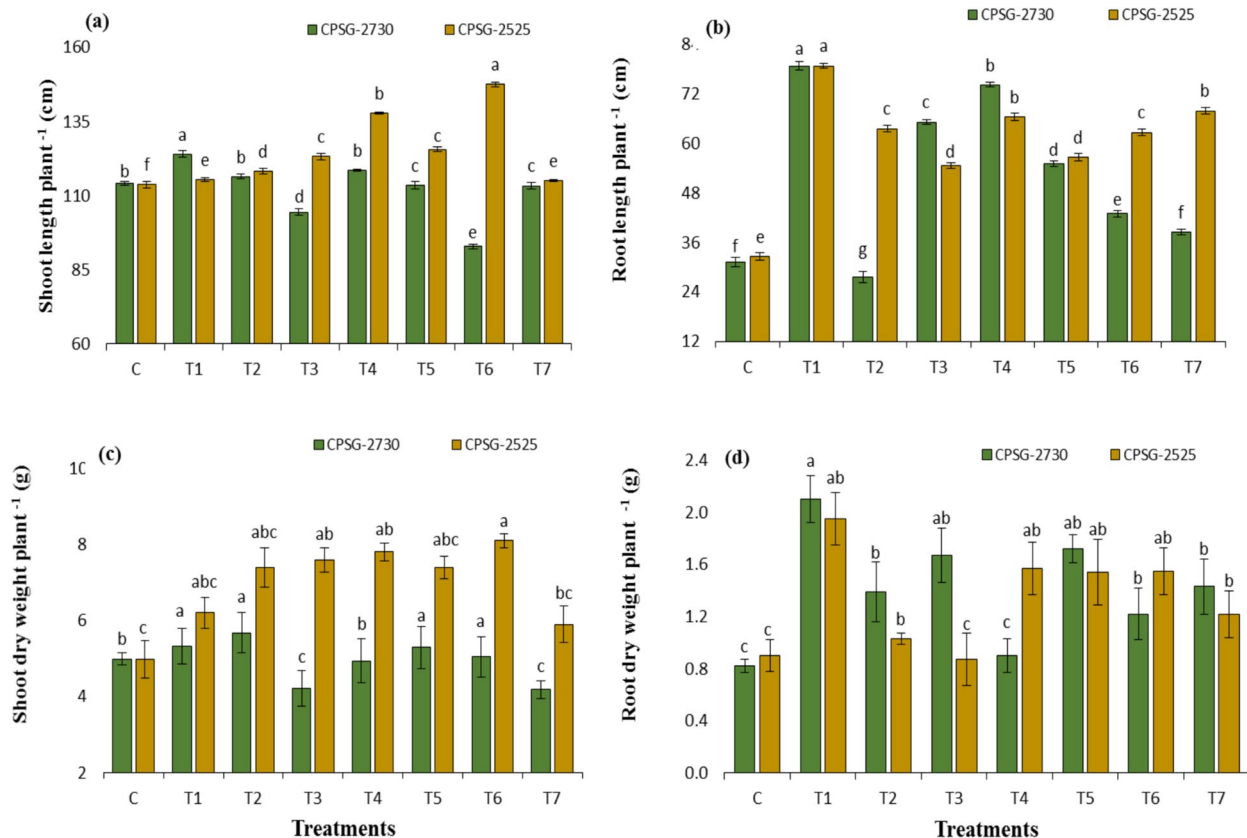


Fig. 5 Effect of rhizospheric application of Plant growth promoting rhizobacteria on (a) shoot length; b root length; c shoot dry weight and (d) root dry weight of sugarcane lines CPSG-2730 and CPSG-2525. Different small letters indicate the significant difference

3.5 Multivariate Analysis

The Pearson's correlation between different agronomical attributes of sugarcane lines exhibited the association at different significance levels, *i.e.*, very strong ($r=0.86–1.00$; $p_{\leq 0.001}$), strong ($r=0.71–0.85$; $p_{\leq 0.05}$) and moderately strong ($r=0.55–0.7$; $p_{\leq 0.01}$) with each other. The shoot length was strongly correlated ($r=0.99–0.95$; $p_{\leq 0.001}$) with shoot fresh weight (SFW), shoot dry weight (SDW), chlorophyll (Chl) and sucrose (Suc) content. Likewise, positive correlation ($r=0.98–0.90$; $p_{\leq 0.001}$) was found between SFW, SDW, Suc and Chl content. SDW showed strong association ($r=0.82, 0.84$; $p_{\leq 0.001}$) with Chl and Suc content, respectively. Furthermore, root length (RL) exhibited very strong correlation ($0.91, 0.93$; $p_{\leq 0.001}$) with root fresh weight (RFW) and root dry weight (RDW). TSS had non-significant ($p > 0.05$) correlation with morphological parameters. Chlorophyll content (Chl) showed very strong positive and significant correlation ($r=1.00$; $p_{\leq 0.001}$) with Suc content followed by total soluble sugars (TSS; $r=0.75$; $p_{\leq 0.05}$), whereas, moderately strong correlation ($r=0.72$; $p_{\leq 0.05}$) was found among Suc and TSS.

