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RESEARCH ARTICLE

Sustainable chickpea (*Cicer arietinum* L.) farming with plant growth-promoting rhizobacteria (PGPR): A novel approach

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Abstract

Sustainable agriculture is paramount to addressing global food security challenges, particularly in the context of climate change and soil degradation. Chickpea (*Cicer arietinum* L.) stands out as a vital legume crop due to its high nutritional value and ability to improve soil health through nitrogen fixation. It's primarily cultivated during the *Rabi* season throughout India. In Tamil Nadu, it's grown in the western districts, where favorable climatic conditions like high relative humidity and cool temperatures prevail. Despite these advantages, chickpea production has declined due to various pests and diseases. Consequently, the use of varieties highly susceptible to wilt (13 %-33.6 %) during the cropping period causes significant yield losses, ranging from 15 % to 40 %. Additionally, existing cultivars are highly susceptible to pathogens, their potential mutations and prevalence. With this background, our study focused on identifying, characterizing and evaluating the growth-promoting and disease-management potential of rhizobacterial strains. In this study, 19 rhizobacterial strains (CPs1-CPs9; CBs1-CBs10) were collected from the chickpea rhizosphere. These strains were further characterized as *Pseudomonas* and *Bacillus* spp. using cultural, morphological, biochemical and molecular methods (MK254689 to MH745128; MH746113 to MK290646). Among these strains, CPs3 (*P. chlororaphis* - MH628219) demonstrated maximum germination (100 %) and a yield of 1194.4 kg/ha. It also showed the lowest wilt incidence (14.3 % in the glasshouse and 21.67 % in the field), achieving a maximum disease reduction of 70.1 %. This was associated with the highest population density (8.2×10^5 cfu/g of soil) observed 35 days after sowing on cv. CO_4 in the root zone (approximately 3 cm to 5 cm from the root).

Keywords: *Bacillus* spp.; chickpea; cool winter; *P. chlororaphis*; root colonizer; 16S rRNA gene

Introduction

Chickpea (*Cicer arietinum* L.), a crucial leguminous crop, holds a noteworthy place in global farming due to its dietary value and ability to enrich soil health through natural biological nitrogen fixation (1). Its flexibility to differing agro-climatic conditions makes it a basic staple in various locales, especially in developing countries where food security and sustainable agricultural practices are progressively critical (2). As worldwide populations rise and climate change intensifies, production losses due to pests and diseases are becoming more severe. This creates a pressing need to enhance crop productivity while guaranteeing sustainability (3, 4). Globally, chickpea is cultivated on 14.5 million hectares, with a production of 14.7 million tons and a productivity of 1014.6 kg/ha. In India, it is grown on approximately 15.0 million hectares, yielding 15.8 million tons with an average yield of 1058 kg/ha. Generally, it is farmed during the cool winter season (*Rabi*) from November to February (5). Commonly, chickpea has been affected by climatic changes, which are often accompanied by an increase in fungal and viral diseases, causing severe yield losses of up to 100 % (6). Among these, *Fusarium* wilt, caused by the ubiquitous soil-borne pathogen *Fusarium oxysporum* f. spp. *ciceris*, leads to yield losses of up to 40 % -60 % (7). Consequently, its impact on yield loss across all cultivars, combined with the pathogen's mutation, virulence and survival abilities, has transformed into a catastrophic challenge in chickpea cultivation (8). Additionally, in regions where chickpea is a staple crop, the increased disease occurrence due to climate change poses a dual challenge (9). Farmers face declining yields and increasing management costs as they attempt to combat emerging diseases, which, in turn, threatens the economic feasibility of chickpea cultivation (10). Under these circumstances, we are highly

focused on the application of plant growth-promoting rhizobacteria (PGPR) in chickpea farming to reduce soil-borne diseases and enhance plant growth (11). Recently, PGPR have been recognized as a promising approach in crop production and protection due to their antagonistic abilities, survival and colonization capabilities, adaptation to stress habitats and production of antibiotics, antimicrobial compounds, secondary metabolites and growth hormones. These attributes play a crucial role in triggering disease resistance and promoting plant growth and development (12-14). With this background in mind, the present work was carried out to isolate, identify and characterize the potential of these PGPR strains for growth promotion and disease management in chickpea cultivation.

Materials and Methods

Sampling sites

An extensive survey was undertaken in major chickpea-growing districts of Coimbatore, Dharmapuri, Dindigul and Tiruppur in Tamil Nadu during the *Rabi* season of 2016-17. Rhizosphere soil samples were collected from the root zone of infected plants, specifically within a 5 cm diameter around the roots (Fig. 1) (10).

