Journal of Agriculture and Food Research 26 (2026) 102742

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Integrated application of plant growth–promoting rhizobacteria and organic amendments enhances growth and nutrient uptake in maize

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ARTICLE INFO

Keywords:
Bacillus cereus
Bacterial consortium
Organic amendments
Plant growth-promoting rhizobacteria (PGPR)
Plant growth-promoting traits (PGPTs)
Pseudomonas putida

ABSTRACT

Nutrient deficiency is a major constraint to maize production and is commonly addressed using synthetic fertilisers. Sustainable alternatives such as plant growth-promoting rhizobacteria (PGPR) combined with organic amendments offer an eco-friendly strategy to improve nutrient uptake and productivity. In this study, two rhizobacterial isolates were characterised based on biochemical attributes and 16S rRNA gene sequencing and identified as *Bacillus cereus* (RB-1) and *Pseudomonas putida* (RB-2). Both isolates exhibited multiple plant growth-promoting traits (PGPTs) including ammonia production, starch hydrolysis, phosphate solubilisation, indole-3-acetic acid synthesis, and siderophore production. The complementary expression of these traits is likely to enhance nutrient availability and root development, thereby contributing to the improved performance observed under co-inoculation. In pot experiments, co-inoculation of the PGPR and a nine-strain bacterial consortium with organic amendments (cow dung and vermicompost) significantly improved seed germination, seedling vigour, and maize growth parameters compared with untreated and fertiliser controls. These improvements were accompanied by increased biomass, higher chlorophyll and carotenoid contents, and enhanced nutrient accumulation (N, P, K, Mg, and Fe) in shoots and roots. A strong positive correlation was observed among fresh and dry shoot and root weights. In summary, these findings indicate that co-inoculation of PGPR with organic amendments enhances maize growth and nutrient uptake under greenhouse conditions and may contribute to more sustainable and eco-friendly nutrient management strategies.

1. Introduction

Maize (*Zea mays* L.) is a major cereal crop of global economic and nutritional importance, ranking third after rice and wheat in terms of cultivation and consumption [1–3]. It serves as a significant source of carbohydrates, vitamins, and essential minerals such as iron, calcium, phosphorus, magnesium, and zinc [4], and contributes substantially to both human and livestock diets [5]. In Pakistan, particularly in Punjab and Khyber Pakhtunkhwa, maize cultivation has expanded rapidly in recent years, increasing agricultural exports by nearly 80% [6]. Despite this progress, maize yields remain below potential due to various biotic and abiotic stress factors [7]. With the global population projected to reach 10 billion by 2050, food demand is expected to rise by 35–56% [8, 9]. Therefore, improving maize productivity through sustainable nutrient management is essential to ensure future food security.

Inorganic fertilisers have long been used to boost crop yield [10], but their excessive and unbalanced use leads to nutrient depletion, particularly of phosphorus (P) and potassium (K) [11]. Nitrogen-based fertilisers often disturb the natural nitrogen cycle, cause leaching, salinity, and greenhouse gas emissions, and reduce soil organic matter, ultimately leading to biodiversity loss [12]. Moreover, the residual chemicals can leach into groundwater and contaminate drinking water

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https://doi.org/10.1016/j.jafr.2026.102742
Received 27 October 2025; Received in revised form 26 January 2026; Accepted 1 February 2026
Available online 3 February 2026

sources [13]. The availability of nutrients from inorganic fertilisers also decreases over time due to microbial immobilisation and physicochemical changes in the soil [2]. To counter these problems, integrating inorganic fertilisers with organic amendments has been shown to enhance soil health and crop productivity [14]. Organic farming provides a sustainable alternative that enriches soil fertility through the use of natural inputs derived from plant and animal residues and improve soil productivity, enhance soil carbon sequestration [14], maintain nutrient balance [15], and reduce the negative environmental effects of chemical fertilisers [3]. Common organic amendments include cow dung, farmyard manure, compost, bark, sawdust, and other biodegradable wastes [16], all of which improve crop growth and yield [17].

Sustainable agriculture also relies on the use of beneficial soil microorganisms such as bacteria, fungi, and algae that enhance plant tolerance to stress [18,19] and improve nutrient acquisition [20]. Among these, plant growth-promoting rhizobacteria (PGPR) are particularly effective in improving plant growth and soil fertility without ecological harm [21–23]. PGPR directly promote plant growth by solubilising soil nutrients and producing growth regulators, and indirectly by suppressing phytopathogens [24]. They include nitrogen-fixing (*Rhizobium, Azospirillum, Azotobacter, Burkholderia*), phosphate- and potassium-solubilising species (*Bacillus, Pseudomonas, Burkholderia*), and other beneficial genera [25,26]. These bacteria have been shown to enhance growth in crops such as wheat, barley, rice, maize, and sugar beet [27–31]. Several studies have demonstrated that bacterial inoculation improves nitrogen and phosphorus availability and enhances crop yield [32]. For example, co-application of phosphorus-solubilising rhizobacteria with bio-organic phosphate significantly improved soil organic matter and increased wheat yield [33]. Thus, integrating PGPR with organic amendments can improve soil fertility, nutrient uptake, and plant productivity sustainably.

Given the environmental concerns associated with synthetic fertilisers, integrating PGPR with organic amendments has been proposed as an alternative approach for improving plant nutrient availability and growth. Although the individual effects of PGPR and organic fertilisers are well documented, existing studies often focus on yield outcomes or on individual inputs applied independently. Comparatively fewer studies have examined how specific PGPR functional traits relate to plant physiological responses and nutrient uptake when PGPR are applied in combination with organic amendments. Moreover, direct comparisons between individual PGPR strains and multi-strain PGPR consortia within the same experimental framework remain limited. Therefore, this study evaluated the individual and combined effects of selected PGPR strains and organic amendments on maize growth, chlorophyll (Chl) and carotenoid contents, and nutrient uptake in roots and shoots. By integrating strain-level and consortium-level responses, the study focuses on linking microbial functional traits with plant growth and nutrient acquisition responses.

2. Materials and methods

2.1. Soil samples collection

Rhizospheric soil samples (200 g each) were collected from three randomly selected maize field locations at Chak 60 JB Shahbaz Pur (31°26′50″ N, 72°56′49″ E). Samples were collected from the rhizosphere of healthy maize plants, sealed in polyethylene bags, transported to the laboratory, and stored at 4 °C until further processing for rhizobacterial isolation.

2.2. Isolation, purification, and preservation of rhizobacterial strains

Rhizospheric soil samples were used for the isolation of rhizobacteria following the serial dilution technique described by Joseph et al. [34]. Briefly, 1 g of soil from each sample was suspended in 9 mL of sterile distilled water, and serial dilutions were prepared up to 10^{-9}. From the 10^{-7} dilution, 1 mL was aseptically spread onto Petri plates containing nutrient agar (NA) medium and incubated at 28 ± 2 °C for 24 h. Multiple morphologically distinct bacterial colonies were obtained and purified, yielding several bacterial isolates. Among these, two representative rhizobacterial isolates, designated RB-1 (*Bacillus cereus*) and RB-2 (*Pseudomonas putida*) were selected for detailed characterisation and subsequent greenhouse experiments. The purified strains were preserved in nutrient broth (NB) supplemented with 40% glycerol and stored at −80 °C for subsequent experiments.

2.3. Collection of bacterial consortium

A bacterial consortium comprising nine strains was obtained from the National Culture Collection of Pakistan (NCCP), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The consortium was included in this study as a comparative reference treatment alongside the individual PGPR isolates. It was previously developed and characterised through systematic screening of multiple bacterial isolates for key plant growth–promoting traits (PGPTs) including phosphate and zinc solubilisation, the presence of nitrogen fixation–related genes (*nifH*), and ACC deaminase activity (*acdS*) [35–37]. During consortium development, strain compatibility was assessed using cross-streak assays, and no antagonistic interactions among the selected strains were reported [35].