among treatments of both sugarcane lines separately according to DMRT at 5% level of significance. Control (C) means non-inoculated plants. T1: PB-St2, T2: SB-1, T3: CRN8, T4: PB-St2+SB-1, T5: PB-St2+CRN8, T6: SB-1+CRN8 and T7: PB-St2+SB-1+CRN8

Nonetheless, root parameters (RL, RFW and RDW) were non-significantly ($p > 0.05$) and negatively correlated with shoot (SL, SFW, SDW) and physiological (Chl, TSS and Suc content) parameters (Fig. 7).

3.6 Principle Component Analysis (PCA)

The first biplot (Fig. 8a) presents the interaction of different treatments and observed variables. In this combined plot, PC1 described the maximum (60%) variation, while second biplot (PC2) showed 13.41% contribution. The observed variables (morpho-chemical) distributed adjacent to each other in the same quadrante showing significantly positive association than the distant observations. Among all treatments, T1 (PB-St2) exhibited significant effect on measured plant growth parameters followed by T2 (SB-1) in both sugarcane lines. T4 (PB-St2+SB-1) and T7 (PB-St2+SB-1+CRN8) in CPSG-2730, whereas T4 in CPSG-2525 present in the same quadrante as that of control, indicated a non-significant response in comparison with control. V1-T1 and V2-T6 showed the positive correlation with RL, RFW and RDW. Shoot parameters (SL, SFW and SDW) observed to