Isolation of PGPR

Ten grams of soil were added to 100 mL of sterile water in conical flasks and shaken on a rotary shaker at 150 rpm for 30 minutes. Then, 0.1 mL of the suspension from serial dilutions (10^{-5} and 10^{-6}) was plated on King's B and Nutrient agar media. The Petri plates were incubated at 28 °C for 48 hours (15).

Characterization of PGPR

Cultural and biochemical characterization

Colonies obtained were purified by streaking. Furthermore, single colonies selected based on their distinct phenotypic (colour) and morphological characteristics, were collected and stored at 4 °C for subsequent studies (16). For bacterial strain identification, various biochemical tests were performed, including HCN production, KOH testing, siderophore production, gelatin hydrolysis, assessment of growth at different NaCl concentrations, catalase activity, starch hydrolysis and citrate utilization. This identification process also incorporated an analysis of morphological and cultural characteristics on agar plates (17, 18).

Molecular characterization

DNA extraction: Twenty bacterial cultures were grown in nutrient agar broth and incubated at 150 rpm on a rotary shaker at 28 °C for 24 hours. After incubation, 1 mL of each culture was taken and centrifuged at 3000 rpm for 5 minutes. The cell pellets were then resuspended in 500 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The pellets were incubated at 55 °C for 30 minutes after adding 50 µL of SDS (10 %) and 25 µL of proteinase K (20 mg/mL). Subsequently, 500 µL of phenol:chloroform (1:1) was added. The tubes were inverted and centrifuged at 14000 rpm for 10 minutes.

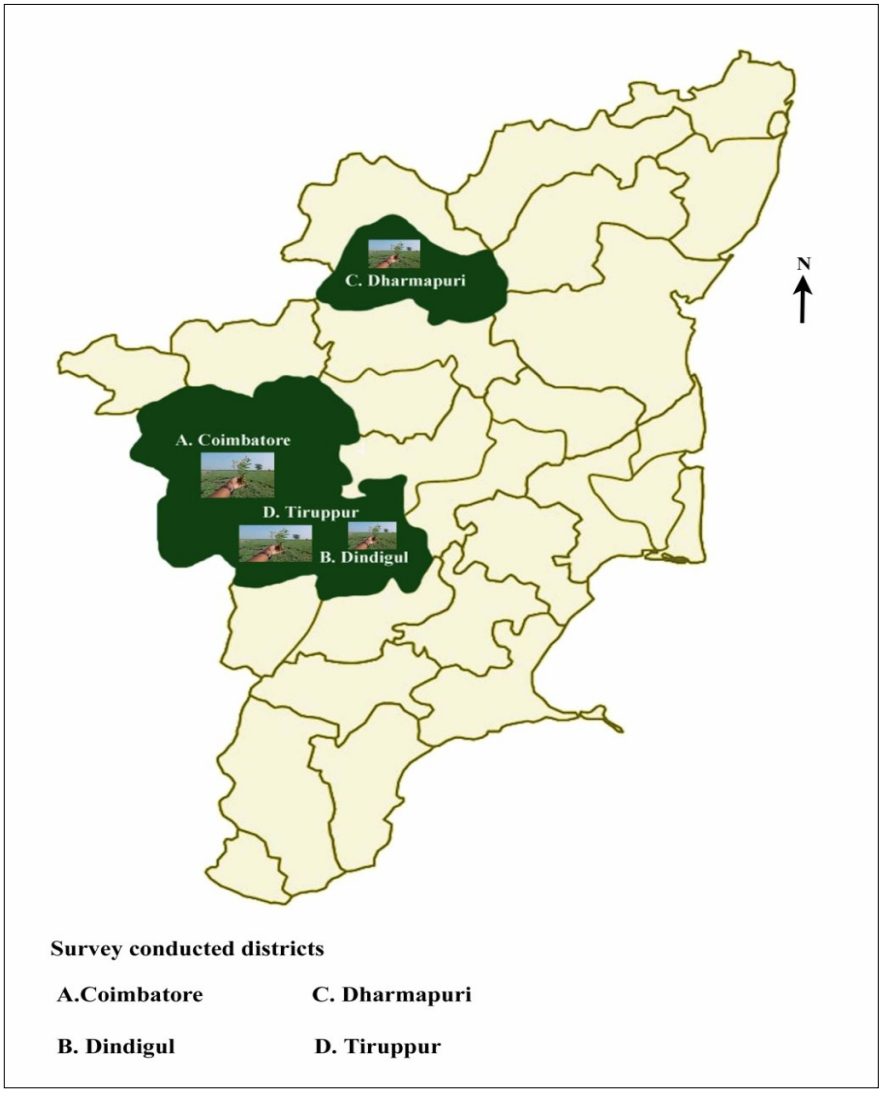

Fig. 1. Details of soil samples collected from rhizosphere of chickpea plants from different locations of Tamil Nadu.

