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Research article

Sphingomonas panaciterrae PB20 increases growth, photosynthetic pigments, antioxidants, and mineral nutrient contents in spinach (*Spinacia oleracea* L.)

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) have been intensively investigated in agricultural crops for decades. Nevertheless, little information is available on the application of *Sphingomonas* spp. as a PGPR particularly in vegetables, despite of potential plant growth promoting traits of this group. This study investigated the role of *Sphingomonas panaciterrae* (PB20) on growth and nutritional profile of spinach applied through seed priming (SP), soil drenching (SD), foliar application (FA), and bacterial culture filtrate foliar (BCF) applications. The results showed that, depending on different methods of application, PB20 significantly increased plant height (19.57–65.65 %), fresh weight (7.26–37.41 %), total chlorophyll (71.14–192.54 %), carotenoid (67.10–211.67 %) antioxidant (55.99–207.04), vitamin C (8.1–94.6 %) and protein content (6.7–21.5 %) compared to control in the edible part of spinach. Among the mineral nutrients, root nitrogen (N) showed greater response to bacterial application (18.65%–46.15 % increase over control) than shoot nitrogen (6.70%–21.52 % increased over control). Likewise, in all methods of application, phosphorus (P) content showed significant increase over control both in root (42.79–78.48 %) and in shoot (3.57–27.0 %). Seed priming and foliar application of PB20 increased the shoot calcium (Ca) content compared to control. BCF foliar application yielded maximum magnesium (Mg), iron (Fe) and zinc (Zn) in shoot. However, seed priming resulted in maximum Fe in root. Overall, seed priming outperformed in growth, vitamin C, antioxidants, N and P uptake, while BCF foliar application resulted in better uptake of several nutrients. Multivariate analysis validated the positive association of most of the growth parameters with SP while several nutrients with FA and BCF. Based on the findings it is evident that this rhizobacteria PB20 has the potentiality to be applied as a biofertilizer to produce nutrient-enriched spinach with an improved yield. Farmers can conveniently incorporate PR20 through seed priming before planting of spinach, with additional benefits through foliar spray.

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1. Introduction

Agricultural production is increasingly constrained because of infertile and contaminated soil, less availability of nutrients, and attacks by pests. Farmers depend on the use of agrochemicals to enhance crop production. Nevertheless, the over dependence of farmers on agrochemicals henceforth restricts their agricultural productivity. The overuse of fertilizers and pesticides leads to a shortage of available nutrients, soil and water contamination, and the loss of the natural microbiome that inhabits soil, and ultimately affects human health through direct exposure of agrochemicals or through food chain contamination [1–3]. Additionally, the over-application of chemical fertilizers is a contributing factor to both climate change and greenhouse gas emissions [4,5]. The increasing need for healthy food on a worldwide scale has pushed researchers to look for new ways to boost the nutritional content and yield of their crops and to reduce the negative effects associated with the excess application of chemical inputs [6]. Scientists and researchers have been looking into plant growth-promoting rhizobacteria (PGPR) as a possible answer to this problem in recent years.

PGPRs are beneficial bacteria that colonize plant roots and are an essential component of the millions of rhizospheric biota [7–9]. PGPRs help plants through numerous mechanisms. These bacteria are usually associated with the plant rhizosphere or colonize the plant cell through endophytism. PGPR are also reported to produce indole acetic acid [10,11], siderophore [12], and other phytohormones that promote plant growth [13,14]. They combat the limited availability of nutrients and biotic and abiotic stress through increasing root growth and various other processes such as biological nitrogen fixation [15], phosphate solubilization [16], indole acetic acid (IAA) production [17], ACC deaminase activity [18], antipathogenic activity [19], hydrogen cyanide (HCN) production, metal tolerance, and induction of systemic resistance [20,21]. Some bacteria can increase plant antioxidant enzyme content and activity [22–24]. These attributes of PGPR make them useful as biofertilizers and biopesticides and reduce the reliance on agrochemicals [25,26].

Among the well-documented PGPR, several isolates of *Bacillus* [27–30], *Pseudomonas* [31], and *Rhizobium* [32–34] have been extensively investigated and strains from these groups have recently become commercially available as biofertilizers [35]. Aside from these large groups, *Azotobacter* [36,37], *Bradyrhizobium* [38,39], *Mesorhizobium* [40,41], *Azospirillum* [42], *Stenotrophomonas* [43], *Actinobacter* [44], and *Lactobacillus* [45] have been reported to possess a variety of plant growth promoting traits, and their effect on plant growth promotion in a wide range of crops has also been determined.

The genus *Sphingomonas* is composed of aerobic yellow-pigment producing bacteria that is ubiquitous in nature and belongs to α-Proteobacteria [46]. Species of *Sphingomonas* were isolated from soil as poly aromatic hydrocarbon (PAH) degrading bacteria [47,48], xenobiotic degrading bacteria [49], and nitrogen fixing bacteria [50]. Previous studies have documented the impact of *Sphingomonas* spp. on cadmium accumulation [51], tomato plant growth [52], drought resistance in Arabidopsis [53], metal phytoremediation and plant growth promotion in rice [54], and increasing nodulation in pea [55]. It has been documented that some species in the genus promote the growth of plants in stressful environments such as salt, drought, and heavy metals in agricultural soil [56,57]. While the use of PGPR in agriculture is now a well-established concept, research on the use of *Sphingomonas* spp. as plant growth promoting bacteria is relatively unexplored and new. We have previously isolated *S. panaciterrae* (PB20) from the rice ecosystem. This strain has remarkable potential as a plant growth promoter because it can produce indole acetic acid (IAA), fix nitrogen, solubilize phosphate, and improve seed germination and root growth promotion in rice seedlings in vitro [58]. To our knowledge, there is no information regarding the application of *S. panaciterrae* to vegetable crops, particularly leafy vegetables.