The consortium consisted of the following NCCP-accessioned strains: NCCP-246 (*Sphingobacterium pakistanensis*), NCCP-525 (*Pantoea* sp.), NCCP-11 (*Cellulomonas pakistanensis*), NCCP-668 (*Citrobacter* sp.), NCCP-673 (*Exiguobacterium* sp.), NCCP-675 (*Raoultella* sp.), NCCP-680 (*Acinetobacter* sp.), NCCP-607 (*Enterobacter* sp.), and NCCP-650 (*Alcaligenes pakistanensis*). This previously validated consortium was used in the pot experiments described in the present study and is hereafter referred to as the “consortium” treatment. Earlier studies conducted under controlled conditions, primarily in wheat, demonstrated that inoculation with this consortium resulted in improved plant growth responses compared with single-strain applications [36,37].

2.4. Morphological and biochemical characterisation of rhizobacteria

Bacterial isolates grown on NA medium were subjected to morphological characterisation. Cellular morphology including motility and shape was examined under a light microscope [38]. Gram staining was performed following the method of Vincent and Humphrey [39]; the appearance of dark purple colouration indicated Gram-positive, whereas a red colouration indicated a Gram-negative reaction. Hydrogen cyanide (HCN) production was detected using the procedure described previously [40]. Briefly, each isolate was streaked on NA plates supplemented with glycine (4.4 g L^{-1}). Whatman No. 1 filter paper, soaked in a solution containing 0.5% picric acid [$(O_2N)_3C_6H_2OH$] and 2% Na_2CO_3, was placed over the plates, which were sealed with parafilm and incubated at 28 ± 2 °C for 48 h. A colour changes from orange to red confirmed HCN production. Fluorescence was assessed by culturing isolates on King's B medium and incubating at 25 ± 2 °C for 48 h. Plates were examined under a UV transilluminator (10–400 nm), and the appearance of blue fluorescence indicated a positive response [41].

For the potassium hydroxide (KOH) solubility test, a 3% KOH solution was mixed with bacterial culture on a clean glass slide. The formation of a viscous thread confirmed KOH solubility [42,43]. Catalase activity was tested by mixing a loopful of bacterial inoculum with a drop of 3% H_2O_2; the immediate formation of oxygen bubbles (within 5–10 s) indicated a positive catalase reaction. Levan production was determined by streaking bacterial isolates on NA medium supplemented with 5% sucrose and incubating for 3 days at 25 ± 2 °C. The development of convex, mucoid, white, and domed colonies confirmed levan production [44]. Carbohydrate fermentation was assessed described earlier [45]. The fermentation medium consisted of 1 g trypticase, 0.5 g NaCl, 0.02 mg phenol red, and 0.5 mg carbohydrate dissolved in 100 mL distilled water. After autoclaving, the medium was inoculated with bacterial culture and incubated at 37 °C for 24 h. A colour changes from red to yellow indicated a positive fermentation response. Hydrogen sulphide (H_2S) production was tested on SIM medium incubated at 37 °C for 24 h; the formation of black ferrous sulphide precipitates indicated a positive reaction. The oxidase test was performed following Hayward [46]. Fresh cultures were inoculated on Whatman No. 1 filter paper saturated with 1% N,N,N′,N'-tetramethyl-p-phenylenediamine (TMPD). The development of a dark purple colour within 10–30 s indicated oxidase activity. The oxidative–fermentative (O/F) test was carried out as described by Hugh and Leifson [47]. In this assay, bacterial cultures were inoculated into basal medium tubes by stabbing halfway to the bottom. One of each pair of tubes was overlaid with a 1 cm layer of mineral oil, while the other remained exposed to air. Tubes were incubated at 37 °C for 48 h, and a colour change from green to yellow indicated a positive oxidative or fermentative response.

2.5. *Molecular characterisation of rhizobacteria*

Genomic DNA was extracted from bacterial isolates using the GeneJet Genomic DNA Purification Kit (Thermo Scientific, Waltham, USA) according to the manufacturer's instructions. The 16S rRNA gene region was amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) [48,49]. Polymerase chain reaction (PCR) amplification was carried out in a 50 μL reaction mixture containing 1.25 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer, 0.5 U μL^{-1} Taq polymerase (1 μL), and 25–150 μL of DNA template. The PCR conditions consisted of an initial denaturation at 90 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 55–60 °C for 20–40 s, and extension at 72 °C for 7 min. Amplified PCR products were separated on a 1% (w/v) agarose gel stained with ethidium bromide and visualized under a UV transilluminator. The amplicons were purified using the Gel and PCR Clean-Up System (Promega) and quantified using a NanoDrop spectrophotometer. Purified PCR products were sequenced by Microgen, Korea. The obtained sequences were assembled using DNASTAR software and compared with related sequences in the NCBI database using the BLAST algorithm. Multiple sequence alignment was performed with CLUSTALW. Phylogenetic analysis was conducted to determine evolutionary relationships between the isolates and closely related sequences.

Phylogenetic analyses were performed separately for the two rhizobacterial isolates based on 16S rRNA gene sequences. For isolate RB-1, a phylogenetic tree was constructed using the Maximum Likelihood (ML) method with the Kimura two-parameter model with invariant sites (K2+I) including reference sequences of *B. cereus*. *Bacillus subtilis* was used as an outgroup to root the tree. For isolate RB-2, a separate ML phylogenetic tree was generated using the Hasegawa–Kishino–Yano (HKY) substitution model with reference sequences of *P. putida*, and *P. fluorescens* was used as an outgroup. All phylogenetic analyses were conducted in MEGA version 12 [50]., and the robustness of the inferred trees was assessed using 1000 bootstrap replications. Bootstrap values ≥ 50% were considered significant and are shown at the corresponding nodes. The 16S rRNA gene sequences obtained in this study were deposited in the GenBank database, and accession numbers were assigned.

2.6. *Plant growth-promoting traits (PGPTs) of PGPR*

PGPTs including ammonia production, starch hydrolysis, phosphate solubilisation, indole-3-acetic acid (IAA) production, and siderophore production, were evaluated using standard qualitative and quantitative assays as described previously [51–56]. For detailed experimental procedures, see Supplementary Methods. Briefly, ammonia production was assessed using peptone water and Nessler's reagent [51], starch hydrolysis was evaluated on starch-amended LB agar [52], and phosphate solubilisation was determined using Pikovskaya's agar [53]. IAA production was quantified colorimetrically using Salkowski's reagent [54], and siderophore production was assessed using the Chrome Azurol S (CAS) assay [55,56].

2.7. *Effect of bacterial inoculation on seed germination and vigour index in maize*

The effect of bacterial inoculation on maize seed germination and seedling vigour was evaluated under *in vitro* conditions [57], with full methodological details provided in Supplementary Methods. Briefly, surface-sterilised seeds were inoculated with bacterial suspensions and incubated under controlled conditions. After 15 days of incubation, germination percentage, shoot and root length, and fresh and dry biomass were recorded.

2.8. *Effect of co-inoculation of rhizobacteria and soil amendments on PGPTs and nutrient uptake*

2.8.1. *Collection of soil and organic waste material*

Soil used for the pot experiment was collected from agricultural land in Machikhokhar, Sialkot, Pakistan. The soil was air-dried and subjected to physicochemical analysis following the methods described by Okalebo et al. [58]. Organic waste materials, namely cow dung (CD) and vermicompost (VC), were collected from Kallupayara, Sialkot, and a commercial supplier in Faisalabad, Pakistan, respectively. Representative bulk samples of CD and VC were analysed for macro- and micronutrient composition prior to use [59,60]. The organic amendments were applied without sterilisation to preserve their native physicochemical and microbial properties.

