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CONTENTS

Sequential functional screening and trait-based association of chickpea rhizobacterial isolates using multiple correspondence analysis

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ABSTRACT: The rhizosphere microbiome plays a vital role in enhancing plant health, productivity and resilience under diverse climatic stress conditions. In the present study, 52 isolates of bacteria were obtained from the rhizosphere of four Chickpea (*Cicer arietinum* L.) genotypes and thoroughly screened for plant growth-promoting (PGP) traits, biochemical characteristics, and multi-stress tolerance. A stepwise screening strategy was employed, in which preliminary qualitative screenings of PGP characteristics narrowed down to the selection of 16 active isolates testing positive for all the examined traits. These were then subjected to biochemical characterization, yielding seven isolates that consistently demonstrated the varying metabolic activities (*viz*., Catalase, Cellulase, Urease, MR-VP, Oxidase, Amylase, Caseinase, Gelatinase and Citrate utilization). Multi-stress tolerance screening revealed three extremely stress resistant strains i.e., PG5-4, PG5-12 and PG186-35, that showed admirable adaptive behaviours towards salinity, drought, temperature and pH stresses. To determine the trait-isolates correlations, Multiple Correspondence Analysis (MCA) was utilized at every selection step, attaining robust statistical confirmation of functionally proficient bacterial strains. This trait-based, multivariate approach not only streamlined the selection process but also facilitated the formulation of ecologically effective PGPR biofertilizers for sustainable agriculture. Thus, the research proposes that the above strains have substantial potential for the development of a climate-smart PGPR consortium biofertilizer, particularly suited for legume-based cropping system under very adverse environmental conditions.

Keywords: Chickpea rhizosphere, biochemical traits, climate resilience, PGPR, plant growth promoting traits, Multiple Correspondence Analysis, stress tolerance

Chickpea (*Cicer arietinum* L.) is a nutritionally beneficial leguminous crop that is extensively grown across the world. It is the essential source of human nutrition as it has highly proteinaceous seeds and important as well because of its ability to enhance soil health through symbiotic nitrogen fixation with *Mesorhizobium* species (Samal *et al*., 2023). This dual benefit of protein source and ecological sustainability enhances the importance of chickpeas in agriculture. However, sudden shift in the climatic conditions the chickpea cultivation has been drastically affected by the rising intensity and frequency of stresses such as drought, salinity, temperature extremes and nutrient deficiencies (Rani *et al*., 2020). The stressors have adverse effects on germination, root growth, nodulation, photosynthesis and eventually crop yield as well as quality. Thus, stress situations being faced with climate change is a strong threat to food and nutritional security.

In this regard, the plant-associated microbiome, particularly the rhizosphere microbiota, played a significant role in the development of plant health and yield (Qu *et al*., 2020). Specifically, Plant Growth-Promoting Rhizobacteria (PGPR), which is a functional group of beneficial microorganisms, inhabit the rhizosphere and promote plant growth by both direct (e.g., nitrogen fixation, phosphate solubilization, phytohormone production) and indirect (e.g., suppression of phytopathogens, induction of systemic resistance, and modulation of plant stress responses) mechanisms (Gupta *et al*., 2025). These PGPRs have varied biochemical functions, including siderophore secretion, hydrolytic enzymes (e.g., proteases, cellulases), organic acids and volatile compounds, which not only help in mobilization of nutrients but also defend plants against biotic and abiotic stresses (Compant *et al*., 2019).

Despite these functional traits, ecological specificity, host genotype interactions and environmental heterogeneity, still make PGPR application in the field inconsistent (Sharma *et al*., 2025). The majority of the previous research has depended on conventional screening strategies that tend to examine a single trait at a time, rather than giving consideration to the integrative ability of strains that possess multiple beneficial traits simultaneously (Hossain *et al*., 2017). This fragmented methodology restricts our capacity to find and harness elite PGPR strains with multifunctionality and high stress-prone environment adaptability (Vishwakarma *et al.,* 2024).

To enhance the accuracy, efficiency, and reliability of selecting promising PGPR strains, researchers are increasingly shifting toward trait-based, multivariate, and integrated screening approaches. These advanced strategies allow a more comprehensive understanding of microbial functions and their potential benefits under varying conditions. In the present study, we applied this broader framework by conducting a systematic, step-wise functional screening of rhizobacterial isolates obtained from multiple chickpea cultivars, ensuring a more robust selection of effective strains (Alemneh *et al*., 2021). The research involves a stepwise characterization protocol, firstly evaluating PGP traits like phosphate solubilization, siderophore and ammonia production, HCN release and phytohormone production, secondly biochemical profiling (e.g., catalase, protease, cellulase activity and utilization of substrates etc.), and lastly screening stress-tolerant characteristics under controlled abiotic stress conditions.