The aqueous layer (500 µL) was carefully transferred into a fresh tube and treated again with 500 µL of phenol:chloroform (1:1). The tubes were inverted a few times, incubated on ice for several minutes and then centrifuged at 14000 rpm for 10 minutes (18). Following this centrifugation, the upper layer (500 µL) was transferred into a new Eppendorf tube and treated with 50 µL of sodium acetate (3M, pH 5.2) and 300 µL of isopropanol (100 %). The tubes were gently inverted and the DNA was precipitated by centrifugation at 14000 rpm for 10 minutes. This was followed by washing with 70 % ethanol and air drying. Finally, the DNA was resuspended in 100 µL of TE buffer containing 2 µL of RNase (19). The DNA was then checked on a 0.8 % agarose gel in TBE buffer at 70 volts for 45 minutes, visualized under a UV transilluminator after EtBr staining. DNA quantification was performed spectrophotometrically by measuring the OD at A260/A280 on a spectrophotometer (20).

PCR reactions : The PCR reaction mixture consisted of 10 µL of 2X PCR master mix, 1 µL of forward primer, 1 µL of reverse primer, 2 µL of template DNA and 5 µL of sterile water. Amplification was carried out in a Thermocycler. The universal 16S rRNA gene primers, 9F (5'-AGTTTGATCCTGGCTCAG-3') and 1510R (5'-GGCTACCTTGTTACGA-3'), were used to amplify the 16S rRNA gene regions of the rhizobacteria. The PCR reaction was performed with an initial denaturation step at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 2 minutes, primer annealing at 55 °C for 1 minute and primer extension at 72 °C for 2 minutes. A final extension at 72 °C for 10 minutes was performed in a PCR Palm Cycler (Corbett Research, Australia). The amplified PCR products were run on a 1.5 % agarose gel in Tris-Acetate Buffer. The gel was stained with ethidium bromide, visualized on a UV-transilluminator and photographed in a gel documentation unit (Alpha Innotech Corp, USA) (21). The 16S rRNA gene region from the isolated strains were further sequenced and subjected to the Nucleotide Basic Local Alignment Search Tool (BLASTn). Additionally, 16S rRNA gene sequences were deposited in GenBank for accession number (22).

Screening of wilt

Selection of PGPR strains

To assess the ability of PGPR under glasshouse and field conditions, selected strains were chosen based on their antagonistic potential against a virulent isolate of *Fusarium oxysporum* f. sp. *ciceris* (Foc 4; MF803741). For these studies, the following rhizobacterial strains were chosen: CPs3 (*P. chlororaphis*, MH628219), CBs5 (*B. subtilis*, MH746091) and the fungal antagonist CTs2 (*T. harzianum*, MH744120). Additionally, Pf1 (*P. fluorescens*) and Tv1 (*T. viride*) were included as checks in the glasshouse and field experiments (23).

Method of applications

In *Rabi* (2017-18), powder-based bioformulations of plant growth-promoting rhizobacteria (PGPR) were utilized for the management of wilt on chickpea cv. CO_4. Seeds were drenched in twice their volume of sterile distilled water containing the talc-based formulation: 10 g/kg of seeds for bacterial treatments (*P. chlororaphis* - CPs3, *B. subtilis* - CBs5 and *P. fluorescens* - Pf1) and 4 g/kg of seeds for fungal antagonists (*T. harzianum* - CTs2 and *T. viride* - Tv1). After 24 hours, the suspension was drained and the seeds were shade-dried for 30 minutes before sowing. Carbendazim, at a rate of 2 g/kg of seeds, was applied as a chemical check through both seed treatment and soil drenching.

Under glasshouse and field conditions, PGPR were also applied through soil application at 5 g/kg of soil (glasshouse) and 2.5 kg/ha blended with 22.5 kg of FYM (field), 30 days before sowing in the field. Salicylic acid at 3 mM was used as a seed treatment, with seeds soaked for 3 hours prior to sowing. Biometric measurements, including the number of pod-bearing branches per plant, number of pods per plant, 100-seed weight, percent wilt incidence and yield data, were recorded. The study was conducted in a randomized block design (Completely Randomized Design for glasshouse and Randomized Block Design for field) and replicated thrice. Wilt incidence was recorded as the percentage of disease occurrence, evaluated using the following formula (24): (Eqn. 1).

$$\text{Per cent disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Results and Discussion

Isolation of PGPR

Twenty rhizobacterial isolates (CPs1 to CBs10) were successfully isolated from chickpea rhizosphere soils collected from various locations across Tamil Nadu. These isolates exhibited significant diversity in their phenotypic and morphological characteristics, as well as their colonization patterns (Table 1). Our findings align with previous research; for instance, a study reported that isolated 118 rhizobacterial strains from the chickpea rhizosphere, many of which demonstrated high levels of indole-3-acetic acid (IAA) production (25). Similarly, researcher reported collecting fifty bacterial strains from the rhizosphere of healthy chickpea plants (26). Furthermore, effective antagonistic bacterial isolates have been consistently obtained from the chickpea rhizosphere during crucial growth stages, from flowering to pod formation (27).