2.8.2. *Greenhouse pot assay*

A greenhouse pot experiment was conducted to evaluate the effects of rhizobacterial inoculation and organic amendments on maize growth. The experiment was arranged in a completely randomised design (CRD) and comprised 13 treatments including individual PGPR inoculations, a multi-strain PGPR consortium, organic amendments, their combinations, and appropriate controls: T1, CD + RB-1; T2, CD + RB-2; T3, CD + Consortium; T4, VC + RB-1; T5, VC + RB-2; T6, VC + Consortium; T7, CD alone; T8, VC alone; T9, RB-1 alone; T10, RB-2 alone; T11, Consortium alone; T12, untreated soil (negative control); and T13, DAP fertiliser (positive control). Each treatment was replicated four times, and each replicate consisted of one pot. Sieved soil was thoroughly mixed with CD or VC according to the treatment plan before being placed into pots. Maize seeds (*var*. Spring 1608) were inoculated with respective rhizobacterial suspensions or consortium for 2 h prior to sowing. Fifteen treated seeds were sown per pot, and uniform agronomic practices were maintained across all treatments. Germination percentage was recorded 14 days after sowing. Plant growth parameters including shoot length, root length, leaf width, and fresh and dry biomass of shoots and roots were recorded biweekly until 12 weeks after germination. The entire experiment was conducted twice under identical greenhouse conditions to confirm reproducibility.

2.8.3. *Estimation of chlorophyll (Cha) contents*

At the maturity stage, healthy leaves were collected from each replicate to determine Chl content. Fresh leaf tissue was extracted in 10 mL of solvent consisting of 80% acetone and 20% distilled water (v/v 8:2) in test tubes. After 24 h of incubation, the samples were analysed using a spectrophotometer at wavelengths of 663, 645, and 480 nm. Concentrations of chl *a*, chl *b*, and carotenoids were calculated described previously [61,62].

2.8.4. *Nutrient analysis of harvested plant samples*

After harvest, maize plants were separated into roots and shoots (including leaves), oven-dried at 65 °C for 72 h, and ground into a fine powder using an electric grinder. For digestion, 0.5 g of plant material was placed in a conical flask with 10 mL of an acid mixture ($HClO_4$: HNO_3, 3:7 v/v) and heated at 100–300 °C on a hot plate until the solution became colourless. The digests were cooled to room temperature, diluted with 50 mL of distilled water, and filtered through Whatman No. 1 filter paper. Phosphorus concentration was determined by mixing 5 mL of the digest with 5 mL of a colour reagent (ammonium vanadate + nitric acid + ammonium molybdate) and measuring absorbance at 470 nm using a spectrophotometer [60]. Total nitrogen concentration was determined by the Micro-Kjeldahl method [63]. The concentrations of Mg, Zn, Mn, Cu, Ca, and Fe were determined using atomic absorption spectrophotometry [64]. Potassium and sodium concentrations were measured using a flame photometer. Nutrient uptake was calculated by multiplying the plant's dry biomass by the corresponding nutrient concentration [65].

2.9. Statistical analyses

Analysis of variance (ANOVA) was performed to evaluate treatment effects, and means were compared using the least significant difference (LSD) test at $P \leq 0.05$. Standard error (SE) was calculated for each treatment. All statistical analyses were conducted using R software (version 4.2.0). Principal Component Analysis (PCA) was used to assess the effects of individual and combined applications of rhizobacterial strains with VC and CD on maize growth and nutrient uptake. Correlation patterns and treatment clustering among plant growth parameters were further examined using heat map visualisation and Pearson correlation analysis.

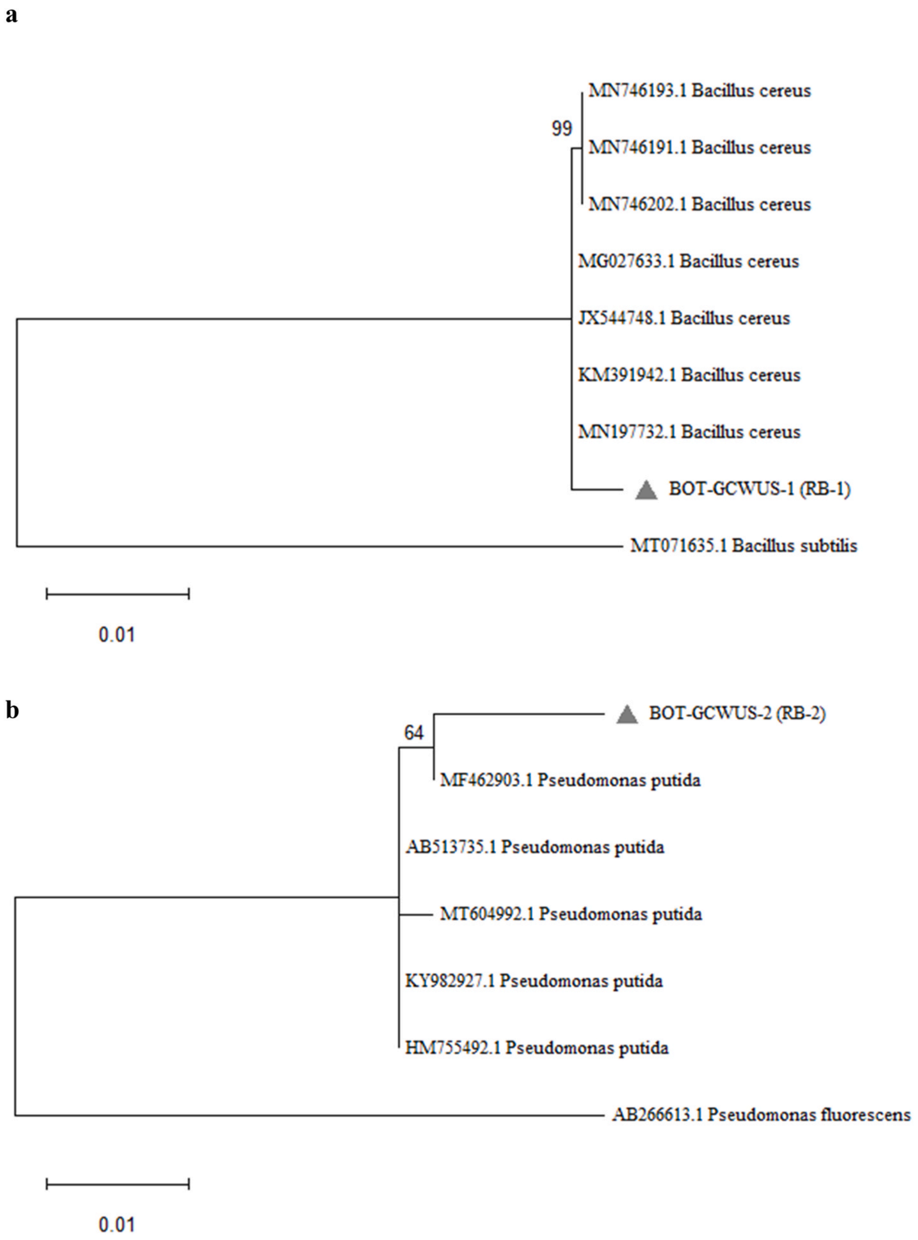

Fig. 1. Maximum Likelihood (ML) phylogenetic trees based on 16S rRNA gene sequences showing the relationships of rhizobacterial isolates with closely related reference taxa. (a) Isolate Bot-GCWUS-1 (RB-1) clustering within the *Bacillus cereus* group. (b) Isolate Bot-GCWUS-2 (RB-2) clustering within the *Pseudomonas putida* group. Isolates obtained in this study are indicated by filled triangles (▲). Bootstrap values (≥50%) based on 1000 replicates are shown at branch nodes, and scale bars represent nucleotide substitutions per site.