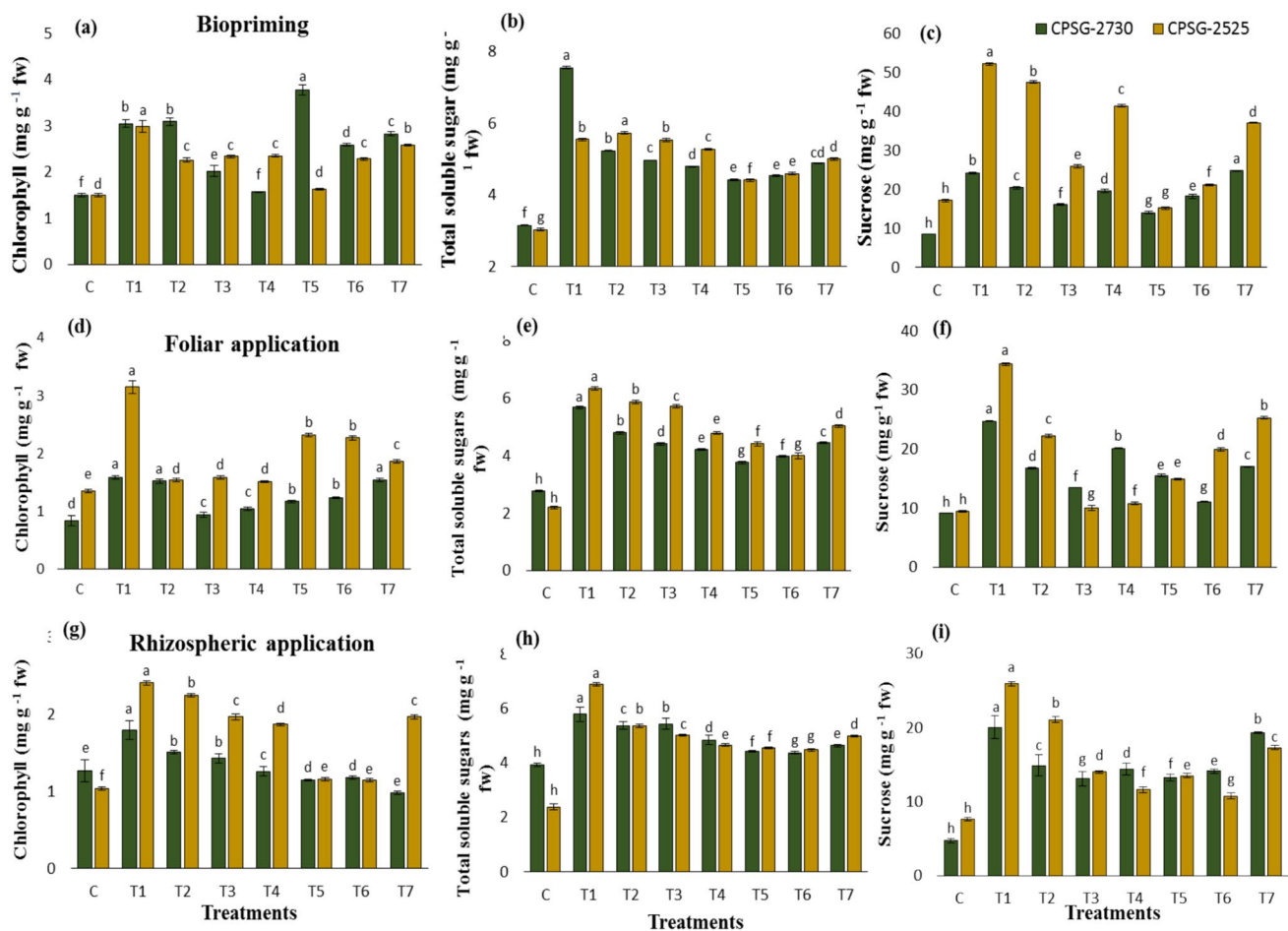


Fig. 6 Effect of plant growth promoting rhizobacteria on chlorophyll content, total soluble sugars and sucrose content under three application methods, i.e., biopriming (a-c), foliar (d-f) and rhizospheric (g-i) applications, respectively in two sugarcane lines CPSG-2730 and CPSG-2525. Different small letters indicate significant difference

be significantly influenced and correlated with V2-T5 and V2-T7, whereas TSS, Suc and Chl content showed strong association with V2-T1 followed by V2-T2 and V1-T2 when compared with control.

The interaction of different application methods in selected sugarcane lines along with observed variables is described in second PCA-based biplot (Fig. 8b). The combined biplot showed 83.92% of total variation in which the maximum variation (62.27%) was contributed by PC1, while PC2 showed 21.65%. The correlation analysis within the PCA highlights the biological significance of various inoculation methods on sugarcane lines. Biopriming was found to be the most effective method, showing positive correlations with TSS, Suc, SFW, and SL. Rhizospheric application showed positive correlation with RL, RFW and RDW and have significant results for root growth in both sugarcane lines. On the contrary, foliar application in CPSG-2730 correlated negatively with all variables, while CPSG-2525 had

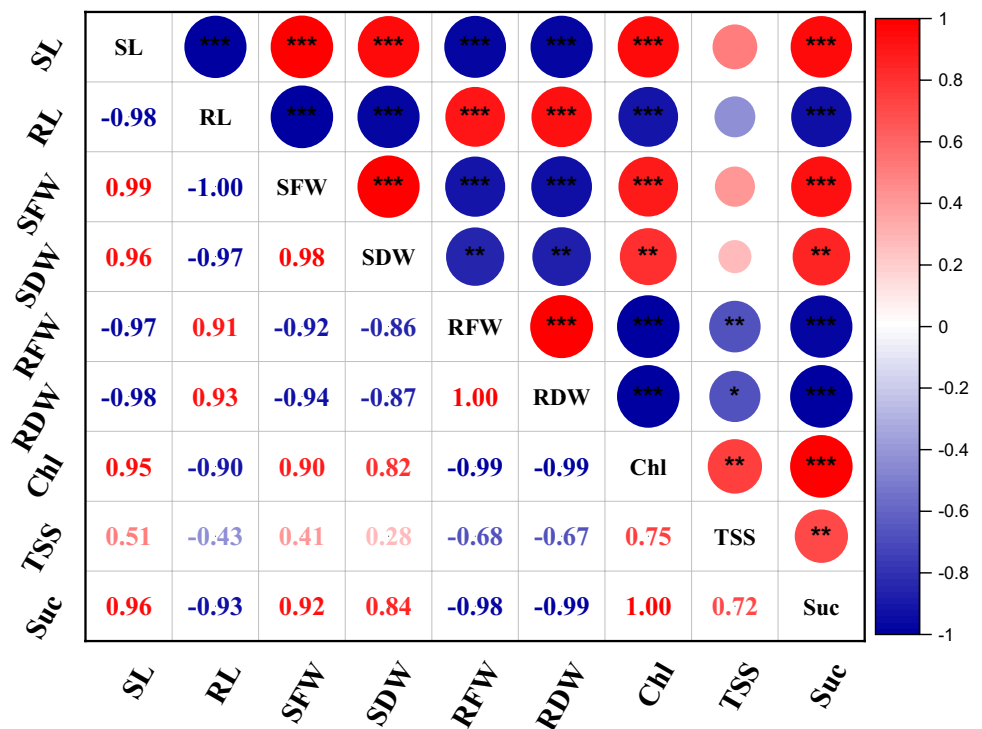
among treatments of both sugarcane lines separately according to DMRT at 5% level of significance. Control (C) means non-inoculated plants. T1: PB-St2, T2: SB-1, T3: CRN8, T4: PB-St2+SB-1, T5: PB-St2+CRN8, T6: SB-1+CRN8 and T7: PB-St2+SB-1+CRN8