Cultural and biochemical characterization

Based on morphological examination, all isolated *Pseudomonas* species generally exhibited pale, rounded edges. However, upon cultural characterization, strains CPs 5 and CPs 9 specifically exhibited a pale white, translucent and slimy consistency. The *Bacillus* species, in contrast, presented serrated and wavy margins with a pale brown to pale white, slimy appearance (Table 1). These findings align with several previous reports. For instance, a study identified 76 fluorescent pigmented *Pseudomonas* isolates and 98 *Bacillus* species isolates from the chickpea rhizosphere (28). Another investigation characterized 16 isolates from each genus (*Pseudomonas* and *Bacillus* spp.) out of 140 total rhizobacterial isolates also from chickpea (29). Furthermore, *Bacillus* species, specifically gram-positive, spore-producing rhizobacteria, constituted 66.6 % of the bacterial population identified from the rhizosphere of the leguminous tree *Dalbergia sissoo* (30).

Biochemical characterization confirmed the identity of the isolates (Table 2). Specifically, results from tests such as the KOH test, hydrogen cyanide (HCN) generation, gelatin hydrolysis, growth at 7 % NaCl and siderophore synthesis were positive for all ten isolates from Pf1 to CPs9, thereby confirming them as *Pseudomonas* species. Similarly, the remaining ten isolates exhibited positive findings in starch hydrolysis, citrate utilization and catalase tests, indicating their classification as *Bacillus* species. These findings are consistent with previous research. For example, research states that plant growth-promoting rhizobacteria, specifically *Paenibacillus illinoisensis* (RH-31), *Bacillus subtilis* (RH-32) and *P. psychrotolerans* (RH-33), produced siderophores under stress conditions (31). Further supporting our results, a study isolated 17 rhizobacterial strains from

Table 1. Cultural, morphological and molecular characterization of antagonistic rhizobacteria from chickpea

S. No.	Locations	GPS Coordinates		Isolates Name	Cultural characteristics	Morphological characteristics	No. of Nucleotides obtained (bp)	Identified as	Accession No.
		Lat (°N)	Long (°E)						
1.	Idigarai	10.6	77.1	Pf1 (TNAU)	Creamy, translucent, slimy	Rounded	700	*P. fluorescens*	(TNAU)
2.	Gomangalam pudur	10.4	77.8	CPs1	Creamy, light translucent, slimy	Rounded	1513	*S. maltophilia*	MK254689
3.	Thippampatti	12.1	78.4	CPs2	Pale yellowish, translucent, slimy	Rounded	1528	*P. kilonensis*	MK263024
4.	Mukkonam	10.5	77.1	CPs3	Milky white, translucent, slimy	Rounded	382	*P. chlororaphis*	MH628219
5.	Modakkupatti	10.6	77.1	CPs4	Brownish, translucent, slimy	Rounded	1532	*P. thivervalensis*	MK267298
6.	Valzavadi	10.5	77.1	CPs5	Pale white, translucent, slimy	Rounded	1502	*P. brassicacearum*	MK267297
7.	Ramachadra Puram	10.5	77.0	CPs6	Creamy yellow, translucent, slimy	Rounded	641	*P. fluorescens*	MH746464
8.	Periyanakan Palayam	10.6	77.1	CPs7	Creamy white, translucent, slimy	Rounded	1518	*P. mandelii*	MK271276
9.	Poolankinaru	10.5	77.1	CPs8	Brownish, translucent, slimy	Rounded	1542	*P. fredriksbergensis*	MK272774
10.	Konnur	10.6	77.1	CPs9	Pale white, translucent, slimy	Rounded	735	*P. psychrotolerans*	MK745128
11.	Gomangalam pudur	10.4	77.8	CBs1	Pale brown slimy	Serrated point margin	655	*B. cereus*	MK746113
12.	Thippampatti	12.1	78.4	CBs2	Pale brown slimy	Thick serrated margin	925	*B. halotolerans*	MK277217
13	Mukkonam	10.5	77.1	CBs3	Pale white slimy	Wavy winged margin	1001	*B. megaterium*	MH746763
14.	Modakkupatti	10.6	77.1	CBs4	Pale brown thick slimy	Light serrated margin	1503	*B. tequilensis*	MK272850
15.	Vazlavadi	10.5	77.1	CBs5	Pale brown powdery slimy	Serrated margin	717	*B. subtilis*	MH246091
16.	Ramachadra puram	10.5	77.0	CBs6	Pale white slimy	Wavy branched margin	1030	*B. licheniformis*	MK275635
17.	P.N.palayam	10.6	77.1	CBs7	Pale white slimy	Wavy branched margin	756	*B. amyloliquefaciens*	MK282427
18.	Poolankinaru	10.5	77.1	CBs8	Pale brown slimy	Wavy branched margin	1308	*B. vallismortis*	MK290421
19.	Konnur	10.6	77.1	CBs9	Pale brown thick slimy	Wavy branched margin	1094	*B. pumilus*	MK290602
20.	Pannaikinaru	10.5	77.1	CBs10	Dull white slimy	Serrated margin	1231	*B. mojavensis*	MK290646