Spinach (*Spinacia oleracea* L.), known as a superfood, is an extremely popular leafy vegetable. It is enriched in vitamin A, B2, B6, B9, C, folic acid, dietary fiber, and minerals [59,60]. It is a very good source of antioxidants [61]. Enhancing the growth and nutrient uptake of spinach plant can significantly improve its nutritional quality and contribute to meeting the dietary requirements of a growing population. Utilizing PGPR in this regard offers a lot of promise for producing spinach with a higher yield and nutrition.

PGPR may be applied for crop improvement in a variety of ways, including seed treatment, soil inoculation, and foliar spray. Each approach has benefits and disadvantages, including compatibility with strain, simplicity of application, and colonization effectiveness. Consequently, finding the best technique for *S. panaciterrae* inoculation in spinach plants is crucial to achieving the best outcomes. Therefore, in this study, we applied our in-house *S. panaciterrae* (PB2) strain to spinach using four methods: namely seed priming, soil drenching, foliar application, and bacterial culture filtrate (BCF) foliar application to investigate the growth promoting efficiency of *S. panaciterrae* PB20 on spinach and finding out the most effective method of application of this PGPR in spinach.

2. Materials and methods

2.1. Plant and soil

The spinach seeds were immersed in bacterial solution for approximately 24 h. A separate batch of seeds (control) was treated with warmed distilled water only. Subsequently, the seeds were wrapped with sterilized tissue paper and placed in a warm environment for three days. Afterwards, the seeds were carefully sown in the soil to initiate germination.

Fresh field soil was collected from 0 to 15-cm depth. Unwanted materials like stones, gravels, pebbles, and plant roots were removed from the bulk soil. Then, the soil was air-dried for several days, and the clods were broken and sieved. Soil pH and EC were measured in a 1:2.5 suspension of soil and water, as described by Tan [62]. Before being used for the pot experiment, the soil was analyzed for initial nutrient status. Total N was determined from the soil extract by the semi-micro Kjeldahl method as described by Bremner [63]. Available P was determined from the initial soil extracted following the method of Olsen et al. [64]. Exchangeable K was determined from the IN NH_4OAc (pH 7.0) extract of the soil as described by Black [65] using a flame photometer. Exchangeable Ca and Mg were also determined form NH_4OAc extract by the titrimetric method [66]. The physicochemical characteristics of the soil is shown

in Table 1. Pots of 18 cm deep, 15 cm diameter at the top, and 13 cm diameter at the bottom were used for transplanting the seedlings. Each pot contained 4 kg of soil mixed with urea, Triple Super Phosphate (TSP), Muriate of Potash (MOP), Gypsum ($CaSO_4$) at the recommended dose for spinach according to the Fertilizer Recommendation Guide of Bangladesh [67].

2.2. Bacterial isolate

The bacterium was isolated from barnyard grass rhizosphere collected from a rice field in our previous experiment. The 16s partial sequence data of the isolate PB20 (NCBI accession MZ540036) and matched reference species were retrieved from National Center for Biotechnology Information (NCBI) database. The Geneious V.11's MUSCLE plug-in was used for multiple alignments. Phylogenetic tree was reconstructed by maximum likelihood analysis using Geneious Prime Version 2023.1.2 RAxML plug-in using rapid bootstrapping and searching for the best scoring ML tree from 1000 bootstrap replicates in the GTR-GAMMA model. The reconstructed phylogenetic tree is presented in Supplementary Fig. S1. The bacterial solution was prepared at approximately 10^8–10^9 CFU/mL for seed priming and for other methods of bacterial application.

2.3. Experimental procedure

The spinach seeds, with and without bacterial priming, were grown in the pots for 45 days after the emergence of the seedlings from the soil. The usual time for harvesting most varieties of spinach (40–60 days) was taken into consideration while choosing the growth period. After 7 days of sprouting, thinning was done, and eight seedlings were kept in each pot. There were four treatments of bacteria *viz.*, seed priming (SP), soil drenching (SD), foliar application (FA), and bacterial culture filtrate (BCF) foliar application, along with the control. The experimental was done in a completely randomized design (CRD) with four replications. After 15 days of sowing, the seedlings were treated with bacterial solution through soil drenching, foliar application, and BCF foliar application. The seedlings were sprayed with water, and weeding was done as and when necessary. The seedlings were harvested after 45 days of sowing. The above-ground edible part of spinach as well as the root of the spinach were harvested separately. The root and shoot of the seedlings were washed with tap water, followed by distilled water. After taking the fresh weight and plant height, the sample was placed in an oven for 48 h at 70 °C, and the subsequent dry weight was taken.

2.4. Determination of total antioxidant, vitamin C, chlorophyll and carotenoid contents

Total antioxidant was determined by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging method [68]. The DPPH solution was added to the methanolic plant extract and to the blank. The absorbance of methanolic extracts of the plant samples and the blank was measured at 517 nm. Antioxidant activity was measured as % inhibition by calculating the change in absorbance in the plant extract from blank absorbance.

Vitamin C was determined by the indophenol dye extraction method [69]. This procedure is based on the quantitative discoloration of 2,6 dichlorophenol indophenol by ascorbic acid. Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid were determined spectrophotometrically with the procedure developed by Arnon [70] and Lichtenthaler and Wellburn [71].