positive correlation with SDW only. Notably, treatment T1 (PB-St2) exhibited the most significant impact on measured plant growth parameters in both lines, followed by T2 (SB-1). These findings offer valuable insights for optimizing PGPR application methods in sugarcane production.

4 Discussion

The successful application of bioinoculants for plant growth improvement mainly relies on their ability to show promising results in control conditions and retain their attributes in the field as well (Sessitsch et al. 2019). Many PGPBs have been observed with growth-promoting results in controlled conditions (Maqsood et al. 2021; Hu et al. 2017), while some studies have been extended to the field applications (Ferrarezi et al. 2021; Calvo et al. 2017; Kumar et al. 2017). The effective inoculation is contingent on the successful

Fig. 7 Pearson's correlation plot between different growth and physiochemical traits (SL: shoot length, RL: root length, SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, Chl: chlorophyll content, TSS: total soluble sugars and Suc: sucrose content) with individual and co-inoculation of *Pseudomonas* (PB-St2) and *Bacillus* (SB-1 and CRN8) strains in sugarcane lines CPSG-2730 and CPSG-2525



establishment and proliferation of the inoculants in the host plant's rhizosphere. Colonization of these inoculants in the rhizosphere is a complex process that depends upon plant-microbe interaction, bacterial compatibility, and various environmental factors. During this process, rhizobacteria spread from the initial inoculum, such as seed treatments to the actively growing area and then multiply within the rhizosphere (Ramaekers et al. 2010). The intrinsic capability of bioinoculants to adhere to the plant root surface and their interaction with other rhizospheric bacteria may be positive or negative (Ren et al. 2015). Nonetheless, the establishment of positive interaction among bacterial strains is a prerequisite for their co-inoculation and successful colonization in the rhizosphere.

