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Isolation and Evaluation of Halotolerant Plant Growth-Promoting Rhizobacteria for Enhancing the Growth of Triticum dicoccum under Salt-Affected Soil Conditions

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Abstract

A significant abiotic stressor that reduces agricultural production is soil salinity, which hinders plant growth through osmotic stress, ion toxicity, and nutritional imbalance. In Kolhapur District, Maharashtra, India, saline agricultural soils (EC 11.3 dS/m; pH 8.4) were used to isolate and evaluate halotolerant plant growth-promoting rhizobacteria (HT-PGPR). Fifteen of the twenty-five bacterial isolates that were acquired showed halotolerance, and four elite isolates (HT-1, HT-4, HT-7, and HT-13) were chosen for their superior plant growth-promoting characteristics. The isolates demonstrated the capacity to fix atmospheric nitrogen, create siderophores, solubilize phosphate, synthesize indole-3-acetic acid, and exhibit ACC deaminase activity in saline environments. Up to 20% NaCl, HT-7 maintained growth and plant growth-promoting activities, demonstrating the best salt resistance. These strains were used to create a talc-based consortium that greatly improved *Triticum dicoccum* development in salty environments. The 40-ppm treatment produced the biggest improvements in root length, shoot length, and plant height when compared to the untreated control. The bioformulation remained viable for up to 12 months under refrigerated storage. These findings demonstrate that halotolerant PGPR consortia, particularly *Bacillus paralicheniformis* HT-1, have strong potential as sustainable bioinoculants for improving crop productivity in salt-affected soils.

1. INTRODUCTION

Soil salinity is defined as the accumulation of water-soluble salts in the soil profile to levels that hinder plant growth and reduce agricultural yield. According to estimates from the Food and Agriculture Organization of the United Nations (FAO, 2021), 418 million hectares of land globally are affected by salt. This includes secondary saline soils that are caused or made worse by human activity, particularly irrigation in arid and semi-arid regions, as well as primary saline soils that are formed by natural geological and hydrological processes. The accumulation of salts in the root zone of plants has a negative effect on plant growth due to ion toxicity which is caused by high concentration of sodium (Na^+) and chloride (Cl^-) ions, disruption of nutrient uptake, excessive production of reactive oxygen species (ROS) and reduction of soil water potential. The secondary but very damaging outcome of ionic and osmotic damage is oxidative stress. Lipid peroxidation of membrane phospholipids, protein carbonylation and oxidation, and single- and double-strand breaks in organellar and nuclear DNA are all caused by ROS (Hasanuzzaman *et al*,2020). These biochemical and physiological disturbances have an impact on agricultural crops' photosynthetic efficiency, root development, seed germination, biomass buildup, and ultimately result in significant yield losses (AbuQamar *et al*.2024; Etesami & Beattie, 2018).

Chemical modifications, such as gypsum, widespread irrigation, drainage improvements, and the creation of salt-tolerant crop kinds are among the traditional ways for restoring salt-affected lands. Even if these approaches have helped with salinity management, they are frequently costly, time-consuming, and difficult to maintain in the face of shifting environmental circumstances. As a result, there has been a rise in demand for eco-friendly and bio-based techniques that increase crop output while preserving soil health. Of these, plant growth-promoting rhizobacteria (PGPR) have become one of the most promising biological tools for sustainable agriculture since they may promote plant development under both normal and stressful circumstances (AbuQamar *et al*.2024).

Halotolerant Plant Growth-Promoting Rhizobacteria (HT-PGPR), a specialized and expanding subgroup of PGPR, has garnered a lot of research interest during the previous 10 years. These organisms have developed molecular and physiological adaptations that let them maintain metabolic function and rhizosphere colonization competence at NaCl concentrations that would entirely inhibit salt-sensitive strains, in addition to the plant-beneficial actions of conventional PGPR. They can function as effective bioinoculants in agricultural areas where traditional PGPR strains would be ineffective due to their salt tolerance mechanisms, which include the accumulation of compatible solutes like ectoine and glycine betaine, sophisticated ion homeostasis systems, membrane lipid remodelling, enhanced antioxidant enzyme production, and exopolysaccharide biosynthesis (Etesami and Beattie, 2018). Despite the global growth of HT-PGPR research, the variety, traits, and agricultural potential of halotolerant rhizobacterial communities in Maharashtra's Sangli & Kolhapur District's particular saline agricultural soils were still completely unexplored. In order to address this, the current research program was designed and carried out. This geographical and ecological uniqueness is significant because native HT-PGPR that were isolated from the same salty environment as the target crop may be better suited for rhizosphere establishment and plant growth promotion in that particular soil matrix than strains that were sourced from geographically and edaphically different environments (Ramadoss *et al*.2013).