2.5. Extraction of plant sample for nutrient analysis

Spinach plant samples were extracted through the wet-oxidation method [72] using di-acid mixture (concentrated nitric acid (HNO_3) and 60 % perchloric acid ($HClO_4$) in 2:1 ratio). Exactly 0.5 g of plant sample was taken in a conical flask. A volume of 10 ml of di -acid mixture was added to it and kept overnight. The sample was then digested in a sand bath in a digestion chamber at 100 ± 5 °C until white fumes evolved, and the solution became clear. When approximately 1 ml of clear liquid remained at the bottom of the conical flask, the aliquot was washed with distilled water, and volumed up to 100 mL, and kept for nutrient analysis. For total N analysis, the plant sample was extracted separately in a block digester at 450 °C using concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) in presence of a catalyst mixture of potassium sulphate (K_2SO_4), copper sulphate ($CuSO_4.5H_2O$), and selenium powder [63].

Table 1
Physicochemical characteristics of initial soil.

Soil Parameters	Values
Texture	Silty loam
pH (1:2.5 soil/water)	6.80 ± 0.31
EC (μs/cm)	101.6 ± 4.9
Organic matter (%)	0.99 ± 0.075
Total Nitrogen, N (%)	0.1 ± 0.01
Total P (mg kg^{-1})	273.33 ± 19.1
Exchangeable potassium, K (meq/100g)	0.14 ± .03
Exchangeable Ca (meq/100g)	4.9 ± .98
Exchangeable Mg (meq/100g)	1.50 ± 0.37

2.6. Analysis of the samples for macronutrients

The estimation of total-N was performed by the semi-micro Kjeldahl method [63]. In this method, organic nitrogen in the sample was converted into ammonium sulphate during digestion. Ammonia liberated by making this solution alkaline was distillated into a known volume of boric acid and mixed indicator solutions, which were then titrated against standard H_2SO_4. Phosphorus of the plant samples was determined colorimetrically by the stannous chloride method, as stated by Jackson [72]. Sulphur in the spinach samples was determined turbidimetrically with spectrophotometer (Model: TG-60 U) at 425 nm wavelength, as described by Tandon [73]. Amount of potassium was determined from the aliquot with the help of flame emission spectrophotometer (Model: JENWAY-PFP7) at 768 nm as suggested by Ghosh et al. [74]. The amounts of calcium and magnesium were determined by the complexometric method of titration [66].

2.7. Analysis of the samples for micronutrients

Spinach root and shoot were analyzed for micronutrients Fe and Zn by an atomic absorption spectrophotometer (AAS, Shimadzu AA-700) with a recovery of 0.2 ppb. Standard solutions of AAS-grade Fe and Zn were used as reference. During the analysis a reagent blank was used with the samples. The relative standard deviation (RSD) was set to 2 % prior to analysis. The recovery percentage was 95%–105 %.

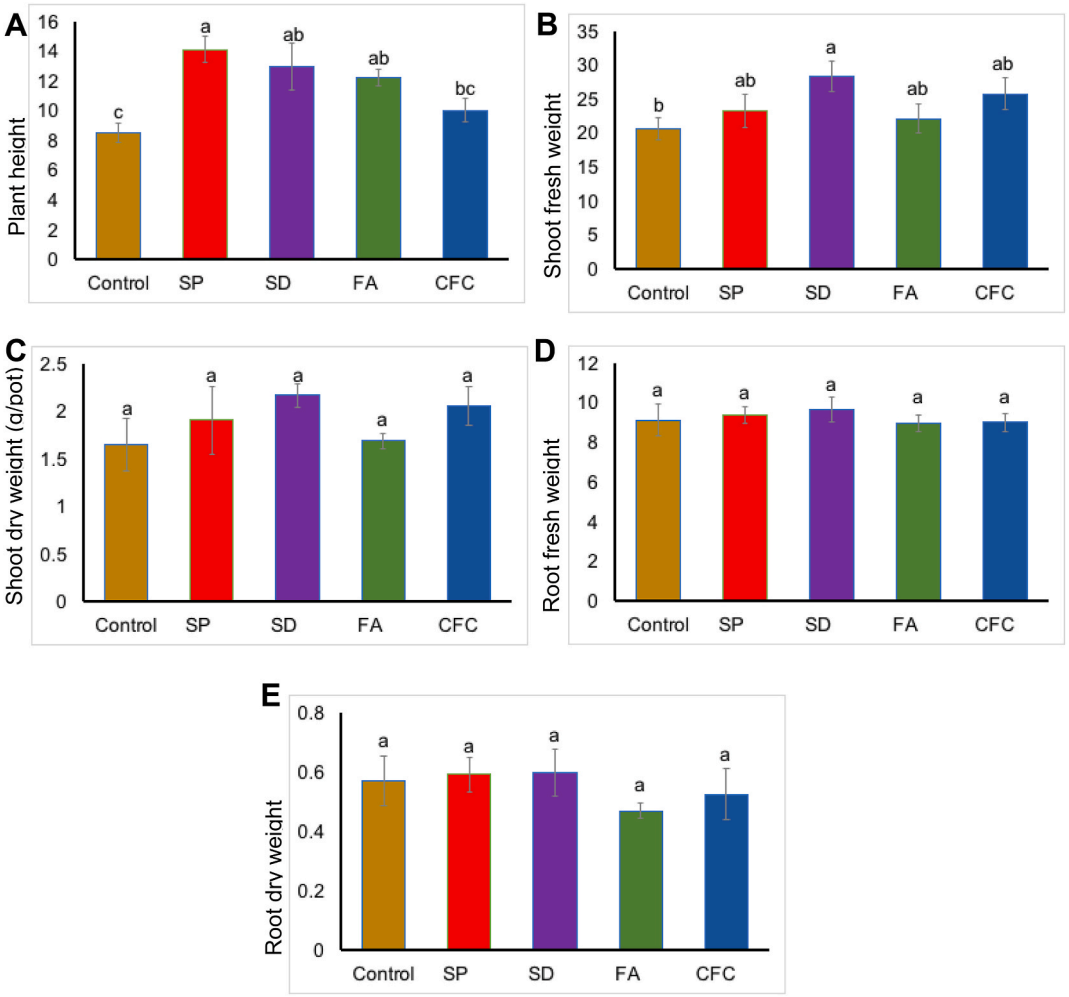

Fig. 1. Growth of spinach as influenced by application of *S. panaciterrae* PB20. A) Shoot fresh weight B) Root fresh weight C) Shoot dry weight D) Root dry weight and E) Plant height. Bars indicate the ±standard error of the means (n = 4). The columns with the same letter are not significantly different at $P < 0.05$ as determined by Tukey's test. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