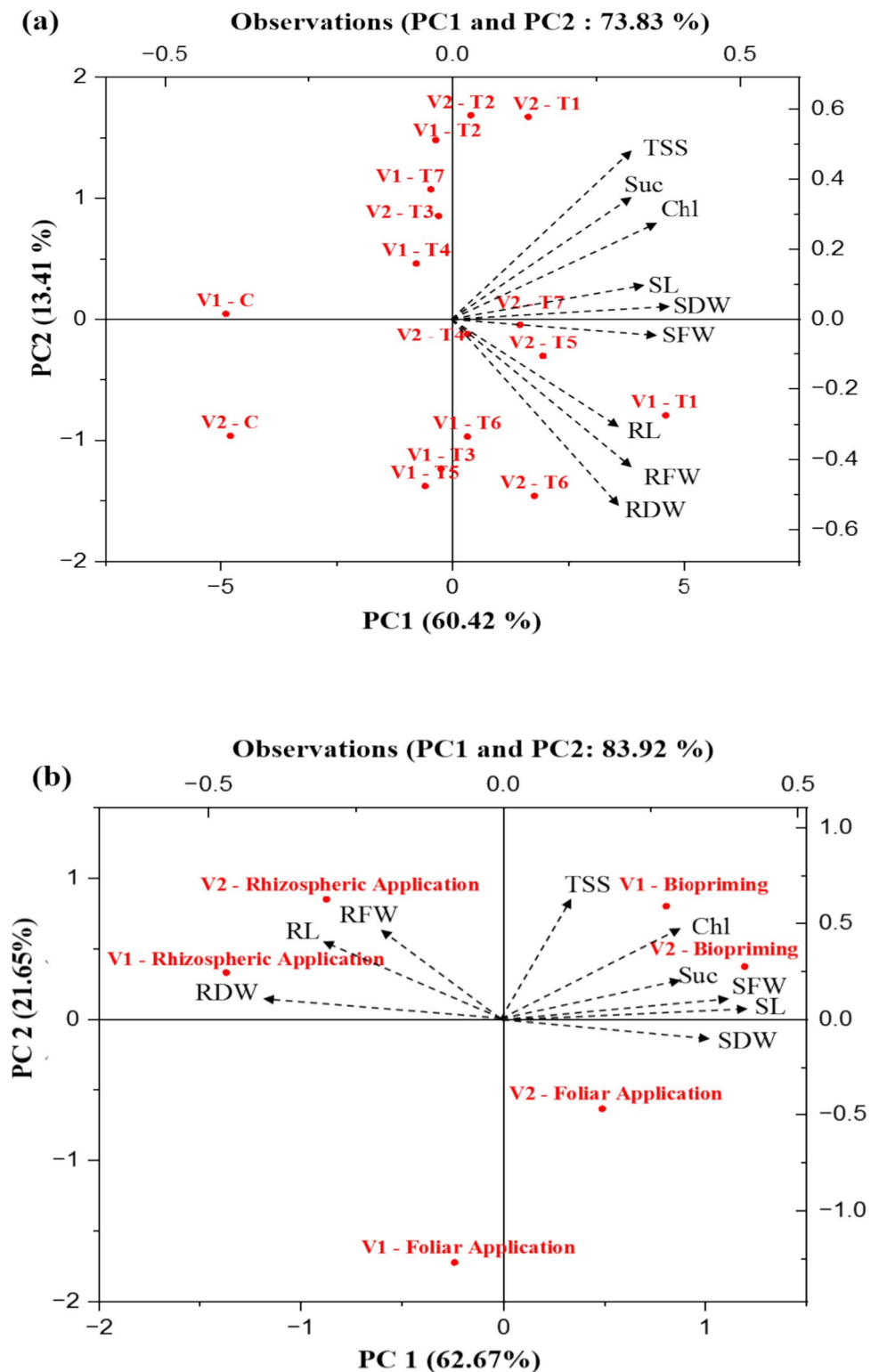
Some bacterial genera with growth-promoting attributes are used in combination. So, before their co-inoculation, it is essential to evaluate their compatibility for successful outcome (Burmolle et al. 2014). To compose consortia, we examined the compatibility of PGPR strains (PB-St2, SB-1 and CRN8) in the in vitro assay with extracellular metabolites of each strain and positive interaction was observed. Our results are similar to the findings of Kumar et al. (2016), who reported the compatibility and synergistic effect of *P. putida* and *B. amyloliquefaciens* in chickpea with their co-inoculation. A positive interaction of fluorescent *Pseudomonas* and *B. licheniformis* was reported in wheat as consortium with PGPR attributes (Ansari and Ahmad 2019). However, a negative interaction among *B. subtilis* and *P. protegens* was also observed, that may depend upon the

strain types, nature and their secondary metabolites (Powers et al. 2015).

In this study, inoculation of plants with rhizobacteria significantly ($p < 0.05$) increased sugarcane plant growth. Among inoculated PGPRs, *Pseudomonas* (PB-St2) was noted as the most effective strain in enhancing most of the observed morpho-chemical parameters, whether applied individually or in consortia than control in both genotypes. *Pseudomonas* and *Bacillus* spp. have also been previously studied and proven as plant growth stimulators (Sutariati et al. 2018). The growth promoting attributes are related to their ability to fix atmospheric nitrogen, production of growth hormones and dissolve phosphate in the soil (Guyasa et al. 2018; Sutariati et al. 2021). In sugarcane production, PGPRs are promising alternatives of chemical fertilizers with low environmental effect and high cost-effective yield (Spolaor et al. 2016; Santos et al. 2020).