(**Source**: NCBI)

Table 2. Biochemical characterization of rhizobacteria (PGPR) from chickpea rhizosphere

Sl. No	Isolates	Biochemical tests									
		Gram's Staining	KOH test	HCN production	Catalase test	Starch hydrolysis	Gelatine hydrolysis	Growth in 7 % NaCl	Citrate utilization test	Siderophore production	Tentatively identified as
1	Pf1	Pink	++	+++	-	-	++	-	-	+++	*Pseudomonas fluorescens*
2	CPs1	Pink	++	+	-	-	++	-	-	+	
3	CPs2	Pink	++	+	-	-	++	-	-	++	
4	CPs3	Pink	++	+	-	-	++	-	-	+	
5	CPs4	Pink	++	++	-	-	++	-	-	+	
6	CPs5	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* Spp.
7	CPs6	Pink	++	+	-	-	++	-	-	+	
8	CPs7	Pink	++	+	-	-	++	-	-	+	
9	CPs8	Pink	++	++	-	-	++	-	-	++	
10	CPs9	Pink	++	+	-	-	++	-	-	+	
11	CBs1	Violet	-	-	++	++	-	+	++	-	
12	CBs2	Violet	-	-	++	++	-	+	+	-	
13	CBs3	Violet	-	-	++	++	-	+	++	-	
14	CBs4	Violet	-	-	+	++	-	+	+	-	
15	CBs5	Violet	-	-	++	++	-	+	++	-	
16	CBs6	Violet	-	-	++	++	-	+	+	-	
17	CBs7	Violet	-	-	++	++	-	+	++	-	
18	CBs8	Violet	-	-	++	++	-	+	++	-	
19	CBs9	Violet	-	-	+	++	-	+	+	-	
20	CBs10	Violet	-	-	++	++	-	+	+	-	*Bacillus* Spp.

chickpea soil, which were demonstrated to produce various plant-growth-promoting compounds *in vitro*, including cellulase, hydrogen cyanide, indole acetic acid, ammonia, siderophores, lipase, protease and solubilized phosphate (32). In a broader investigation, 28 out of 196 growth-promoting bacterial strains from diverse plant species exhibited at least five different growth-promoting traits, such as nitrogen fixation, phosphate solubilization, indole-3-acetic acid production, siderophore production and biofilm formation (33).

Molecular characterization

16S rRNA gene sequence analysis successfully amplified all twenty strains in PCR reactions. Among these, ten strains (Pf1 to CPs9) and the remaining ten (CBs1 to CBs10) yielded amplified fragments size of 550 bp and 700 bp, respectively, confirming their identities as *Pseudomonas* and *Bacillus* species (GenBank accession numbers MK254689 to MK290646) (Table 1; Fig. 2). These results are corroborated by previous studies. For instance, 24 rhizobacterial isolates from pigeon pea and chickpea exhibited 94.5 % to 100 % sequence similarity homology with various *Bacillus*, *Acinetobacter*, *Enterobacter*, *Cedecea*, *Serratia* and *Pseudomonas* species based on 16S rRNA gene analysis (34). Similarly, various rhizobacteria isolated from different root-nodulating crops such as chickpea, redgram, *Sesbania* and *Shola pith* showed high identity with *Rhizobium spp.* (IHSR), *Rhizobium tropici* (IHRG), *Rhizobium multihospitium* (IHAA), *Mesorhizobium spp.* (IHGN-3), *Burkholderia cepacia* (IHCP-1) and *Rhizobium pusense* (IHCP-1) through their approximately 1500 bp amplicon size in 16S rRNA gene sequencing. Furthermore, drought-tolerant strains, identified as *B. velezensis* (ZM39) and *B. amyloliquefaciens* (Cha43), were isolated from walnut and characterized using 16S rRNA genes (35). In another study, two genera of plant growth-promoting rhizobacteria were identified via 16S rRNA gene sequencing: *Micrococcus*, including *Micrococcus luteus* (WI 12, WI 41 and WI 80), *Micrococcus yunnanensis* (WI 30 and WI 60) and *Micrococcus sp.* (WI 11 and WI 91); and *Bacillus*, including *B. subtilis* (WI 63, WI 65), *B. tequilensis* (WI 62), *B. cereus* (WI 36) and *B. licheniformis* (WI 90) (36). It is also notable that *Bacillus* genus 16S rRNA genes commonly produce a fragment size of 536 bp (37).