2.8. Statistical analysis

The data were analyzed using statistical software 'R version 3.4.2'. Results were expressed as the mean of four replicates ± standard error. One-way analysis of variance (ANOVA) and post-hoc analyses were conducted to determine significant variations among the treatments (LSD, $P < 0.05$) using the 'agricolae' package in 'R version 3.4.2'. Hierarchical clustering was carried out using the "pheatmap" package.

3. Results

3.1. PB20 promoted plant height and biomass of spinach

The application of PB20 increased the plant height of spinach regardless of the application method (Fig. 1). Seed-primed spinach produced the tallest plant, with a 65 % increase in plant height over the control (Fig. 1A). The plant height was increased by 52 %, 42 %, and 19 % in the soil drenching, foliar application, and BCF foliar application treatments, respectively, compared to the control. In terms of the fresh and dry weights of the edible portion of spinach, soil inoculation with PB20 produced the highest fresh and dry weights, followed by BCF foliar application, seed priming, and foliar application (Fig. 1B and C). No significant difference was found in the fresh and dry weight of the root (1D, 1E). Similar findings were also reported by Luo et al. [53], who observed that soil inoculation of *Shingomonas* sp. Cra20 in *A. thaliana* under well-watered conditions resulted in an increase of 61.97 % above-ground fresh weight and 74.63 % shoot dry weight.

3.2. PB20 increased the production of photosynthetic pigments in spinach

Fig. 2 showed that bacterial treatments affected the photosynthetic pigments chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids in spinach. All these attributes were significantly increased over the control due to bacterial application. Total chlorophyll and chlorophyll *b* content were found the maximum in bacterial foliar application followed by BCF foliar application, soil drenching and seed priming (Fig. 2B and C). In the case of chlorophyll *a*, soil drenching resulted in the maximum and was statistically similar to foliar application and culture filtrate foliar application (Fig. 2A). The maximum amount of carotenoid was also found in soil drenching, followed by bacterial foliar application and culture filtrate foliar application (Fig. 2D).

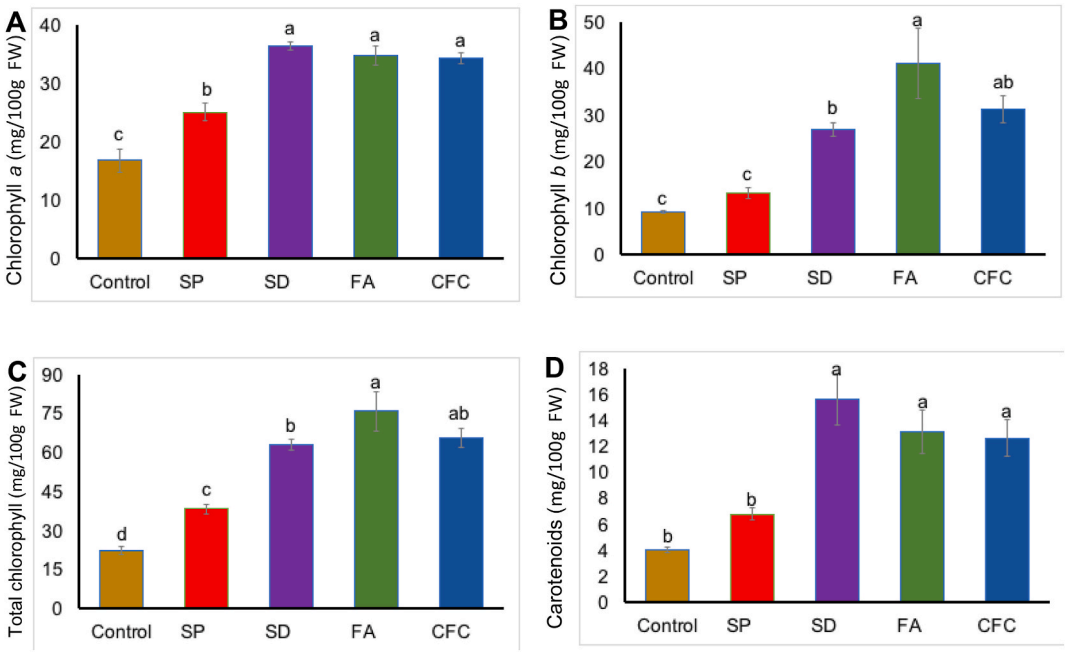

Fig. 2. Effect of *S. panaciterrae* PB20 on photosynthetic pigments of spinach. A) Chlorophyll *a*, B) Chlorophyll *b*, C) Total chlorophyll and D) Carotenoid contents. Bars indicate the ±standard error of the means (n = 4). The columns with the same letter are not significantly different at $P < 0.05$ according to Tukey's test. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

3.3. PB20 upregulated total antioxidant and vitamin C and protein content in spinach

Regardless of the method of application, PB20 increased the amount of DPPH total antioxidant activity in spinach. Fig. 3A represents the maximum amount of antioxidative activity found in seed-primed spinach, followed by foliar application, BCF foliar application, and soil drenching. Seed priming of spinach with PB20 also increased the vitamin C content over the control. However, the maximum amount of vitamin C was found in spinach leaf in the case of BCF foliar application followed by seed priming and foliar application (Fig. 3B). Amount of crude protein increased slightly in spinach shoots due to bacterial treatment especially in seed priming, though the protein contents were statistically similar to the control. However, in all methods of bacterial application, spinach root protein contents were significantly increased compared to contro l(Fig. 3C).