3. Results

3.1. Isolation and identification of rhizobacteria

Based on morphological, biochemical, and molecular characterisation, two rhizobacterial isolates, designated RB-1 and RB-2, were identified as *B. cereus* and *P. putida*, respectively. Hereafter, these isolates are referred to as RB-1 and RB-2. Morphologically, both isolates were rod-shaped, with RB-1 exhibiting a Gram-positive reaction and RB-2 a Gram-negative reaction. Biochemical profiling (Supplementary Table 1) showed that RB-1 was positive for HCN production, catalase, and oxidase activity, but negative for fluorescence, KOH solubility, levan formation, carbohydrate fermentation, H_2S production, and oxidative–fermentative reactions. In contrast, RB-2 tested positive for HCN production, catalase activity, fluorescence, KOH solubility, levan formation, H_2S production, oxidase activity, and oxidative–fermentative reactions, but was negative for carbohydrate fermentation.

Phylogenetic analysis of the 16S rRNA gene sequences (~1500 bp) confirmed species-level identification of the isolates (Fig. 1). Isolate RB-1 showed 99.58% sequence similarity with *B. cereus* (GenBank accession no. MN746193.1), while isolate RB-2 exhibited 99.65% sequence similarity with *P. putida* (GenBank accession no. MF462903.1). The 16S rRNA gene sequences generated in this study were deposited in the GenBank database under accession numbers PX456173 (Bot-GCWUS-1; RB-1) and PX456174 (Bot-GCWUS-2; RB-2).

3.2. Characterisation of rhizobacterial strains for PGPTs

RB-1 and RB-2 exhibited multiple PGPTs including ammonia production, starch hydrolysis, phosphate solubilisation, IAA synthesis (with and without tryptophan), and siderophore production. Both strains tested positive for ammonia production and starch hydrolysis. IAA production (Supplementary Fig. 1a) was detected in both strains, with RB-1 producing higher concentrations both without tryptophan (6.23 ± 0.23 μg mL^{-1}) and with tryptophan supplementation (22.90 ± 1.26 μg mL^{-1}) compared with RB-2, which produced 4.55 ± 0.13 μg mL^{-1} and 17.05 ± 0.33 μg mL^{-1}, respectively. Phosphate-solubilising activity (Supplementary Fig. 1b) was also prominent, with RB-1 showing slightly greater solubilisation (81 ± 3.28 μg mL^{-1}) than RB-2 (75 ± 2.89 μg mL^{-1}). Similarly, both isolates produced siderophores (Supplementary Fig. 1c), with RB-2 exhibiting higher activity (28.55 ± 1.05%) than RB-1 (24.53 ± 0.66%).

3.3. Effect of rhizobacterial inoculation on seed germination and vigour index in maize

All rhizobacterial treatments including RB-1, RB-2, and the consortium, when applied to maize seeds, increased seed germination and early seedling growth compared with the uninoculated control. Seed germination reached 100% in all bacterial treatments (Supplementary Fig. 2a). Shoot length was improved in seeds treated with RB-1 and RB-2 than in the control (Supplementary Fig. 2b). Root length was greatest in RB-1-treated seedlings and lowest in RB-2-treated seedlings (Supplementary Fig. 2c). Both individual strains and the consortium increased shoot and root biomass relative to the control. Maximum fresh shoot weight was recorded in RB-2 and the consortium treatments, whereas dry shoot weight was highest in RB-2 (Supplementary Fig. 2d and e). Fresh root weight was highest in the consortium treatment, followed by RB-2, while dry root weight was greatest in the consortium (Supplementary Fig. 2f and g). The vigour index was highest in seedlings inoculated with RB-1, showing a clear increase relative to the control (Supplementary Fig. 2h).

3.4. Effect of co-inoculation of rhizobacteria and soil amendments on plant growth and nutrient uptake

The combined application of rhizobacteria and soil amendments (CD or VC) resulted in significantly higher maize seed germination and growth responses than either individual treatments or the controls (Supplementary Fig. 3). Seed germination reached 100% in all combined treatments of CD or VC with RB-1, RB-2, or the consortium, compared with 90% in the positive control. Among individual applications, full germination (100%) was recorded in CD, VC, RB-1, and the consortium, whereas RB-2 showed a lower germination percentage (84%) (Fig. 2a). Shoot length was significantly higher in all co-inoculated treatments than in both control treatments (Fig. 2b). The maximum shoot length was recorded in CD + RB-2, followed by VC + RB-1. Among individual treatments, VC alone produced the tallest plants. Maximum root length was observed in VC + RB-1 and VC + RB-2, with values approximately two-fold higher than the negative control. Application of the consortium alone also significantly increased root length compared with the control (Fig. 2c). Leaf number and leaf width were also enhanced under combined applications. The highest leaf number was recorded in CD and VC treatments relative to negative control, while leaf width was greater in all PGPR + organic amendment combinations than in the controls (Fig. 2d and e). Shoot and root biomass responses followed similar trends (Fig. 2f–i). Fresh shoot weight (FSW) was highest in CD + RB-2 and VC + RB-1 treatments, while dry shoot weight (DSW) reached maximum values in CD + RB-2 and VC + consortium treatments (Fig. 2f and g). Fresh root weight (FRW) was higher in CD + RB-2, CD + consortium, VC + RB-1, and VC + consortium treatments, and dry root weight (DRW) was greatest under combined applications compared with individual treatments and controls (Fig. 2h and i). Regression analysis showed strong positive relationships between FRW and DRW ($R^2 = 0.8316$) and FSW and DSW ($R^2 = 0.8759$) (Supplementary Fig. 4a and b).

3.5. Estimation of chlorophyll and carotenoid content

Combined applications of PGPR and organic amendments resulted in higher Chl and carotenoid contents than the control treatments (Table 1). Among the co-inoculated treatments, the highest Chl *a* concentration was recorded in VC + RB-2, followed by VC + RB-1, whereas the lowest Chl *a* value occurred in CD + RB-2. Among individual applications, RB-1 showed the greatest Chl-a content, while the consortium showed the lowest. A similar pattern was observed for Chl *b*. Maximum Chl b concentration was recorded in VC + RB-2, whereas CD + RB-2 showed the lowest value. In individual treatments, VC resulted in the highest Chl-b content (10.83 ± 0.80 mg g^{-1}), while the consortium recorded the lowest value. Carotenoid content also varied among treatments. The highest carotenoid concentration was observed in VC + RB-1, followed by the positive control, whereas the negative control showed the lowest value. Among individual treatments, RB-1 and VC recorded higher carotenoid concentrations than the other treatments.