For analyzing the intricate interplay between several categorical traits and for detecting sets of functionally correlated strains, Multiple Correspondence Analysis (MCA) was employed (Azam *et al*., 2023). MCA is a powerful multivariate method specifically designed for dealing with qualitative data. It helps identify which traits are linked to specific strains and group them into functionally similar clusters, thereby simplifying the selection of suitable PGPR candidates for further use (Moreira *et al*., 2016).

The integrative strategy not only improves the effectiveness of screening and selection but also sets the stage for developing microbial consortia designed for climate-resilient chickpea cultivation. Therefore, this study addresses an important knowledge gap in PGPR research by integrating sequential trait-based screening with multivariate analysis. This combined approach enables a more precise identification and characterization of promising rhizobacterial strains that can contribute to sustainable and climate-resilient chickpea production.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Healthy plants of four chickpea varieties (PG-5, PG-3, PG-186, and PUSA-368) at one to two months of growth were carefully uprooted to isolate plant growth-promoting rhizobacteria (PGPR) from the chickpea rhizosphere of sandy loam soil with near neutral pH (6.86). Under aseptic conditions, the rhizospheric soil around the roots was collected in sterile polybags. In order to evaluate bacterial growth, the collected soil samples were processed using the serial dilution procedure and plated on Plate Count Agar (PCA). Bacterial isolates were isolated, purified and maintained for further analysis. Bacterial isolates were chosen and purified for morphological characterisation, Gram staining PGP traits.

Preliminary PGP Trait Screening

The initial qualitative screening of 52 rhizobacterial isolates from the chickpea rhizosphere was performed to analyse plant growth-promoting (PGP) characteristics. Phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore secretion, hydrogen cyanide (HCN) generation, ammonia production, ACC deaminase activity, potassium solubilization and exopolysaccharide (EPS) formation tests were all part of the screening.

Indole-3-Acetic Acid (IAA) Production

The modified colorimetric approach of Bric *et al*.

(1991) was used to qualitatively analyse IAA production. 100 µg m^{-1} L-tryptophan was added to 200 µL of fresh bacterial cultures, which were then incubated for five days at 28±2°C in rotatory shaker at 100 rpm. Following incubation, the cultures were centrifuged for 15 minutes at 4°C at 5,000 rpm. 2mL of the supernatant was collected and over it 4mL of Salkowski reagent (100 mL of 35% perchloric acid along with 2 mL of 0.5 M $FeCl_3$) was added and at room temperature got incubated for half an hour. Formation of a pink hue indicated positive IAA generation.

Siderophore Production

The siderophores production was examined using Chrome Azurol Sulphonate (CAS) agar medium in accordance with Schwyn and Neilands's (1987) method. 60.6 mg of CAS in 50 mL of distilled water, 10 mL of 1 mM $FeCl_3$ (dissolved in 10 mM HCl), and 52.9 mg of hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 mL of distilled water were combined to create the CAS dye solution. After thorough mixing the dye complex, it was autoclaved. Bacterial cultures were inoculated to nutrient agar plates coated with a 10% CAS dye solution, then incubated at 28±2°C. Siderophore production was indicated by a yellow-orange halo surrounding colonies.

Hydrogen Cyanide (HCN) Production

HCN production was assessed by adding 4.4 g L^{-1} glycine supplement to nutritional agar before streaking bacterial inoculant on it as method oulined by Bakker and Schipper, 1987. A filter paper was placed on the lid of Petri dish after being soaked in 0.5% picric acid and 0.2% sodium carbonate and sealed with Parafilm. Further the plates were incubated for 48-52 hours at 28±2°C. The color of filter paper changed from yellow to orange-brown, indicating the production of HCN.

Ammonia Production

The ammonia production was assessed by culturing in peptone water and incubating at 28±2°C for 48 hours (Hansen, 1930). To this Nessler's reagent (0.5 mL/tube) was added and the development of a yellow to brown color was observed as the positive test for ammonia production.