3.4. Effects of S. panaciterrae strain PB20 on mineral nutrient contents in spinach

Although the shoot or above ground part is the only edible part of spinach, the mineral elements were determined both in the root and shoot to understand the effect of bacteria on nutrient absorption and transport of mineral elements from root to shoot in different methods of application. The results of mineral nutrient content were calculated on a dry weight basis.

3.4.1. Effects of PB20 on primary nutrients N, P and K contents in spinach

N contents was affected significantly by the application of PB20. In both root and shoot N content was increased due to bacterial treatment through all methods of application compared to control (Fig. 4A). In both shoot and root, highest N content was found in seed primed spinach, which was statistically similar to bacterial foliar application, BCF foliar application, and soil drenching. N content was increased by 18–46 % in the root and 6–21 % in the shoot due to bacterial application compared to the control. Likewise, PB20 also positively affected the P content in spinach, irrespective of method of bacterial application, over the control (Fig. 4B). P content was increased by 42%–78 % in root and 3–26 % in shoot as compared to control. In root, the highest P content was found in seed-primed spinach, followed by foliar application and BCF foliar application and soil drenching. On the other hand, shoot P content was

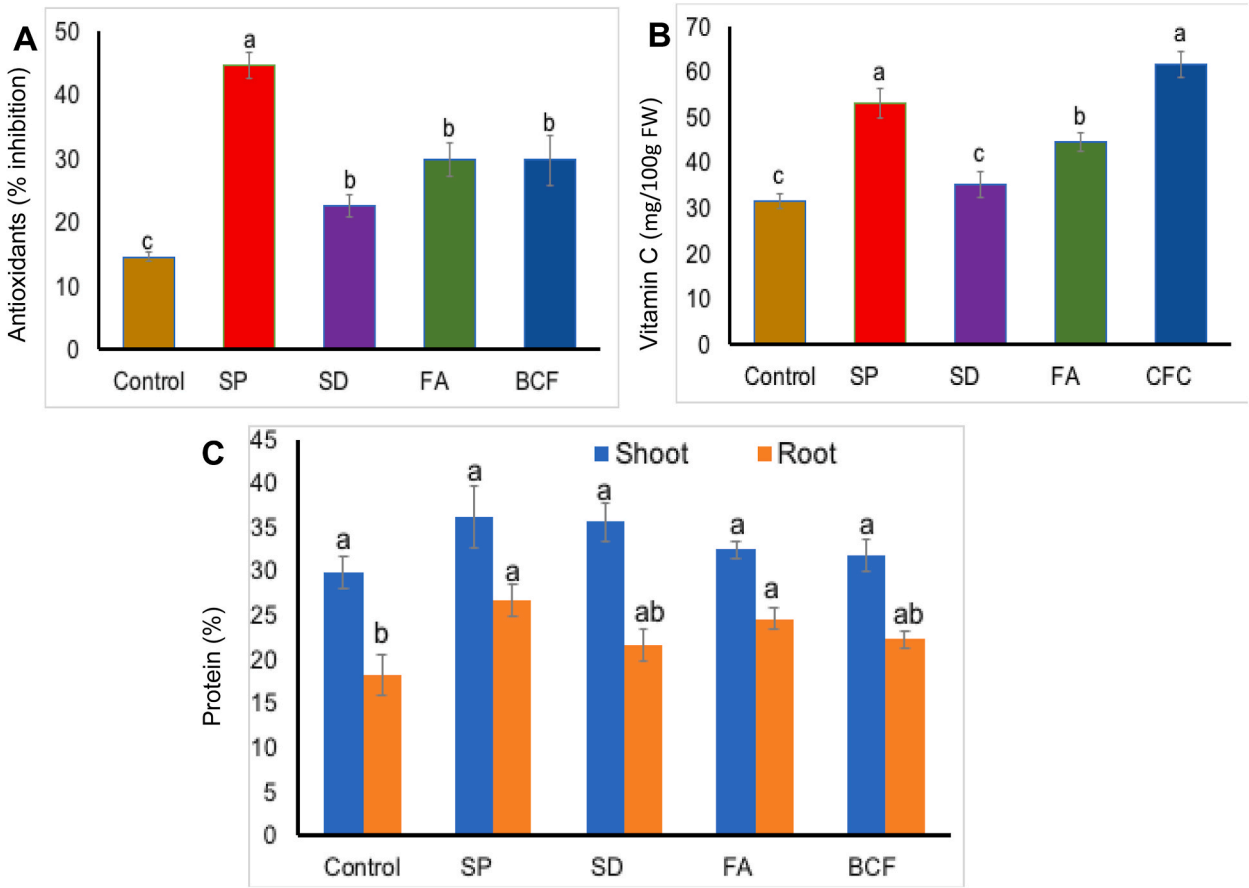

Fig. 3. Effect of *S. panaciterrae* PB20 on A) Vitamin C, B) Antioxidants, and C) Protein contents of spinach. Bars indicate the ±standard error of the means (n = 4). The columns with the same letter are not significantly different at $P < 0.05$ according to Tukey's test. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