Screening of wilt

Under glasshouse conditions, the treatment involving *P. chlororaphis* strain CPs3 (MH628219) was significantly more effective than other rhizobacterial strains. This treatment, applied as both a seed treatment (10 g/kg of seeds) and a soil application (5 g/kg of soil), resulted in 100 % germination and recorded the lowest wilt incidence of 14.3 %, achieving an impressive 80.7 % disease reduction (Table 3). In field studies, the CPs3 (*P. chlororaphis*) treatment consistently demonstrated superior performance. It recorded the highest yield of 1194.4 kg/ha, accompanied by favorable biometric parameters including 13 pod-bearing branches per plant, 32.3 pods per plant and a 100-seed weight of 33.0 g. These results were notably superior to the chemical check, carbendazim (0.1 %), which yielded 1180.0 kg/ha. Furthermore, CPs3 effectively reduced wilt incidence to 21.67 %, representing a 70.18 % disease reduction (Table 4).

These findings corroborate previous research indicating that PGPR strain applications effectively reduce wilt incidence and enhance growth-promoting activities, such as increased pod number, pod weight and overall yield, as observed with *B. amyloliquefaciens* (K3) (38). Other studies have also highlighted the efficacy of bacterial consortia in chickpea wilt management and

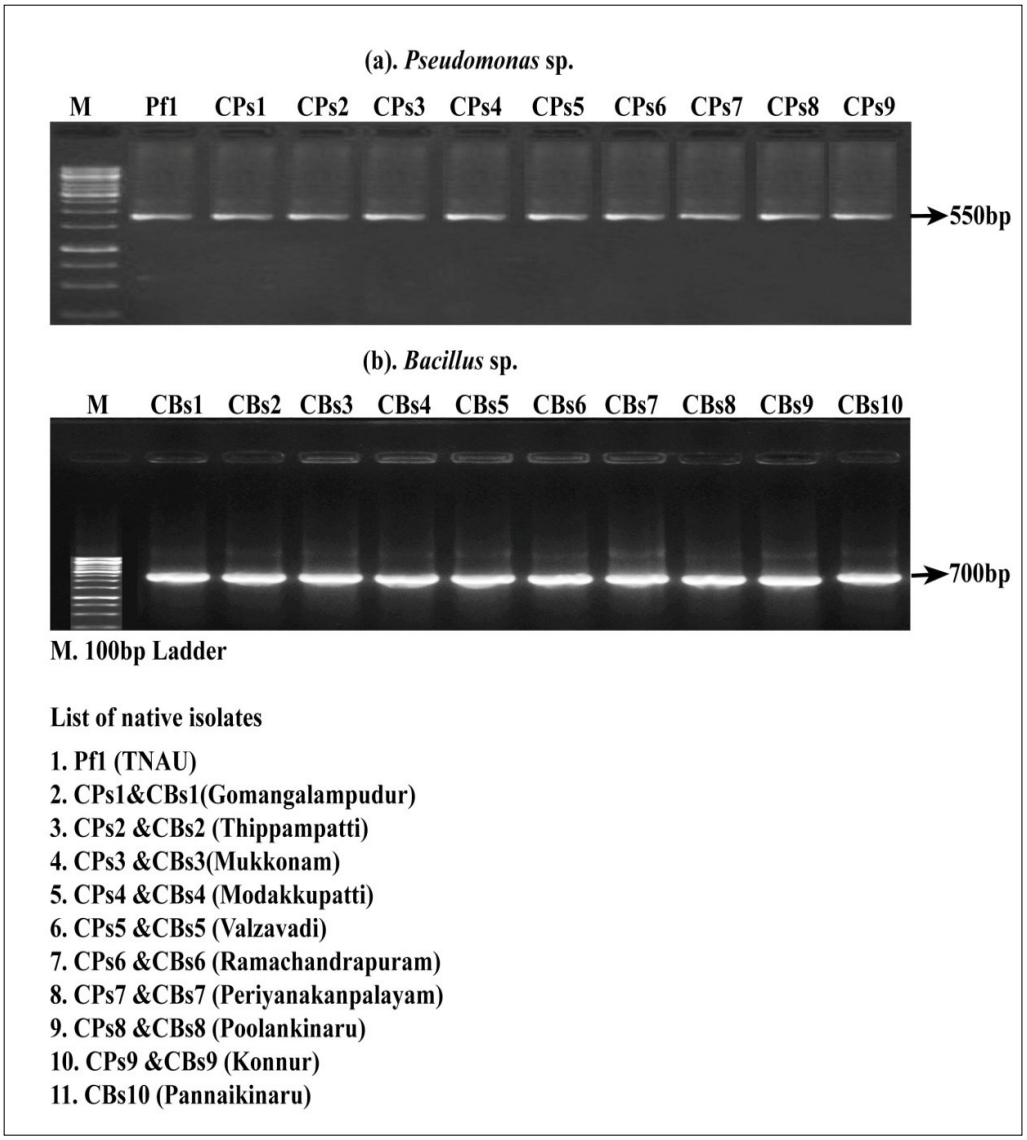

Fig. 2. Molecular characterization of PGPR strains from chickpea rhizosphere by using 16S rRNA gene.

Table 3. Screening of *Fusarium* wilt in chickpea (cv. CO_4) by using PGPR under glasshouse conditions