ACC Deaminase Activity

The method developed by Penrose and Glick (2003) with Dworkin and Foster (DF) minimal salt media (Dworkin and Foster, 1958) adjusted with 3 mM 1-aminocyclopropane-1-carboxylate (ACC) was used to detect ACC deaminase activity. ACC-supplemented DF medium was used to spot-inoculate log-phase bacterial cultures and incubated for 48 hours at 28±2°C. The growth on the prepared medium confirmed ACC deaminase activity as it is necessary for growth on ACC medium but not on DF only.

Phosphate Solubilization

The phosphate solubility was evaluated using Pikovskaya's agar (Pikovskaya, 1948) medium. The bacterial cultures were spot inoculated for 4-5 days at 28±2°C. The distinct halo zone around the colony indicated ability for phosphate solubilisation.

Potassium Solubilization

Aleksandrov agar medium was used to test potassium solubilization (Verma *et al*., 2016). For five to seven days, the cultures on Aleksandrov agar medium were incubated at 28±2°C. The solubilization of insoluble minerals including potassium was ascertained by observing the creation of haloes surrounding colonies.

Exopolysaccharide (EPS) Production

The ethanol precipitation method (Bajpai *et al*., 2016), was used to produce EPS. For 52 hours, bacterial cultures were cultivated in nutrient broth at 28±1°C while being shaken at 150 rpm. The supernatant was mixed with cold absolute ethanol (1:1 v/v) following centrifugation (8,000-10,000 rpm, 15 min, 4°C). The presence of a viscous layer at the contact suggested the development of EPS.

Biochemical Characterization of PGP-Positive Isolates

Selected rhizobacterial isolates were biochemically characterized in order to evaluate their metabolic functionality and enzymatic adaptability. The rapid generation of oxygen bubbles upon the addition of

Table 1: Bacterial Isolates showing result of different PGP traits

Strain name	IAA	Siderophore	P Solubilization	K Solubilization	ACC Deaminase	HCN	Ammonia Production	Exopolysaccharide
PG186-33	+	-	-	-	+	+	+	-
PG186-11	-	+	+	-	+	-	-	+
PG186-19	-	+	+	-	-	+	+	-
PG186-17	+	+	+	+	+	+	+	+
PG186-21	+	-	+	-	-	-	-	-
PG186-20	+	+	+	+	+	+	+	+
PG186-186	-	+	-	-	-	-	+	-
PG186-3	-	-	-	-	-	+	-	+
PG186-14	-	+	-	-	-	-	+	-
PG186-27	+	+	+	+	+	+	+	+
PG186-40	+	+	+	+	+	+	+	+
PG186-41	+	+	+	-	+	+	-	+
PG186-7	+	-	+	+	-	-	+	-
PG186-2	+	+	-	+	-	+	-	-
PG186-43	-	+	-	-	-	-	-	+
PG186-35	+	+	+	+	+	+	+	+
PG3-8 (i)	+	+	+	+	+	+	+	+
PG3-8(ii)	-	-	-	+	+	-	+	-
PG3-9	-	-	-	-	-	+	-	-
PG3-2	+	+	+	+	+	+	+	+
PG3-11	-	-	-	+	+	+	-	-
PG3-7	+	-	+	-	-	-	-	+
PG3-5	+	+	+	+	+	+	+	+
PG3-1	+	+	+	+	+	+	+	+
PG3-6	+	-	+	-	-	+	-	-
PUSA-12	+	+	+	+	+	+	+	+
PUSA-7	+	+	+	+	+	+	+	+
PUSA-16	-	-	-	+	-	-	-	-
PUSA-15	-	+	+	-	-	+	-	+
PUSA-10	+	+	+	+	+	+	+	+
PUSA-3	+	-	-	+	+	+	+	-
PUSA-9	-	+	+	-	+	-	+	-
PUSA-11	+	+	+	+	+	+	+	+
PUSA-5	-	+	+	+	-	+	-	-
PUSA-2	+	+	+	+	+	+	+	+
PUSA-22	+	-	-	-	+	-	-	-
PUSA-3	+	+	+	-	-	+	+	-
PG5-17(ii)	+	+	+	-	-	+	-	+
PG5-15	+	+	+	+	+	+	+	+
PG5-5	-	-	-	-	-	-	-	-
PG5-11(ii)	+	+	+	+	-	+	+	-
PG5-11(i)	+	-	+	+	+	+	-	+
PG5-8	+	-	-	-	-	-	+	+
PG5-19	+	+	+	+	+	+	+	+
PG5-4(i)	+	+	+	+	+	+	+	+
PG5-4(ii)	-	+	+	+	-	-	-	+
PG5-16	+	-	-	-	-	+	+	-
PG5-7	+	-	+	-	+	+	-	-
PG5-13	-	-	+	-	-	-	-	+
PG5-10	+	+	+	+	+	+	+	+
PG5-1	+	+	-	-	-	+	-	+
PG5-12	-	-	-	-	+	+	+	-