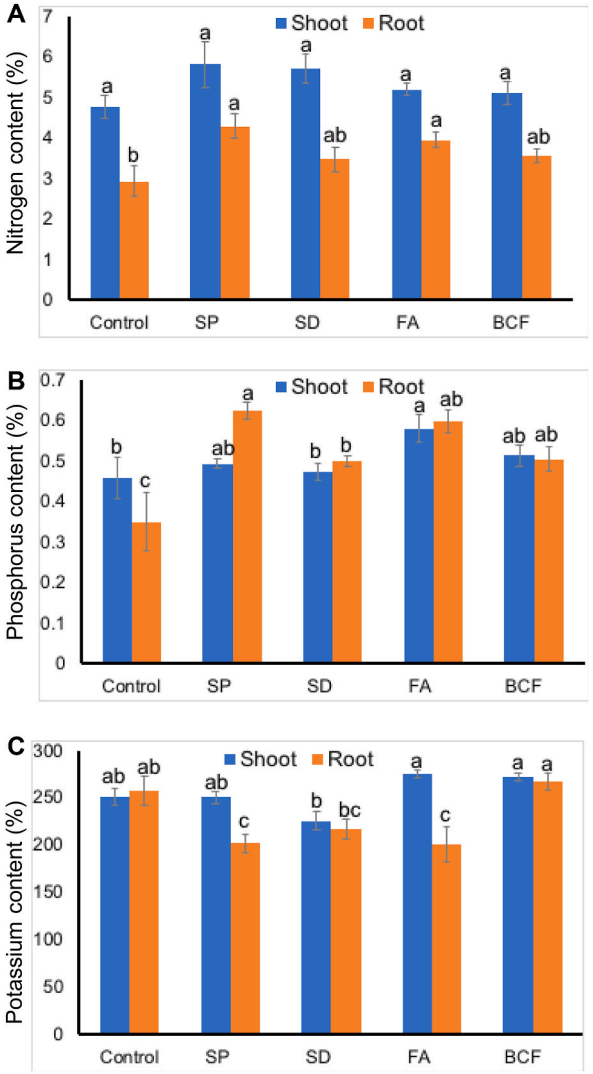

Fig. 4. Effect of *S. panaciterrae* PB20 on A) nitrogen, B) Phosphorus, and C) Potassium contents of spinach. Bars indicate the ±standard error of the means (n = 4). The columns with the same letter are not significantly different at $P < 0.05$ according to Tukey's test. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

maximum in foliar application, followed by BCF foliar application, seed priming, and soil drenching.

Unlike the N and P content, the K content was not affected by the bacterial application (Fig. 4C). Shoot K content was maximum in foliar application followed by BCF foliar application while root K content was maximum in BCF foliar application. Seed priming and soil drenching did not increase the K content significantly as compared to the control.

3.4.2. Effects of PB20 on secondary nutrients ca, Mg, S content in spinach

Among the secondary nutrients calcium (ca), magnesium (Mg) and sulphur (S), PB20 increased the amount of Ca most in spinach shoots in all methods of bacterial applications (Fig. 5). The influence was more prominent in shoot than in root. In both root and shoot, the maximum amount of Ca was found in bacterial foliar application, followed by seed priming. In the case of Mg, BCF foliar application showed the best performance and yielded the maximum shoot Mg content (Fig. 5A). However, root Mg context did not increase due to the BCF foliar application. Next to it, the bacterial foliar application results in the second highest amount of Mg in shoot. Seed priming showed a marginal increase in Mg content in shoots and roots over the control(Fig. 5B). Unlike Ca and Mg, the S content was not influence by the application of PB20. Amount of S was almost similar in control and in bacteria treated spinach through all four methods, though there was a slight increase in S content in seed-primed spinach shoot (Fig. 5C).

3.4.3. PB20 increased the micronutrients Fe and Zn in spinach

Fig. 6A showed that application of PGPR PB20 significantly influenced the amount of micronutrient Fe content in spinach root and

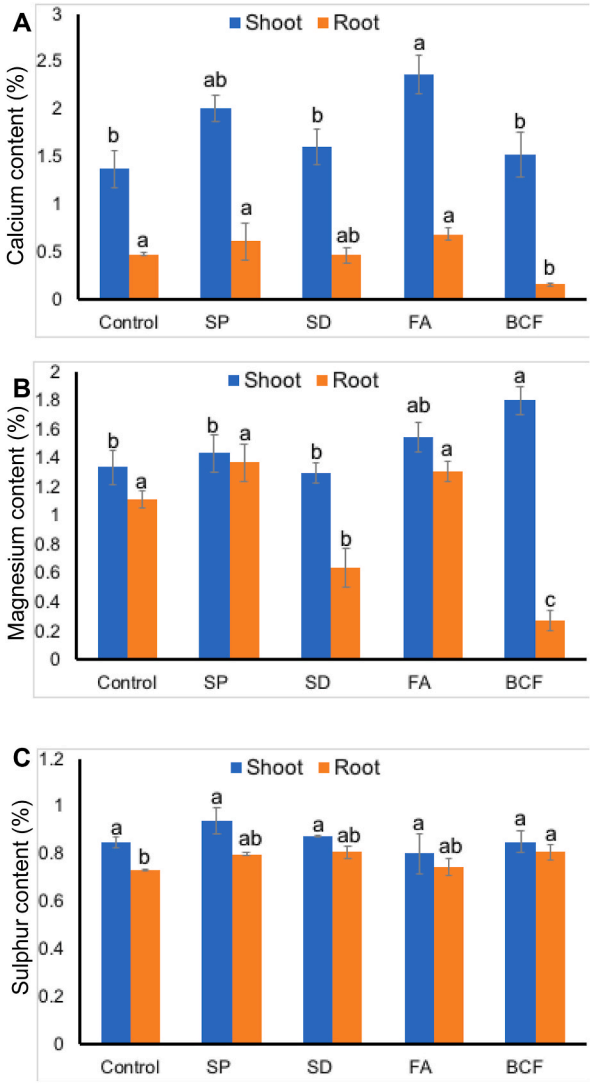

Fig. 5. Effect of *S. panaciterrae* PB20 on A) Calcium, B) Magnesium, and C) Sulphur contents of spinach. Bars indicate the ±standard error of the means (n = 4). The columns with the same letter are not significantly different at $P < 0.05$ according to Tukey's test. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

shoot. Except for soil drenching, bacterial application in the other three methods increased the amount of Fe in the shoot and the root of spinach as compared to the control. In shoot, the maximum amount of Fe found in case of BCF foliar application, followed by bacteria foliar application, and seed priming. On the other hand, seed priming resulted the highest Fe accumulation in root (200 % increased over control). Like Fe, the amount of Zn was also found maximum in both root and shoot in case of foliar application followed by BCF foliar application and seed priming (Fig. 6B). Soil drenching by bacteria did not influence the Fe and Zn content in spinach.