S. No	Treatment details (Seed treatment, Soil application and Soil drenching)	Germination (%)	Percent disease incidence (%)	Per cent disease reduction over control (%)
1.	**T1.** Pf1(*P. fluorescens*) Seed treatment with 10g / kg of seeds + soil application of 5g / kg of soil	88.3 ^{b}	28.3 ^{b} (32.15)	61.8 ^{b}
2.	**T2.** Pf1 (*P. fluorescens* - Liquid) Seed treatment with 10 mL / kg of seeds + soil application of 5g / kg of soil	72.1 ^{e}	35.3 ^{de} (36.47)	52.4 ^{de}
3.	**T3.** Tv1 (*T. asperellum*) Seed treatment with 4g / kg of seeds + soil application of 5g / kg of soil	83.3 ^{c}	27.0 ^{b} (31.19)	63.6 ^{b}
4.	**T4.** CPs3 (*P. chlororaphis*) Seed treatment with 10g / kg of seeds + soil application of 5g / kg of soil	100.0 ^{a}	14.3 ^{a} (22.24)	80.7 ^{a}
5.	**T5.** CBs5 (*B. subtilis*) Seed treatment with 10g / kg of seeds + soil application of 5g / kg of soil	72.1 ^{e}	33.6 ^{cd} (35.46)	54.7 ^{cd}
6.	**T6.** CTs2 (*T. harzianum*) Seed treatment with 4g / kg of seeds + soil application of 5g / kg of soil	78.3 ^{d}	38.6 ^{e} (38.44)	47.9 ^{e}
7.	**T7.** Carbendazim 0.1 % (Seed treatment 2g/ kg of seeds + Soil drenching @ 14 DAS)	83.3 ^{c}	10.6 ^{a} (19.05)	85.6 ^{a}
8.	**T8.** Salicylic acid (Seed treatment @ 3mM (3- hours seeds soaking before sowing)	72.1^{e}	30.6 ^{bc} (33.62)	58.7 ^{bc}
9.	**T9.** Control	50.0 ^{f}	74.3 ^{f} (59.57)	0.00 ^{f}

Values are mean of three replications.

Means followed by a common letter are not significantly at 5 % level by DMRT

*Values in the parenthesis are arscine-transformed values.

Table 4. Management of *Fusarium* wilt of chickpea (cv. CO_4) under field conditions by using PGPR for sustainable crop production