3% hydrogen peroxide on bacterial cell signified the breakdown of H_2O_2 indicative of catalase activity (Cappuccino and Sherman, 1996). The following hydrolytic enzyme activities were assessed by zone clearance on particular media: gelatinase activity through liquefaction of nutrient gelatin medium after incubation at 4°C (Aneja, 2003; Thomas *et al*., 2012), amylase activity via starch hydrolysis on starch agar after Gram's iodine staining (Kasana *et al*., 2008), and caseinase activity on skim milk agar as indicated by halo formation around bacterial colonies (Kasana *et al*., 2008). Citrate utilization was assessed on Simmons citrate medium, where bacterial growth and a color shift confirmed the use of citrate as the only carbon source (MacWilliams, 2009). Urease activity was detected using Christensen's urea agar, where a color shifts from yellow to pink indicated ammonia release and increased pH (Cappuccino and Sherman, 1996). Methyl Red (MR) and Voges-Proskauer (VP) tests were used to identify the fermentation pathways in MR-VP broth. Mixed acid fermentation (MR+) was indicated by a red coloration following methyl red addition, while acetoin production (VP+) was confirmed by the formation of a pink ring following VP reagent treatment (McDevitt, 2009). The presence of cytochrome c oxidase was shown by a purple-blue tint on oxidase discs confirming oxidase activity (Dimri *et al*., 2020).

Stress Tolerance Assays

The pH tolerance was determined by growing bacterial isolates in pH-adjusted nutrient broth at 4, 5, 6, 7, 8, and 10 using 1N HCl or 1N NaOH. Sterilized media were inoculated with 1% (v/v) of actively growing cultures in various pH-adjusted nutrient broth and incubated at 28/ ±/ 2°C for 48 hours. Growth was measured by optical density (OD) at 600 ηm, where higher ODs indicated increased tolerance to adverse pH (Takano and Aoyagi, 2022). Temperature tolerance was ascertained by cultivating the isolates in nutrient broth at six temperatures i.e., 12, 20, 28, 37, 45 and 50°C. Growth at all temperatures was observed after 48 hours of incubation measuring OD at 600 žm (Sarikhani *et al*., 2019). Salt tolerance was measured by growing the isolates on nutrient broth with different NaCl concentrations (0%-30%). Bacterial growth at each salinity point after 48 hours of incubation at 28 ± 2°C was measured through OD at 600 ηm (Yaish *et al*., 2015). Biocontrol activity of the bacterial isolates was ascertained against two fungal species namely *Rhizoctonia solani*, a soil-borne pathogen and *Colletotrichum truncatum*, a seed-borne pathogen by growing them together and observing the inhibition of the fungal growth in the surrounding of PGPR. These tests provided initial information about the tolerance ability of microbes to alkaline/acidity, temperature fluctuation, salt stress and biotic stress which are key to selecting stress-resilient strains for sustainable agriculture under climate change scenario.

Trait Matrix Construction and Statistical Analysis

Three separate trait matrices were developed to systematically screen and categorize the functional diversity among chickpea rhizobacterial isolates. The first matrix included all 52 isolates and recorded the presence or absence of various plant growth-promoting (PGP) traits. Based on their overall PGP potential, 16 promising isolates were selected for further biochemical evaluation, and this information was used to construct the second matrix, which documented key qualitative biochemical characteristics. A third matrix was then prepared for these selected isolates to capture their tolerance levels against different stress factors, including salinity, pH, temperature, and fungal pathogen challenge.

To better understand the complex relationships between isolates and their traits, Multiple Correspondence Analysis (MCA) was performed in R software for each matrix (Abdi and Valentin, 2007). MCA is a multivariate statistical method designed to analyse and simplify large sets of categorical data. It works by reducing the data into a few meaningful dimensions, allowing clearer visualization of how isolates group together and how different traits are associated (Khangar and Kamalja, 2017). Through these component plots, MCA helped reveal clustering patterns, similarities, and correlations between traits and strains.