3.6. Nutrient uptake in maize shoots

Variations in shoot nutrient concentrations were observed among treatments (Table 2). Both combined and individual applications showed higher nutrient concentrations than the controls, with the highest values generally recorded under combined PGPR and organic amendment treatments. Among the combined applications, the highest nitrogen (N) concentration was recorded in VC + consortium, followed by VC + RB-2 and CD + consortium. Among individual treatments, VC resulted in higher shoot N content than both the positive and negative controls. Phosphorus (P) concentrations followed a similar pattern. The highest P content occurred in VC + consortium, followed by CD + consortium. In individual treatments, VC also showed higher P concentrations than the controls. Potassium (K) concentrations varied

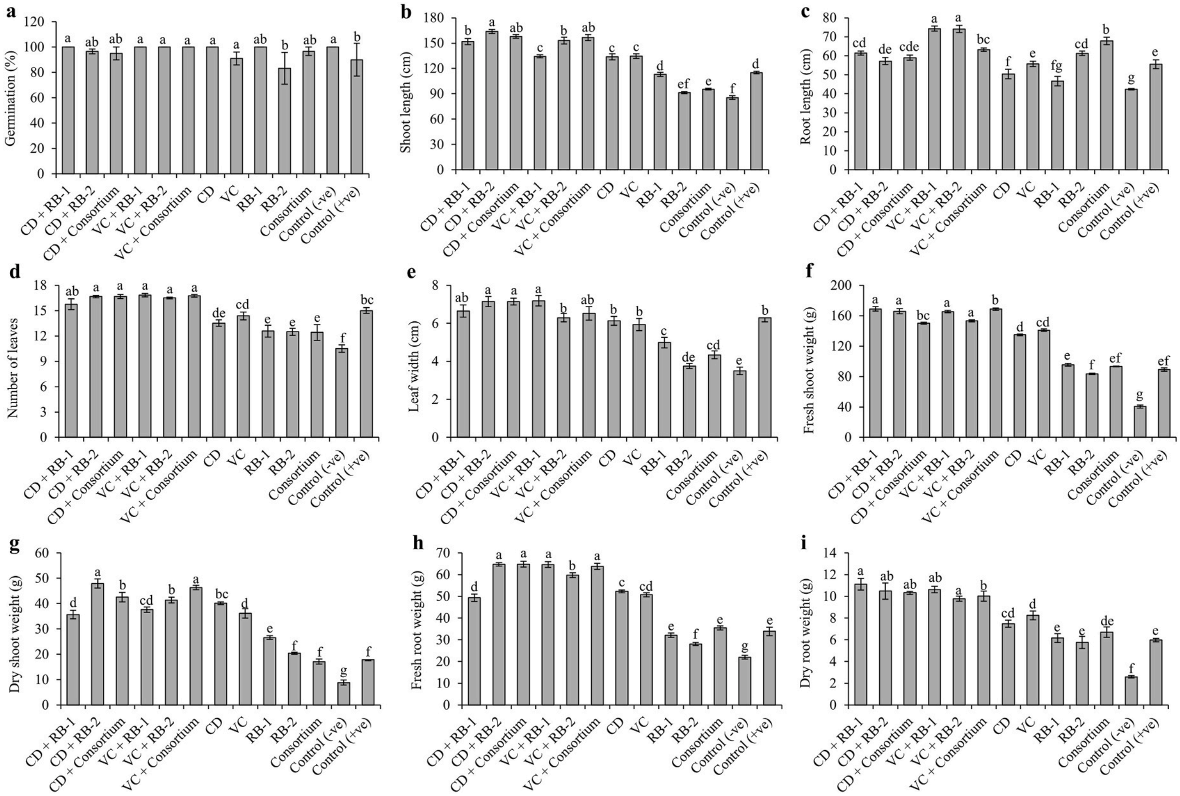

Fig. 2. Effects of individual and combined applications of plant growth-promoting rhizobacteria (PGPR) and organic amendments on maize growth parameters under greenhouse conditions. (a) Germination percentage, (b) shoot length, (c) root length, (d) number of leaves, (e) leaf width, (f) fresh shoot weight, (g) dry shoot weight, (h) fresh root weight, and (i) dry root weight. Error bars represent the standard error (±SE) of four biological replicates. Different letters indicate significant differences among treatment means according to the least significant difference (LSD) test ($P \leq 0.05$).

Table 1
Chlorophyll *a*, chlorophyll *b*, and carotenoid contents in *Zea mays* leaves under combined and individual applications of rhizobacterial isolates and organic amendments.

Treatment	Chl *a* (mg g^{-1})	Chl *b* (mg g^{-1})	Carotenoids (mg g^{-1})
CD + RB-1	14 ± 3.5^{ef}	8 ± 0.5^{ef}	0.2 ± 0.03^{d}
CD + RB-2	13 ± 0.7^{fg}	7 ± 1.0^{gf}	0.2 ± 0.03^{bcd}
CD + Consortium	$14. \pm 1.9^{cd}$	11 ± 1.0^{cd}	0.4 ± 0.02^{ab}
VC + RB-1	19 ± 1.9^{ab}	14 ± 1.1^{ab}	0.5 ± 0.01^{a}
VC + RB-2	20 ± 1.8^{a}	14 ± 1.1^{a}	0.5 ± 0.04^{a}
VC + Consortium	14 ± 0.6^{bc}	11 ± 0.6^{bc}	0.2 ± 0.00^{cd}
CD	12 ± 0.5^{def}	8 ± 0.4^{def}	0.2 ± 0.02^{bcd}
VC	15 ± 1.7^{cd}	11 ± 0.7^{cd}	0.4 ± 0.02^{abc}
RB-1	16 ± 1.4^{cde}	10 ± 0.5^{cde}	0.4 ± 0.05^{ab}
RB-2	$12 \pm 0.6C^{def}$	10 ± 0.4^{cdef}	0.2 ± 0.02^{d}
Consortium	11 ± 0.5^{ef}	8 ± 0.4^{ef}	0.2 ± 0.03^{d}
Control (-ve)	7 ± 0.2^{g}	5 ± 0.4^{g}	0.2 ± 0.01^{cd}
Control (+ve)	14 ± 0.5^{cdef}	9 ± 1.4^{cdef}	0.4 ± 0.02^{abc}

Different letters indicate significant differences among treatment means according to the LSD test ($P \leq 0.05$).

across treatments. Maximum K values were observed in VC + consortium and CD + consortium. Among individual inoculations, RB-1 and RB-2 recorded higher K concentrations than the positive and negative controls. Among secondary nutrients, magnesium (Mg) concentration was highest in VC + consortium, while the consortium applied alone also resulted in higher Mg levels than the controls. Iron (Fe) concentration also differed among treatments. The highest Fe concentration was observed in VC + consortium, whereas in single applications the consortium showed higher Fe uptake than the control (Table 2).

3.7. *Nutrient uptake in maize roots*

Root nutrient concentrations varied among treatments (Table 3). Higher root nutrient levels were observed under combined applications of rhizobacteria and organic amendments compared with individual treatments and controls. In co-inoculated treatments, root N concentrations were higher than those in the positive and negative controls. Among individual applications, VC and CD also resulted in elevated root N levels relative to the controls (Table 3). Additionally, P and K concentrations also differed among treatments. In individual applications, P concentrations ranged from 244 ± 3.34 to 278.25 ± 5.44 mg kg^{-1}, while K concentrations ranged from 204.25 ± 2.93 to 261 ± 4.02 mg kg^{-1}, both exceeding the control values. Higher P and K concentrations were generally observed under combined treatments. Among secondary nutrients, Mg concentration was highest in VC + consortium and lowest in CD + RB-1. In individual treatments, VC also resulted in higher Mg concentrations than the positive and negative controls. Fe concentrations followed a similar pattern. The maximum Fe concentration was recorded in VC + consortium, followed by CD + consortium. In individual applications, the bacterial consortium resulted in higher Fe concentrations than the controls (Table 3).