S. No	Treatments details (Seed treatment + Soil application)	Per cent disease incidence (%)	Per cent reduction over control (%)	No. of pod bearing branches / Plant	No. of pods / Plant	100 seeds weight (g)	Yield (kg / ha)
1.	**T1.** Pf1 (*P. fluorescens*) Seed treatment with 10g / kg of seeds + soil application 2.5kg /ha of field	32.00 ^{c} (34.43)	55.96	11.0 ^{bc}	24.7 ^{c}	30.6 ^{c}	1172.5 ^{c}
2.	**T2.** Pf1 (*P. fluorescens*-Liquid) Seed treatment with 10 mL / kg of seeds+ soil application 2.5kg /ha of field	34.67 ^{cde} (36.05)	52.29	8.7 ^{d}	13.7 ^{f}	26.3 ^{de}	1121.3 ^{d}
3.	**T3.** CPs3 (*P. chlororaphis*) Seed treatment with 10g / kg of seeds + soil application 2.5kg /ha of field	21.67 ^{b} (27.72)	70.18	13.0 ^{a}	32.3 ^{a}	33.0 ^{a}	1194.4 ^{a}
4.	**T4.** CBs5 (*B. subtilis*) Seed treatment with 10g / kg of seeds + soil application 2.5kg /ha of field	33.67 ^{d} (35.46)	53.66	10.0 ^{cd}	23.0 ^{c}	25.6 ^{e}	1113.2 ^{e}
5.	**T5.** Tv1 (*T. asperellum*) Seed treatment with 4g / kg of seeds+ soil application 2.5kg /ha of field	36.67 ^{e} (37.25)	49.53	9.7 ^{cd}	19.7 ^{d}	23.0 ^{f}	1057.0 ^{f}
6.	**T6.** CTs2 (*T. harzianum*) Seed treatment with 4g / kg of seeds+ soil application 2.5kg /ha of field	42.33 ^{f} (40.58)	41.75	10.3 ^{c}	18.7 ^{d}	28.0 ^{cd}	1052.0 ^{g}
7.	**T7.** Carbendazim (0.1 %) Seed treatment with 2g / kg of seeds + soil drenching @ (14 DAS)	18.33 ^{a} (25.34)	74.77	12.0 ^{ab}	27.7 ^{b}	27.3 ^{b}	1180.0 ^{b}
8.	**T8.** Salicylic acid Seed treatment @ 3mM (3- hours seed soaking before sowing)	33.67 ^{cd} (35.45)	53.66	8.7 ^{d}	15.7 ^{e}	22.3 ^{f}	1017.0 ^{h}
9.	**T9.** Control (Untreated)	72.67 ^{g} (58.48)	0.00	7.0 ^{e}	13.7 ^{f}	18.3 ^{g}	859.5 ^{i}

Values are mean of three replications.

Means followed by a common letter are not significantly at 5 % level by DMRT

*Values in the parenthesis are arscine-transformed values.

growth promotion. For instance, a consortium of *Serratia spp.* IN-1, *Serratia spp.* IS-1, *Enterobacter spp.* IN-2 and *Enterobacter spp.* IN-6, along with strains IR-27 and IR-57, exhibited significant inhibition of pathogen growth and controlled disease occurrence. This was coupled with substantial growth-promoting effects, including a 107 % increase in root length, a 23 % increase in shoot length and a 54 % increase in branching (25). Similarly, PGPR strains RHA and RPG, isolated from the chickpea rhizosphere, proved effective in reducing fungal root infection in greenhouse settings and significantly increasing seed yields (39). The intrinsic ability of PGPR for growth promotion, colonization and the synthesis of antifungal compounds plays a crucial role in disrupting wilt pathogen colonization and ensuring plant survival from sowing to harvesting (40).

Conclusion

The integration of plant growth-promoting rhizobacteria (PGPR) into chickpea cultivation represents a highly promising strategy for enhancing agricultural productivity while simultaneously minimizing environmental impact. By harnessing the intricate symbiotic relationships between beneficial microbes and chickpea plants (*Cicer arietinum* L.), farmers can achieve improved plant growth, optimized nutrient uptake and increased resilience to both biotic and abiotic stresses. This sustainable and innovative approach not only contributes to the economic viability of chickpea production but also aligns with the fundamental principles of sustainable agriculture by significantly reducing the reliance on synthetic chemical fertilizers and pesticides.

The strategic utilization of PGPR in chickpea farming fosters healthier soil ecosystems, enhances microbial biodiversity and optimizes rhizobiome function, thereby ensuring the long-term sustainability of cultivation practices. Furthermore, this strategy directly supports global food security initiatives by promoting higher yields in a crop that is nutritionally and economically fundamental across various regions, particularly in developing nations. As research continues to elucidate the complex mechanisms and multifaceted benefits of PGPR, the broader adoption of these beneficial microorganisms in chickpea cultivation can serve as a compelling model for sustainable agricultural practices in other crops as well. Overall, advancing our understanding and practical application of PGPR in chickpea farming outlines a crucial step towards ecological resilience and agricultural sustainability. This makes it a novel and indispensable approach in the ongoing pursuit of nutritional security and responsible environmental stewardship.

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Authors' contributions

PAR supported the research by funding sources (AICRP). KK, MJR & EAAS contributed to data collection. SV supervised the research project. AK provided subject expertise and critically reviewed the manuscript. PJ revised and finalized the manuscript. PMS

conceptualized and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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AI Declaration : Grammarly AI tool was used to improve language and readability, with caution.

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