Overall, this multivariate approach was effective in distinguishing the functionally superior isolates and

provided deeper insights into the connections among PGP traits, biochemical properties, and stress tolerance within the chickpea rhizobacterial community (Zhang *et al.,* 2020).

RESULTS AND DISCUSSION

PGP Trait Distribution

Among the 52 rhizobacterial isolates from the rhizosphere of four chickpea varieties, 16 isolates-PG5-15, PG5-12, PG5-19, PG5-10, PG3-8, PG3-1, PG3-2, PG3-5, PUSA-12, PUSA-7, PUSA-11, PUSA-2, PG186-35, PG186-27, PG186-20 and PG186-17 were found to be positively responsive to all screened plant growth-promoting (PGP) traits. This primary screening served as the basis for selecting top performing traits, which were subsequently tested through biochemical assays to determine their metabolic competence and stress tolerance under fluctuating physiological conditions (Khan *et al*., 2019; Shi *et al*., 2022).

Biochemical Profiles of Selected Isolates

The biochemical characterization of the 16 selected isolates depicted seven strains-PG5-4, PG5-12, PG186-35, PG3-2, PUSA-2, PG186-20 and PUSA-11 showing a consistent positive response in all the biochemical tests (Table 2). These tests indicated their high level of metabolic flexibility and their potential ability to promote plant growth under fluctuating environmental conditions (Singh *et al*., 2020; Wójcik *et al*., 2023). Based on their well-documented PGP traits and biochemical profiles, the seven isolates were further selected for detailed evaluation stress tolerance.

Stress Tolerance of Functionally Competent Isolates

The isolates were analysed in a broad multi-stress tolerance assay to assess their ability to function under stress conditions such as high salt, pH fluctuations, extreme temperatures and biotic stress. Of the seven biochemically proficient isolates, three isolates i.e, PG5-4, PG5-12 and PG186-35 survived and grew luxuriantly under all stress conditions. These isolates showed uniform (0.8-0.9) optical density under higher salt conditions (maximum 30% NaCl), acidic to alkaline pH ranges (4 to 11), and severe temperatures from 12°C to 50°C. They also inhibited the growth of *Rhizoctonia solani* and *Colletrotrichum truncatum*, thus assuring their resistance to varied environmental stresses (Akond *et al*., 2016; Kadapure *et al*., 2025).

Multiple Correspondence Analyses (MCA)

The initial MCA was conducted to illustrate the co-occurrence of PGP traits with 52 bacterial isolates from the rhizosphere of four chickpea genotypes

Table 2: Isolates showing result of different biochemical test

Isolate	Catalase	Amylase	Caseinase	Cellulase	Gelatinase	Urease	Citrate	MR	VP	Oxidase
PG5-4	+	+	+	-	+	-	+	+	+	-
PG5-15	-	-	-	+	+	-	+	-	+	-
PG5-12	+	+	+	-	+	+	-	+	-	-
PG5-19	+	+	+	+	+	-	-	+	-	+
PG5-10	+	+	+	+	+	+	+	+	+	+
PG3-8	+	-	+	-	+	+	+	+	+	-
PG3-1	+	-	-	-	-	+	+	-	-	+
PG3-2	-	+	+	+	-	-	+	+	+	+
PG3-5	-	-	-	+	+	-	+	+	+	+
PUSA-12	-	-	-	-	-	-	-	+	+	-
PUSA-7	+	+	+	-	-	-	+	+	+	-
PUSA-11	-	+	+	+	-	+	+	+	-	-
PUSA-2	+	-	-	+	-	+	+	+	-	+
PG186-35	+	-	+	+	+	-	+	+	+	+
PG186-27	+	+	+	+	+	-	+	-	-	+
PG186-20	+	+	+	+	+	+	+	-	+	-
PG186-17	+	-	-	+	+	-	-	+	-	-

namely PG186, PG3, PG5 and PUSA (Fig 1). From the MCA plot, it was evident that there were clear patterns of clustering among the isolates according to their characteristics. Dimension 1 (Dim1), which explained 30.8% of the total variance, successfully distinguished the isolates based on their functional diversity and Dimension 2 (Dim2), which explained 13.9% of the variation, further resolved their differentiation. Significantly, PG5 and PG3 isolates reflected greater concentration on the positive side of Dim1, implying greater frequency and consistency of PGP traits. By contrast, PG186 and PUSA isolates were more dispersed in the negative and central regions of Dim1, reflecting greater heterogeneity. The overlapping but resolvable ellipses indicate partial convergence of traits across groups, but with some intra-group similarities, especially in PG5 and PG3. These trends justified the selection of 16 isolates (PG5-15, PG5-12, PG5-19, PG5-10, PG3-8, PG3-1, PG3-2, PG3-5, PUSA-12, PUSA-7, PUSA-11, PUSA-2, PG186-35, PG186-27, PG186-20, and PG186-17) that showed consistency across all PGP traits and thus were shortlisted for further biochemical characterization.