3.8. *Multivariate and correlation analysis of plant growth and nutrient uptake*

A heatmap with hierarchical clustering was generated to visualise relationships among maize growth parameters and treatments (Fig. 3). The dendrogram separated variables into four main clusters based on correlation patterns. The first cluster included Chl *a*, Chl *b*, and carotenoids; the second comprised root length; the third contained biomass and nutrient parameters (FSW, DSW, FRW, DRW, N, P, Mg, and Fe); and

Table 2
Nutrient concentrations in maize shoots under combined and individual applications of plant growth-promoting rhizobacteria (PGPR) and organic amendments.

Treatment	N concentration (mg kg^{-1})	P concentration (mg kg^{-1})	K concentration (mg kg^{-1})	Mg concentration (mg kg^{-1})	Fe concentration (mg kg^{-1})
CD + RB-1	1970.5 ± 53.2^{cd}	519 ± 4.16^{e}	533 ± 2.08^{b}	2438.5 ± 44.3^{g}	113 ± 1.96^{d}
CD + RB-2	1937.5 ± 33.2^{d}	533.5 ± 5.45^{cd}	503 ± 8.40^{cd}	2622.25 ± 30.43^{f}	125.5 ± 3.66b$_{c}$
CD + Consortium	2067 ± 47.5^{bc}	585.5 ± 3.66^{b}	541 ± 2.48^{ab}	3008 ± 13.41^{b}	130.25 ± 1.65^{b}
VC + RB-1	1981 ± 32.3^{cd}	524.25 ± 3.50^{de}	521.75 ± 1.89^{bc}	2802.5 ± 26.9^{d}	120.75 ± 2.32^{c}
VC + RB-2	2167.75 ± 35.9^{b}	575 ± 4.04^{b}	487 ± 2.86^{d}	3022.75 ± 16.82^{b}	129.75 ± 2.29^{b}
VC + Consortium	2346.25 ± 50.9^{a}	623 ± 2.12^{a}	560.25 ± 4.77^{a}	3316.75 ± 57.7^{a}	143 ± 2.27^{a}
CD	1640.25 ± 43.5^{f}	516.5 ± 2.99 ^{ef}	397 ± 2.94^{f}	2434.25 ± 9.1^{g}	86.75 ± 2.39^{f}
VC	1779.5 ± 45.2^{e}	536 ± 2.35^{c}	359 ± 4.14^{g}	2631 ± 11.50^{f}	85.25 ± 1.38^{fg}
RB-1	1253 ± 76.3^{gh}	481.5 ± 3.38^{g}	417 ± 3.76^{e}	2430 ± 7.33^{g}	80 ± 1.68^{g}
RB-2	1239 ± 40.9^{h}	475 ± 2.74^{g}	428 ± 20.48^{e}	2711.5 ± 8.77^{e}	83.75 ± 2.14^{fg}
Consortium	1380 ± 46.0^{g}	506 ± 6.49^{f}	410.5 ± 2.60^{ef}	2902.25 ± 3.73^{c}	93.25 ± 2.66^{e}
Control (-ve)	855.75 ± 443^{j}	315 ± 3.19^{i}	267.25 ± 3.79^{i}	1632.75 ± 12.78^{i}	61.75 ± 1.25^{h}
Control (+ve)	1084.25 ± 19.8^{i}	414 ± 2.27^{h}	317.75 ± 2.69^{h}	2243.25 ± 1958^{h}	85.25 ± 1.38^{fg}

Different letters indicate significant differences among treatment means according to the LSD test ($P \leq 0.05$).

Table 3
Nutrient concentrations in maize roots under combined and individual applications of plant growth-promoting rhizobacteria (PGPR) and organic amendments.

Treatment	N concentration (mg kg^{-1})	P concentration (mg kg^{-1})	K concentration (mg kg^{-1})	Mg concentration (mg kg^{-1})	Fe concentration (mg kg^{-1})
CD + RB-1	1032.5 ± 19.2^{c}	306 ± 3.70^{d}	319.75 ± 3.47^{a}	1520.75 ± 4.32^{f}	67.75 ± 2.29^{d}
CD + RB-2	1037.5 ± 23.8^{c}	314.25 ± 2.06^{cd}	299.5 ± 7.03^{b}	1685 ± 5.67^{c}	70.75 ± 1.25^{cd}
CD + Consortium	1285.75 ± 7.21^{a}	341.5 ± 2.10^{b}	310.75 ± 3.38^{ab}	1800.75 ± 7.66^{b}	84.5 ± 1.85^{b}
VC + RB-1	1275.25 ± 35.8^{a}	325.25 ± 3.38^{c}	310.25 ± 3.73^{ab}	1575.5 ± 7.51^{e}	73.75 ± 1.38^{c}
VC + RB-2	1155 ± 38.1^{b}	345.75 ± 2.95^{b}	281.5 ± 8.99^{c}	1690.5 ± 4.80^{c}	81.25 ± 1.49^{b}
VC + Consortium	1330.75 ± 21.2^{a}	371.25 ± 1.89^{a}	322.25 ± 3.25^{a}	1861.75 ± 2.28^{a}	93.5 ± 1.44^{a}
CD	1042.25 ± 25.5^{c}	252.25 ± 2.93^{fg}	261 ± 4.02^{d}	1564.75 ± 1.65^{e}	55 ± 1.47^{f}
VC	1119.75 ± 11.2^{b}	278.25 ± 5.44^{e}	244.5 ± 4.44^{e}	1637.25 ± 8.17^{d}	60.25 ± 1.49^{e}
RB-1	797.75 ± 28.4^{ef}	256 ± 7.99^{f}	204.25 ± 2.93^{f}	1113.25 ± 6.76^{i}	49.25 ± 1.75^{g}
RB-2	845.25 ± 20.7^{de}	244 ± 3.34^{g}	216.75 ± 3.17^{f}	1295.25 ± 5.86^{h}	57.5 ± 2.10^{ef}
Consortium	911 ± 14.3^{d}	273.25 ± 3.42^{e}	216.25 ± 3.17^{f}	1426.25 ± 4.23^{g}	62 ± 1.68^{e}
Control (-ve)	578.5 ± 28.8^{g}	109.5 ± 4.37^{i}	106.25 ± 3.12^{h}	820 ± 18.87^{j}	33.25 ± 1.11^{h}
Control (+ve)	749 ± 14.4^{f}	185.5 ± 3.88^{h}	136.5 ± 2.10^{g}	1100.75 ± 13.14^{i}	48 ± 1.83^{g}

Different letters indicate significant differences among treatment means according to the LSD test ($P \leq 0.05$).