3.4.4. Visualization of data with clustered heatmap

A heatmap was generated for visualizing the performance of different plant parameters under different treatment conditions using colour intensity, and the parameters were further grouped into different clusters using the hierarchical clustering method. The clustered heatmap showed that treatments were grouped into two major groups: A and B (Fig. 7). Group A included the 'Control' treatment and group B included all four bacterial applications, which can be further subdivided into the 'FA (foliar application) and BCF (bacterial culture filtrated)' and 'SP (seed priming) and SD (soil drenching)' groups. Overall, the SP had more positive associations with plant growth parameters, antioxidants, and nutrient acquisition profiles, whereas FA and BCF had more positive associations with photosynthetic pigments, vitamin C, and several nutrients.

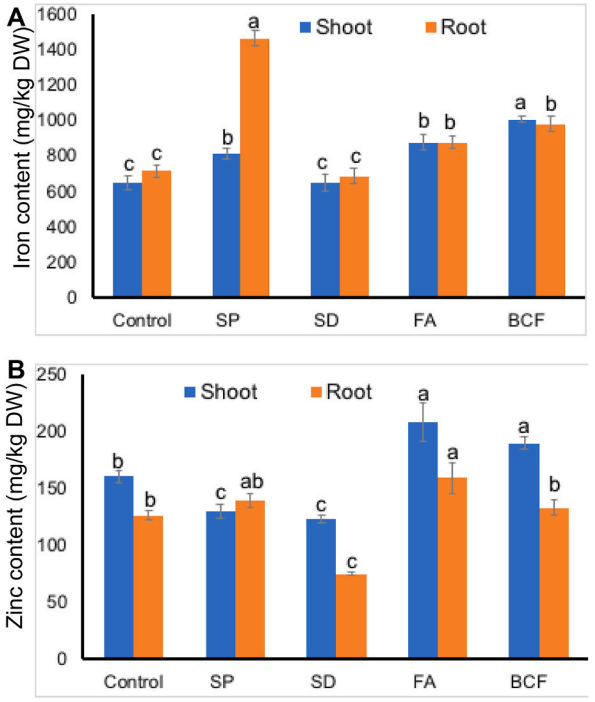

Fig. 6. Effect of *S. panaciterrae* PB20 on A) iron (Fe), and B) Zinc (Zn) contents of spinach. Bars indicate the ±standard error of the means (n = 4). The columns with the same letter are not significantly different at $P < 0.05$ according to Tukey's test. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

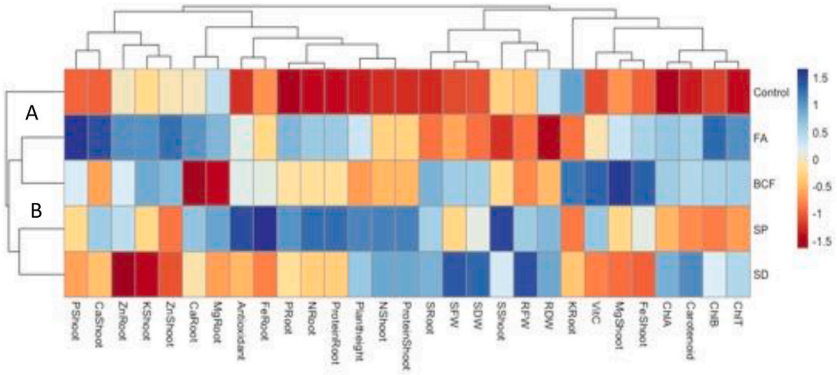

Fig. 7. Heatmap showing the hierarchical clustering among the treatments. Analysis was done by using normalized data of all parameters. SP indicates seed priming, SD indicates soil drenching, FA indicates foliar application and BCF indicates bacterial culture filtrate foliar application.

4. Discussion

Agricultural practice using PGPR on a broad scale begun in the early 20th century through rhizobial inoculation of legumes [75]. During the last century, different PGPRs have been extensively studied, and a few are now being applied commercially. In this research, we investigated the effect of a rhizobacteria from the *Sphingomonas* family, *S. panaciterrae* (PB20), on the growth and nutrient uptake of a popular leafy vegetable, spinach. We also investigated the suitable method of application of this bacterium in leafy vegetable spinach. This strain was reported to have several growth promoting characteristics in our previous research. In spinach, PB20 promoted growth parameters such as the plant height, fresh weight, and dry weight of the edible part, resulting in increased yield. Additionally, PB20 increased the total antioxidants, chlorophyll, and carotenoid of spinach leaves. The increase in photosynthetic pigments also contributed to increasing the biomass and yield of spinach. The higher antioxidant content in spinach, even in small amounts, could significantly contribute to enhancing the human diet through the consumption of antioxidant-rich spinach. PGPR mediated growth improvements have been reported earlier by many researchers in different crops, such as wheat [76], tomato [77, 78], maize [79], sugarcane [80], rice [81–84]. In the present study, bacterial application through seed priming showed the maximum

improvement in plant height. Despite the growth improvement in all the methods of applications, maximum plant height in seed primed spinach suggested that PB20 might increase the plant height through indole acetic acid (IAA) formation, which triggers the apical elongation, and the amount of IAA might be produced in the maximum amount in case of seed priming.