In our study, PGPRs were applied by three different methods to evaluate the best responsive method in terms of sugarcane plant growth and biochemistry. Biopriming has been identified as the most effective and suitable method for PGPRs inoculation followed by rhizospheric and foliar application. Additionally, the results indicate that biochemical parameters such as chlorophyll content, total soluble sugars and sucrose content were observed to be maximum with biopriming in both genotypes than control. Both genotypes exhibited variable responses to the individual and combined inoculations of the applied strains across the different measured parameters. In biopriming, T1 (PB-St2),

Fig. 8 Principal Component Analysis (PCA) biplots representing individual and co-inoculation of *Pseudomonas* (PB-St2) and *Bacillus* (SB-1 and CRN8) strains as treatments (a); and the interactive correlation of different inoculation methods (b) on measured growth and physio-chemical parameters (SL: shoot length, RL: root length, SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, Chl: chlorophyll content, TSS: total soluble sugars and Suc: sucrose content) in two sugarcane lines. V1: CPSG-2730; V2: CPSG-2525, whereas the treatments are; T1: PB-St2, T2: SB-1, T3: CRN8, T4: PB-St2+SB-1, T5: PB-St2+CRN8, T6: SB-1+CRN8 and T7: PB-St2+SB-1+CRN8



T6 (SB-1 + CRN8) and T5 (PB-St2 + CRN8) were more effective treatments with significant increase in plant height and dry biomass. Application of PGPRs through biopriming for a specific time period initiates physiological changes in the seed that helps to increase seed germination (Anitha

et al. 2013). Seed priming with rhizobacterial inoculum is reported to accelerate the germination rate, thereby improving earlier vegetative growth with well-established crop and improved yield (Tahir et al. 2017). Furthermore, this application method has been shown to support plant tolerance

against abiotic stresses like salinity (Ha-Tran et al. 2021). *Bacillus* species are frequently used successfully for priming of different crops like rice, corn, and potato (Brahim et al. 2022). Our findings are similar to the results of Schultz et al. (2014), who applied diazotrophic bacteria (*Azospirillum*, *Gluconacetobacter*, *Herbaspirillum* and *Bulkholderia* spp.) to the commercial sugarcane varieties by immersion of sugarcane mini-setts. The results indicated 13.5% increased stalk yield, 10.9% dry matter along with 13.3% more nitrogen accumulation than control. Increased sugarcane growth along with 40% increase in cane yield was reported with biopriming of endophytic *Pseudomonas* and *Bacillus* strains by Chauhan et al. (2013). However, some studies cited in the literature reported findings that contrast with our observations regarding co-inoculation of PGPRs (Vestberg et al. 2004; Felici et al. 2008; Lobo et al. 2022).

In rhizospheric application, substantial growth enhancement of sugarcane roots was observed with PGPRs inoculation compared with either biopriming or foliar application. Amongst various treatments, T1 (PB-St2) was found to be most effective inoculant, leading to over 100% increase in root growth and biomass. In contrast, other treatments did not show a significant difference compared with the control. Plant roots exude various nutrients and signaling molecules that regulate plant–microbe interactions. Microbes involved in these interactions can affect root development through phytohormone production (Win et al. 2018). In sugarcane, enhanced root growth with PGPRs may result from IAA production, which stimulates root proliferation and promotes the formation of adventitious roots in monocots (Sutariati et al. 2020; Santos et al. 2020). The production of this growth hormone in plants has been shown to increase biomass, leading to improved nutrient uptake and overall plant growth, especially in stressed conditions (Kumar et al. 2016; Sun et al. 2023). Our findings with *Pseudomonas*, either alone or in combination with *Bacillus* are consistent with the findings reported in the literature. González et al. (2015) observed that among various rhizobacteria, *Pseudomonas* strain produced the highest root dry matter in sugarcane. Santos et al. (2018) reported 13% increase in root dry matter with rhizospheric application of *B. subtilis* and *B. pumilus* in sugarcane. *Pseudomonas* is reported to have indoleacetic acid (IAA) production ability, which is a plant growth regulator and used in many crops at field level (Shahid et al. 2017). However, Baharlouei et al. (2011) reported contrasting findings, showing non-significant differences in root dry weight between inoculated and non-inoculated plants.

In foliar application, most of the growth parameters were significantly improved with T1 (PB-St2), while T7 (PB-St2 + SB-1 + CRN8) yielded maximum shoot dry biomass in both sugarcane genotypes. However, plants of T3 (CRN8) showed non-significant results in comparison to

the non-inoculated control plants. Combined application of *Pseudomonas* and *Bacillus* in foliar application on sugarcane variety RB92579 showed 87% increase in total dry mass (Rosa et al. 2020). Effect of *Bacillus* spp. was studied in sugarcane crop by Chandra et al. (2018), who reported increased root growth due to the production of IAA. Rhizospheric bacteria produce secondary metabolites like auxin in response to the substrates released from the roots as compared to the non-rhizospheric soil. As a result, plants inoculated with PGPRs show better and early root growth that supplies more nutrients and water from the rhizosphere and hence helps the tall crops like sugarcane in better anchorage in the soil (Hassan 2009; Vacheron et al. 2013). However, Lobo et al. (2022) studied the co-inoculation of plant growth promoting bacteria on different IAA sensitive and insensitive tomato genotypes and reported contrasting results with no increase in plant dry biomass and in fact reduction in plant development. This could be attributed to the negative interaction among interacting partners. Plant development is influenced by multiple factors and to assess the impact of plant–microbe interaction is a complex process. This process is controlled by various factors such as microbial metabolites and plant exudates, which can lead to altered gene expression among interacting partners and eventually affect plant growth (Sasse et al. 2018).