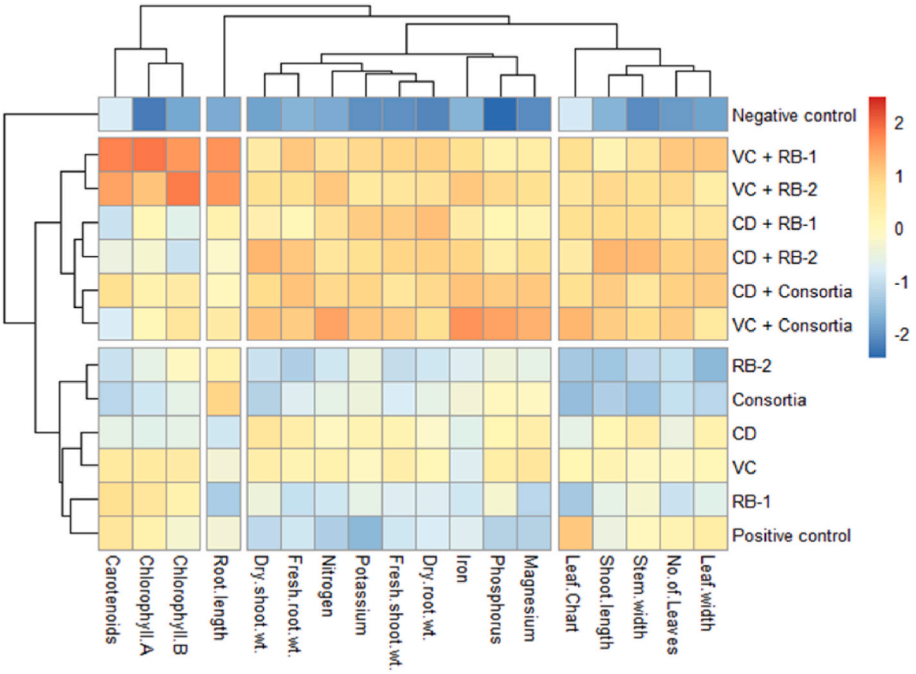

Fig. 3. Heatmap with hierarchical clustering illustrating relationships among morphological, physiological, and nutrient uptake parameters of maize under different treatments. The dendrogram groups treatments and variables into major clusters based on correlation patterns. The colour gradient represents correlation intensity, ranging from −2 (blue) to +2 (red), with higher values indicating stronger positive associations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the fourth group consisted of shoot length, stem width, number of leaves, leaf width, and leaf Chl content. Among treatments, VC + RB-1 and VC + RB-2 were associated with higher Chl and carotenoid values, while VC + consortium was associated with higher Mg, P, and Fe values.

PCA further summarised variation in maize growth and nutrient uptake among treatments. The first two principal components (PC1 and PC2) explained 92.3% and 3.9% of the total variance, respectively, with a cumulative variance of 96.2% (Supplementary Table 2). Among nutrient variables, Fe showed the largest contribution to total variance, followed by P, N, and Mg. The combined treatment VC + consortium showed the greatest dispersion along PC1, followed by CD + consortium, whereas the controls clustered separately (Fig. 4).

Pearson correlation analysis showed positive associations among pigments, growth traits, and nutrient uptake parameters (Supplementary Fig. 5). Chl *a* exhibited strong positive correlation with several morphological and nutrient variables, followed by Chl *b* and

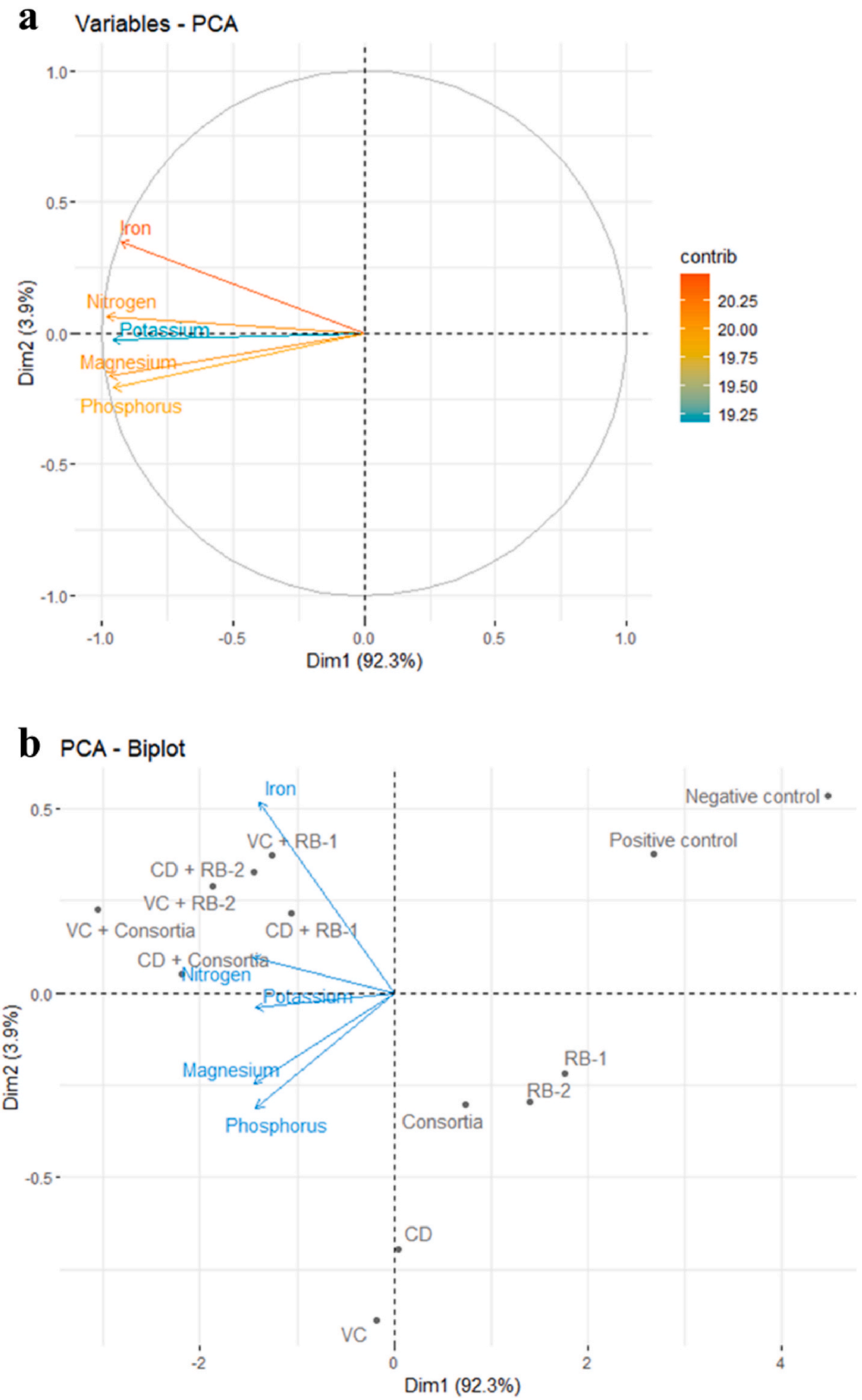

Fig. 4. Principal component analysis (PCA) illustrating relationships between nutrient uptake variables and treatment effects in maize. (a) Variable contribution plot showing the loadings of nutrient elements (Fe, P, N, Mg, and K) on the first two principal components (PC1 = 92.3% and PC2 = 3.9%). (b) PCA biplot showing the distribution of treatments based on their association with nutrient uptake parameters. Combined treatments (VC + RB-1, VC + RB-2, and VC + consortium) are separated from the controls along PC1, indicating enhanced nutrient uptake.

carotenoids. Shoot length was positively associated with stem width, number of leaves, and both fresh and dry biomass. Fresh shoot weight showed positive correlations with nutrient concentrations (K $<$ Mg $<$ Fe $<$ N), while dry root weight was associated with K, N, Fe, Mg, and P accumulation. Carotenoids showed negative correlations with shoot and root length, shoot biomass, and nutrient uptake.

4. Discussion

This study evaluated the potential of two rhizobacterial isolates, RB-1 and RB-2, applied individually and with organic amendments, to enhance maize growth and nutrient uptake under greenhouse conditions. Both isolates expressed multiple PGPTs including IAA and siderophore production, phosphate solubilisation, catalase activity, and ammonia generation. These functional traits are commonly associated with improved plant physiological performance and nutrient acquisition, and may help explain the growth and nutrient uptake responses observed in maize. These findings are consistent with the well-established roles of *Bacillus* and *Pseudomonas* as effective PGPR capable of stimulating root growth, improving nutrient availability, and suppressing phytopathogens through phytohormone production and iron chelation [21–23,25,66]. In particular, IAA-mediated root development and siderophore-associated Fe acquisition have been reported as contributing to biomass accumulation and Chl synthesis [67,68].