PGPRs promote plant growth directly or through indirect mechanisms. In direct mechanism, the bacteria improve plant growth by facilitating resource acquisition (nitrogen, phosphorus, and essential minerals) or modulating plant hormone levels, or indirectly by decreasing pathogen inhibitory effects on plant growth and development as biocontrol agents [85,86]. On the other hand, *Sphingomonas* mediated growth improvement also been reported by several studies [52,53,56,57]. In the current research, PB20 might enhance the plant growth directly through improvement of growth regulating plant hormones such as IAA. It could also improve the growth of spinach through accumulation of two essential plant macronutrients, N and P, which are crucial for plant growth enhancement. The bacterium PB20 was reported as an N-fixing and P-solubilizing bacterium in our previous study [58]. In spinach, it increased the root and shoot N and P contents. Furthermore, it is also evident that the spinach plant could accumulate more Ca and Mg, especially in the shoot, when it is inoculated with PB20. Ca is an integral part of cell division, and Mg is the essential component of chlorophyll, influencing plant photosynthesis, food production and ultimately the growth of the plant. Therefore, the mechanism by which PB20 improved plant height, fresh and dry weight could be explained through its superior IAA production and better acquisition of two primary essential elements, N and P, and two secondary vital essential elements Ca and Mg, along with its higher antioxidant and photosynthetic pigment production.

Spinach is a very good source of iron (Fe). An improved Fe content is very vital for a proper Fe-rich diet. The application of PB20 improved the amount of Fe in the root and shoot of spinach. Another very important micronutrient Zn, was also increased in the root and shoot of spinach in all methods of bacterial application. The bacterium might be capable of solubilizing the insoluble Fe and Zn in soil, that might boost their amount in the root and shoot. Another reason for the improved plant growth could be because bacterial application might enable the plant to uptake more water and nutrients from the soil.

Among the four methods of application studied, seed priming showed better efficacy over the other three methods in terms of growth improvements, especially the plant height, antioxidants, vitamin C and N contents in the root and shoot. On the other hand, the root drenching showed the highest fresh and dry weight of the plant and maximum chlorophyll *a* and carotenoid content. Foliar application of bacterial solution and its culture filtrate helped the spinach plant absorb nutrition better. The efficacy of the method of application of PGPR depends on the colonization potential of the bacteria. The rhizospheric conditions conferred by plant root exudates in the rhizosphere, microbial activities in response to root exudates and the rhizospheric environment, and mutual interactions of both rhizospheric bacterial communities and plant roots all contribute to effective bacterial colonization in plant roots [87–89]. While in the case of seed priming, bacterial endophytism begins before germination and sprouting of the seedling, in soil drenching, it starts with the colonization of the bacteria in root. Therefore, the colonization potential of bacteria in soil drenching is more dependent on the rhizospheric environment as compared to seed priming. That is why seed priming showed the better performance than soil drenching, especially in the case of plant height. Foliar application of the bacteria and BCF showed good performance in the case of total chlorophyll content and enhanced the ionic absorption, especially P, Ca, Mg, and Fe in both the root and shoot. Foliar treatment of nitrogen-fixing bacteria was reported to increase growth and yield of pea [90,91], apple [92], mulberry [93], and apricot [94]. In a study, Esitken et al. [95] showed that the administration of floral and foliar bacteria during the full bloom and cell division phases of sweet cherries resulted in enhanced fruit set and development, and the effect was explained by the presence of IAA and *trans*-zeatin. In our study, foliar application of bacteria and BCF over the canopy probably stimulated the IAA synthesis in young leaves, which might enhance the absorption and transport of ions into the root and shoot. The increased nutrient contents in foliar application can also be explained by the higher production of photosynthetic pigments that enhanced the nutrient absorption by the plants. Further investigation is necessary to reveal the plant-microbe interaction while applying *S. panaciterrae* to leaf surface or across the canopy of the plant.

5. Conclusion

In conclusion, the application of rhizobacteria *S. panaciterrae* (PB20) increased the plant height, growth, chlorophyll content, antioxidant, vitamin C, and several mineral nutrient contents of spinach compared to the control. In all four methods of application, most of the growth and quality attributes were enhanced due to bacterial application. Each of the four methods of application showed the best performance in one or more growth and nutrient improvement parameters and therefore certainly added value to the harvested spinach. It is evident that seed priming of spinach using PB20 is a promising way to improve growth and antioxidants, while foliar application and BCF foliar application could increase photosynthates and mineral nutrient contents. However, a combination of bacterial seed priming along with foliar application of bacteria would be the best practices for the application of PB20 in spinach. The findings of this research could contribute to sustainable agriculture by practicing nutrient-rich vegetable production, which will improve the spinach yield as well as enhance nutritional security for consumers. Further research is needed to investigate the effects of the application of *S. panaciterrae* PR20 or other strain of *S. panaciterrae* on soil properties and on different leafy vegetables. Performing field experiments in various soil conditions is necessary prior to recommending this PGPR as a bioinoculant to farmers for vegetable cultivation.

Data availability

All data are included in tables, figures, and supplementary materials.

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Ethics declarations

Informed consent was not required for this study because this study did not include any human and animal trial.

CRediT authorship contribution statement

Razia Sultana: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Shah Mohammad Naimul Islam:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Conceptualization. **Nurjahan Sriti:** Formal analysis, Data curation. **Mysha Ahmed:** Formal analysis, Data curation. **Sourav Biswas Shuvo:** Formal analysis, Data curation. **Md Habibur Rahman:** Investigation, Data curation. **Asif Iqbal Ibne Jashim:** Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25596.

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