Higher chlorophyll content was observed in plants inoculated with bacteria than in non-inoculated control. This change can be due to higher nitrogen (N) availability and uptake by the growing tissues from the soil with PGPR inoculation (Chandrasekhar et al. 2005). Similar findings of increased chlorophyll content were observed by Chauhan et al. (2013) in sugarcane with *Pseudomonas* and *Bacillus* spp. inoculation. Higher chlorophyll content in sugarcane ratoon crop was also reported with inoculation of diazotrophic bacteria that could be related with more nitrogen availability and increased plant dry mass. However, contrasting results were observed among different genotypes, with increased N content but without significant increase in chlorophyll content (Matoso et al. 2020). Inoculation of the PGPRs to sugarcane leads to enhanced physiological parameters due to increase in water and nutrients use efficiency, availability of fixed N and solubilized P in the roots. This increase can also be attributed to greater below-ground carbon sink in plants and strong source-sink relationship with PGPR application (Vafadar et al. 2014). Additionally, N availability by nitrogen fixing rhizobacteria also has a positive effect on the chloroplast formation that results in enhanced chlorophyll content in leaves (Chandrasekhar et al. 2005; Singh et al. 2016).

The productivity of photosynthesis is closely linked to the efficiency of chloroplast function, the conductance of stomata, and the rate of net CO₂ assimilation (Seema et al. 2018). As a consequence of high chlorophyll content in

leaves, the photosynthetic rate becomes high and production of photosynthates, *i.e.*, total soluble sugars and sucrose also increases. Variable total soluble sugars were observed among different treatments of this study, which indicated pronounced effect of bio-inoculants on carbohydrates. Improved sugar level in the leaves is of particular value due to their importance in photosynthesis, translocation and respiration (Rao et al. 2007). Thus, inoculation of *Pseudomonas* and *Bacillus*, individually or in consortium, possibly provided the sugarcane with more nutrients' uptake that in turn, improved CO₂ fixation through photosynthesis. It may be inferred that in sugarcane, higher carbon assimilation through photosynthesis leads to greater sucrose accumulation in the stem (Souza et al. 2008; Kleingesinds et al. 2018).

The variable results observed in plant growth and physiology in the current study align with existing literature on the impact of PGPR inoculation on plants (Saad et al. 2020; Ferrarezi et al. 2021). This consistency with previous findings highlights the importance of considering different PGPR application methods in order to achieve optimal outcomes. In our study, most of the physiological parameters were observed significantly improved with individual PGPR inoculation as compared to the use of consortia and biopriming was found to be the most effective method for both genotypes. These variations with PGPRs treatments might be attributed to several factors, such as type of application methods, different mechanisms and plant development influenced by PGPRs inoculation, biotic and abiotic stress, and interaction with soil microbiota. Moreover, certain studies indicate that the response of PGPRs as growth stimulators may also be influenced by the plant genotype (Vidotti et al. 2019). Contrasting effect of denitrifying PGPR was observed among different soil types (Florio et al. 2017). Marks et al. (2015) compared seed inoculation and foliar spray as application methods of lipo-chito oligosaccharides (LCOs) metabolites and found variable results regarding maize grain yield. Another study conducted by Fukami et al. (2016) reported difference in nitrogen content with different application methods of nitrifying bacteria and concluded that each application method has some limitations under certain conditions.