Molecular identification by 16S rRNA sequencing confirmed the isolates as *B. cereus* and *P. putida*, consistent with earlier reports describing these taxa as efficient rhizosphere colonisers across diverse environments [69–71]. The seed bioassays further showed that inoculation with either single specie or the consortium enhanced germination, vigour, and early seedling growth without phytotoxic effects, which is consistent with previous studies reporting improved seedling performance following PGPR inoculation [72–74].

An important outcome of this study is the apparent synergy between PGPR and organic amendments. Co-application with CD or VC generally outperformed individual treatments in enhancing shoot and root growth, leaf development, Chl and carotenoid contents, and overall biomass accumulation under greenhouse conditions. These responses were observed across treatments involving individual PGPR strains as well as the consortium. These improvements may be explained by mechanisms proposed in previous studies including gradual nutrient release and improved soil conditions associated with organic amendments [75]. Such conditions have been suggested to facilitate PGPR colonisation and support their functional roles in phosphate solubilisation and siderophore-mediated iron acquisition [75,76]. This is consistent with studies showing that integrating bio-inputs with organic fertilisers can enhance soil organic carbon, nutrient cycling, and plant productivity [15,16,77–80].

The nutrient profiles further support this interpretation. Co-inoculated treatments showed higher N, P, K, Mg, and Fe concentrations in shoots and roots than the controls and most single inputs including treatments involving the consortium, consistent with PGPR-mediated solubilisation and mobilisation of sparingly soluble nutrient pools and with improved root architecture increasing uptake area. The concurrent rise in Chls and carotenoids suggests enhanced photosynthetic capacity, which is an expected downstream response to improved N and Mg supply and increased Fe bioavailability for Chl biosynthesis. These outcomes are in line with broader evidence that PGPR, especially when paired with organic substrates, can improve nutrient use efficiency in cereals [75,77,78].

Multivariate analyses provide integrative support for the observed treatment effects. The heatmap clustered pigments with growth and biomass traits, while nutrients (such as Fe, P, N, and Mg) grouped with biomass metrics, indicating coordinated variation in resource capture and growth-related parameters under co-inoculation. PCA (PC1 and PC2 explaining 92.3% and 3.9% of the variance, respectively; cumulative 96.2%) separated co-treated plants from controls and highlighted Fe, followed by P, N, and Mg, as major contributors to overall variance. This pattern is consistent with nutrient-related responses observed across treatments including those associated with siderophore production and nutrient solubilisation traits measured *in vitro*. Pearson correlation analysis further showed strong associations between Chl *a* and, to a lesser extent, Chl *b* and carotenoids with morphological and biomass traits. Fresh and dry biomass were also positively associated with nutrient accumulation. Together, these multivariate relationships support the interpretation that combined PGPR and organic amendment treatments were associated with coordinated changes in growth, physiological traits, and nutrient uptake under greenhouse conditions.

Importantly, these results contribute to the growing body of evidence suggesting that integrated nutrient management approaches may help address some limitations associated with heavy reliance on synthetic fertilisers, such as soil organic matter decline, nutrient imbalance, and environmental externalities [12]. By showing that RB-1 and RB-2, applied individually and in combination with CD or VC, were associated with improved maize growth and nutrient uptake under greenhouse conditions, this study highlights the potential of PGPR–organic amendment combinations as a complementary approach to synthetic fertiliser use in controlled environments [15,16,25,66].

In summary, co-inoculation of RB-1 (*B. cereus*) and RB-2 (*P. putida*) with organic amendments improved maize growth and nutrient uptake under studied conditions. These responses are likely associated with complementary processes commonly attributed to PGPR, such as microbial nutrient solubilisation and phytohormone-mediated root development. Rather than directly demonstrating yield or soil fertility improvements, the present findings highlight physiological and nutritional responses at the plant level that may be relevant to integrated nutrient management strategies. Although a multi-strain PGPR consortium was included as a comparative reference treatment, this study did not aim to resolve strain-specific functional contributions within the consortium. Accordingly, the observed responses may reflect additive or functionally overlapping effects among consortium members. Because the study was conducted under controlled greenhouse conditions, future field-based investigations across diverse agroecological settings are required to evaluate agronomic performance, yield responses, and longer-term soil effects, as well as to optimise inoculum–amendment combinations and application rates. In addition, the present study did not assess antimicrobial resistance genes in PGPR strains or antibiotic residues in organic amendments, which represents an important consideration for future research on PGPR–organic amendment applications.

5. Conclusions

This study indicates that co-inoculation of RB-1 (*B. cereus*) and RB-2 (*P. putida*) with organic amendments (i.e., CD or VC) enhanced maize growth and nutrient uptake under greenhouse conditions. The combined application was associated with increased Cha and carotenoid contents, greater biomass accumulation, and improved uptake of key nutrients (N, P, K, Mg, and Fe). Overall, these findings suggest that PGPR–organic amendment interactions can support plant growth and nutrient acquisition under controlled conditions, while further evaluation under field conditions is required to assess broader agronomic relevance.

CRediT authorship contribution statement

Mushfaq Ul Nisa: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Zarrin Fatima Rizvi:** Supervision, Resources. **Sajjad Hyder:** Writing – original draft, Validation, Supervision, Resources, Project administration, Conceptualization. **Amjad Shahzad Gondal:** Validation, Software, Formal analysis. **Iftikhar Ahmed:** Resources, Methodology. **Nadia Riaz:** Visualization, Software. **Iqra:** Visualization, Validation, Software. **Amelia Cristina Montoya Martínez:** Validation, Methodology,

Conceptualization. **Sergio de los Santos-Villalobos:** Validation, Resources, Methodology. **Mudassir Iqbal:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization, Resources.

Ethics approval and consent to participate

Not applicable.

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

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors, however, the research was supported by the institutions involved in the study.

Declaration of competing interest

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

Acknowledgements

The authors would like to express their sincere gratitude to the Department of Plant Protection Biology, Swedish University of Agricultural Sciences (SLU), Sweden, for providing the necessary resources and support throughout the research. The authors also acknowledge the handling editor, John P. Brooks, and the four anonymous reviewers for their constructive feedback, valuable suggestions, and time, which substantially improved the quality of the manuscript.

List of abbreviations

BLAST	Basic Local Alignment Search Tool
CAS	Chrome Azurol S
CD	Cow Dung
CFU	Colony-Forming Units
Chl	Chlorophyll
Chl-a	Chlorophyll *a*
Chl-b	Chlorophyll *b*
CRD	Completely Randomised Design
DNA	Deoxyribonucleic Acid
DRW	Dry Root Weight
DSW	Dry Shoot Weight
Fe	Iron
FRW	Fresh Root Weight
FSW	Fresh Shoot Weight
$\mathbf{H_2O_2}$	Hydrogen Peroxide
$\mathbf{H_2S}$	Hydrogen Sulphide
HCN	Hydrogen Cyanide
IAA	Indole-3-Acetic Acid
K	Potassium
KOH	Potassium Hydroxide
LSD	Least Significant Difference
MEGA	Molecular Evolutionary Genetics Analysis software
Mg	Magnesium
ML	Maximum Likelihood
N	Nitrogen
NA	Nutrient Agar
NARC	National Agricultural Research Centre
NB	Nutrient Broth
P	Phosphorus
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PGPR	Plant Growth-Promoting Rhizobacteria
PGPTs	Plant Growth-Promoting Traits
RB-1	*Bacillus cereus* strain (Bot-GCWU-1)
RB-2	*Pseudomonas putida* strain (Bot-GCWU-2)
rRNA	Ribosomal Ribonucleic Acid
VC	Vermicompost

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2026.102742.

Data availability

Data will be made available on request.

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