The second MCA of 16 selected isolates was performed in order to explain their patterns of association according to the biochemical characteristics. Dim1 and Dim2 accounted for 20.4% and 16.6% of the total variation, respectively, between the strains. The MCA diagram depicts a dispersed pattern of isolates, representing a wide spectrum of biochemical abilities (Fig 2). A few isolates, i.e., PG186-35, PG5-12 and PUSA-2, were placed prominently on the positive side of Dim1 and Dim2, showing strong correlations with several biochemical characteristics. The others, e.g., PG5-10 and PG186-17, grouped in the negative or central quadrants, exhibiting comparatively medium or differential trait sets. Though inter-varietal in origin isolates from PG186, PG3, PG5 and PUSA were showed overlapping but spatial distributions, pointing toward their convergent potential under biochemical analysis. The variability of distribution justified the selection process and also allowed determination of seven extremely potent isolates i.e, PG5-4, PG5-12, PG186-35, PG3-2, PUSA-2, PG186-20 and PUSA-11, showed positive responses to all biochemical tests thus were shortlisted for stress tolerance tests.

Table 3: Isolates showing stress tolerance function

Isolate	Low pH (4)	High pH (11)	Temp (12- 50°C)	*Biocontrol ability	Salinity (30%)
PG5-4	+	+	+	+	+
PG5-12	+	+	+	+	+
PG186-35	+	+	+	+	+
PG3-2	+	+	+	-	-
PUSA-2	-	+	-	+	-
PG186-20	+	+	-	-	+
PUSA-11	+	+	+	+	-

Note: Biocontrol ability test was performed against *Rhizoctonia solani* and *Colletrotrichum truncatum*

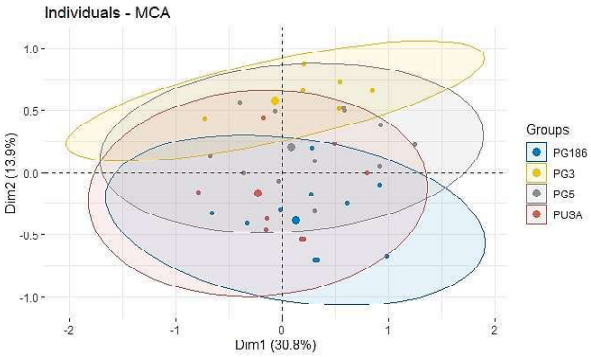

Fig. 1: MCA of 52 Chickpea Rhizobacterial Isolates Based on PGP Traits

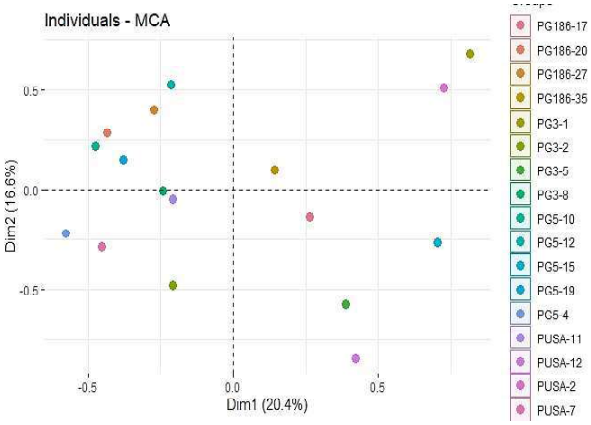

Fig. 2: MCA Plot of 16 Selected Isolates Based on Biochemical Characteristics

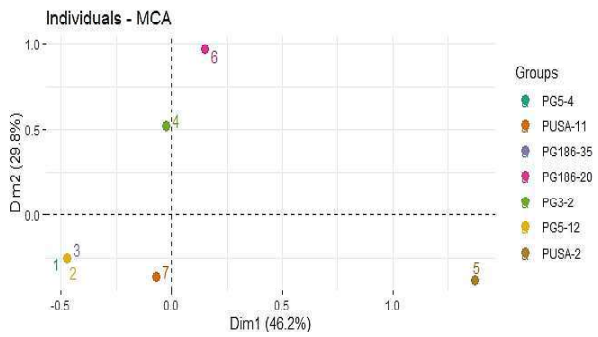

Fig. 3: Final MCA of 7 Isolates Under Climate Stress Conditions Highlighting Multi-Stress-Tolerant Isolates