Survival of inoculated plant growth promoting rhizobacteria in the soil is a major concern for positive plant–microbe interaction and growth improvement. Their establishment in the rhizosphere is the first step that is necessary for the root colonization of host plants. It is a multifarious progression that involves bacterial traits, genes and rhizospheric environment (Jha and Subramanian 2013). Among concerned inoculants, *P. aurantiaca* (PB-St2) showed the highest survival rate followed by *B. amyloliquefaciens* (SB-1) and *B. subtilis* (CRN8). Moreover, their population density was decreased, as the sugarcane plants mature. The survival of the inoculants is reported to be higher in the rhizosphere during early

plant development stages than maturity (Mukhtar et al. 2017). Ability of the bacterial strains to interact with plants for colonization is probably more important than their count. The competent strains survive better and are also efficient in root colonization (Santos and Rigobelo 2021). This trend is expected to be influenced by the depletion of nutrients and unfavorable environmental conditions that hinder bacterial survival (Shoaib et al. 2020). Moreover, the metabolites produced by the microorganisms themselves contribute to the decrease in bacterial count in the rhizosphere, indicating a general behavior within the microbial community (Bogino et al. 2013). Furthermore, physico-chemical properties of soil, plant root exudates, and microbial population are some of the other factors that dictate the plant–microbe interaction, rhizobacterial growth and extent of their survival in the rhizosphere (Glick 2014).

The positive impact of PGPR inoculation has been observed on both the aboveground as well as belowground plant parts. However, above-ground plant growth gained more importance due to their usage as food and fodder, whereas root plays a key role in providing support, anchorage, nutrient mobilization and biomass accumulation (Bardgett et al. 2014). Furthermore, roots also contribute to the nutrient cycling and rhizospheric microbiome conservation (Grover et al. 2021). Exploring PGPR-mediated modulation of root traits could offer benefits for enhancing agroecosystem efficiency and sustainable agricultural production in the future. In developing countries like Pakistan, farmers lack an authentic biofertilizer for sugarcane in local market. Consequently, they rely on chemical fertilizers and other agrochemicals to enhance crop yield and management. To address this issue in sugarcane, PGPRs showing promising results with most suitable application method were further evaluated in an independent study to assess their field implications (Idrees et al. 2024, unpublished). This endeavor aims to identify the PGPRs capable of effectively competing with indigenous microflora in real-world application. Eventually, these alternatives seek to reduce the use of synthetic fertilizers and promote sustainable sugarcane farming practices.

Plant growth promotion by PGPRs is a complex phenomenon that probably adheres to the “additive hypothesis” (Amaya-Gómez et al. 2020). Thus multiple PGPR mechanisms interact within the soil ecosystem to enhance plant growth and yield. However, PGPRs that perform better under controlled conditions may not replicate similar results in the field conditions. Moreover, PGPRs response may vary across different soil types and even for the same crop. Thus, evaluating the compatibility among PGPRs and their effectiveness in relation to specific soil conditions and crops is decisive for attaining consistent outcomes. Some rhizobacterial strains produce growth-promoting compound in response to some external stimuli, for instance, in response to the root exudates, thereby establishing a positive

relation with the host. Thus, only competent microbes that survive with constant vying in rhizosphere and effectively colonize the plant roots are suitable for use as successful PGPR in the field (Nazari and Smith 2020). The screening of such PGPRs is a technical process that involves isolation, identification and then in vitro testing for their role in plant growth enhancement or as biocontrol agent. Additionally, different bacterial genera may exhibit antagonistic interaction or synergistic effect when applied together. Therefore, it is imperative to assess the compatibility of selected strains before their co-inoculation as bioformulation.

5 Conclusion

Our study explored the competency of one *Pseudomonas* and two *Bacillus* spp. in the growth promotion of sugarcane with different application methods under natural conditions. Amongst various treatments, *Pseudomonas aurantiaca*; PB-St2 was found to be more competent plant growth promoting rhizobacterium as compared to the two tested *Bacillus* spp. in sugarcane. Among the application methods, biopriming was a better choice compared with foliar and soil application. It increased chlorophyll content, enhanced photosynthetic activity and improved carbohydrate assimilation in sugarcane, leading to an increased plant biomass. However, plant root growth parameters were significantly improved through rhizospheric application. Co-inoculation of plant growth promoting rhizobacteria has shown potential to enhance the beneficial effects of different microbial species, which could facilitate the production of biofertilizers for future commercialization. Consequently, this approach could potentially decrease the reliance on chemical fertilizers in sugarcane farming.

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Author Contribution All authors contributed in the conception and design of this study. Experiment conduction, data collection and analysis were performed by SI under the supervision of SM and FA. The first draft was written by SI while further improvement and proof reading were done by SM and FA. The final manuscript was approved by all authors.

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Data Availability The datasets used and analyzed in the current study are available on reasonable request from the corresponding author.

Declarations

Conflict of Interest The authors have no competing financial or personal conflict in the presented research work.

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