The third and last Multiple Correspondence Analysis (MCA) conducted on the seven biochemically positive-tested isolates effectively demarcated the variation in stress tolerance, with Dimension 1 (Dim1) explaining 46.2% and Dimension 2 (Dim2) explaining 29.8% of the overall variation thereby elucidating more than 76% of the phenotypic variation (Fig 3). In the final MCA plot, the three selected strains PG5-4, PG5-12 and PG186-35 clustered together in Quadrant IV (positive Dim1, negative Dim2), indicating a consistent and strong association with key biochemical traits linked to stress resilience. Additional isolates like PG186-20, PG3-2, PUSA-11, and PUSA-2 were placed nearer to the centre or in opposite quadrants, indicating partial or variable responses under specific stress treatments. This concluding MCA also confirmed the reliability of the screening approach for identifying most resistant strains for further potential use in climate challenging agricultural systems.

The wide variation in PGP traits observed among the chickpea rhizobacterial isolates reflects the strong adaptive capacity of these microbes within the rhizosphere (Kumari *et al*., 2025). Such diversity arises because different bacteria adjust their metabolic and functional responses according to the chemical, physical, and biological conditions surrounding the plant roots (Kai *et al.,* 2016). This selective expression of traits, such as IAA production, phosphate solubilization, siderophore release, and ACC deaminase activity indicates that many isolates are functionally equipped to help plants cope with specific environmental constraints (Singh *et al*., 2011).

Using MCA in this study provided a powerful way to visualize and interpret these functional variations. MCA groups isolates based on shared traits, helping identify natural clusters that may not be obvious through conventional trait-by-trait evaluation (Baki *et al*., 2022). For example, isolates with high IAA production often appeared in close proximity to those showing strong phosphate-solubilizing ability and siderophore production (Tewari and Singh, 2015). This clustering points toward a functional synergy, where multiple traits collectively enhance root elongation, improve nutrient acquisition, and strengthen plant stress tolerance (Prashar *et al.,* 2014). Such relationships are consistent with known plant–microbe interactions in legumes, where certain microbial traits tend to co-evolve to meet the nutrient and physiological demands during early plant development (Panke *et al.,* 2016).

This pattern-based approach to PGPR evaluation enhances the accuracy of selecting bioinoculants isolates. Instead of assessing traits individually which can overlook how traits operate together, MCA provides a broader ecological perspective, showing how microbial strategies are organized in nature (Ter Braak, 1990). This holistic understanding is particularly valuable for chickpea cultivation under variable field conditions, where plants benefit more from microbial consortia exhibiting complementary and mutually reinforcing functions. Overall, MCA-supported functional grouping helps identify isolates that are not only strong in individual traits but also functionally consistent across multiple beneficial activities, increasing their potential effectiveness as bioinoculants for sustainable agriculture production.

CONCLUSION

This standardized, step-wise trait approach, coupled with multivariate analysis, articulates the significance of guided microbial selection for precision bioinoculants production. This structured trait-based screening, strengthened by MCA,

demonstrated the value of a guided and data-driven approach for selecting efficient PGPR strains. MCA clearly separated isolates based on their PGP traits, biochemical activities, and abiotic-biotic stress tolerance, allowing precise identification of functionally superior candidates. Among all isolates, PG5-4, PG5-12, and PG186-35 consistently clustered as the most robust performers across all functional matrices, indicating strong and stable plant-beneficial potential. These isolates represent the most promising candidates for developing individual or consortium-based bioinoculants aimed at enhancing legume productivity under climate-induced stresses. Molecular identification through 16S rRNA gene sequencing and field testing to evaluate their performance and ecological suitability is warranted. Formulation optimization and carrier material compatibility will also be critical for future downstream commercial use. These combined approaches would accommodate the development of climate-smart biofertilizer based on mechanistic microbial traits.

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