An emerging approach that reflects the awareness that no one bacterial strain, no matter how metabolically adaptable, can completely address the numerous growth-limiting variables plants experience under salt stress is the usage of PGPR as multi-strain consortia rather than single-strain inoculants. A properly designed consortium can provide complementary and potentially synergistic PGPR activities; for example, one strain may excel at IAA synthesis while another excels at phosphate solubilization; one may maintain high ACC deaminase activity under extreme salinity while another is more effective at siderophore synthesis under alkaline conditions. Particularly when the component strains avoid competitive interference with one another and occupy separate ecological niches in the rhizosphere, the net impact of a complementary consortium might surpass the total of the individual strain contributions (Yang *et al.*2009). In order to make microbial bioinoculants more commercially viable for long-term crop production, carrier-based formulations with a longer shelf life are also included (AbuQamar *et al*. 2024).

Consequently, the present research followed an integrated experimental program: the systematic isolation of HT-PGPR from the highly salty soils of Kolhapur , comprehensive morphological, biochemical, and molecular characterization, quantitative evaluation of all key PGP traits under salt stress, antifungal biocontrol screening, consortium formulation with shelf-life validation, and, finally, proof-of-concept plant growth promotion trials utilizing *Triticum dicoccum* (Khapli wheat), an economically and culturally important crop variety in Maharashtra. *Triticum dicoccum* Schrank (emmer wheat, also known as Khapli wheat in Maharashtra) is an old tetraploid wheat species (2n = 4x = 28; AABB genome) that was one of the first cereals grown in the Fertile Crescent and has been continuously farmed in the Deccan Plateau of peninsular India for thousands of years.

Recent research and commercial interest have focused on the nutritional properties of *T. dicoccum*. Compared to *T. aestivum*, Khapli wheat has higher concentrations of total protein (17–20% vs. 12–14%), dietary fibre, carotenoids (especially lutein and zeaxanthin), antioxidant phenolics, and minerals such as iron and zinc (Lata *et al*. 2018). It is being sought after by health-conscious customers and those managing metabolic disorders like diabetes mellitus because of its lower glycaemic index and greater concentration of slowly digestible starch. Despite the fact that its yields are lower than those of contemporary wheat kinds, its traditional culinary function in Maharashtrian cuisine (making of 'khapli rotis,' porridges, and artisan breads), along with these nutritional characteristics, has generated a high-end market niche that makes its growth economically appealing. From an agronomic perspective, *T. dicoccum* exhibits moderately improved resilience to certain abiotic stresses and biotic stresses (smut, rust) in comparison to *T. aestivum*, even though it is still vulnerable to salinity at EC levels observed in the study area. The HT-PGPR consortia created in this study showed that they can encourage the development of *T. dicoccum* in saline settings.

2. METHOD AND MATERIALS

Taking a Samples of saline soil from agricultural fields in Maharashtra, India's Kolhapur District's Argunwad region was conducted. The samples were put into sterile polythene bags, moved to the lab, and kept at low temperature until they were processed and analysed. Evaluation of the Soil's Physico-Chemical Characteristics Physicochemical characterization of the soil was done after sample collection to measure factors like pH and electrical conductivity (EC). Soil samples with high pH and high electrical conductivity were chosen for further study based on the analysis.

2.1 Isolation of HT PGPR

The soil from the rhizosphere was weighed (1 Kg moist weight) and placed in 250 mL Erlenmeyer flask with 99 mL of sterile physiological saline (0.85% NaCl). Suspension was shaken for 30 minutes on an orbital shaker at 200 rpm and 28°C, to help break up soil aggregates and release soil bacteria. 1 mL aliquots were then transferred to 9 mL of sterile physiological saline (0.9% NaCl) to prepare serial ten-fold dilutions (10^{-3}–10^{-6}) and slightly vortexed (10 s) prior to the next dilution. Appropriate dilutions (10-3 to 10-5) were carried out for plating on the nutrient media (Luria-Bertani (LB) agar) with the required (5% NaCl) concentration of NaCl for isolation of halotolerant bacteria (Mir, M. I., Hameeda.*et.al*, 2022).

2.2 Morphological, Biochemical, and Molecular Characterization of PGPR

The four bacterial isolates that were found to be halotolerant were cultured on a nutrient agar (NA) plate for 24 h, at 37 ± 2°C, for morphological characterization. Incubation was followed by a study of the colony morphology by its size, shape, colour, margin, elevation, consistency, and opacity. The isolates were subjected to gram staining to assess the gram reaction, cell morphology and to check for motility using the standard motility test. The screened halotolerant isolates were tested for catalase activity, citrate utilization, Glucose, Sucrose, Methyl red, Voges-Proskauer, Nitrate reduction, urease production, Amylase Production for further characterization of the isolates. (Pallavi *et.al* 2023). Isolates are recovered from the water sample and used for Molecular identification using 16srRNA sequencing method.

2.3 Qualitative Analysis of Indole-3-Acetic Acid (IAA) by HPLC

The selected halotolerant plant growth promoting rhizobacterial (HT-PGPR) isolates were qualitatively confirmed for their IAA production activity by using high performance liquid chromatography (HPLC). The bacterial isolates were cultured in nutrient broth containing L-tryptophan (0.5 g L^{-1}) and then shaken at 150 rpm and 28 ± 2 °C for 48–72 h. The cultures were then centrifuged at 10,000 × g for 10 min and the supernatant was collected after centrifugation for IAA extraction.

A reverse-phase C18 column (250 mm × 4.6 mm, 5 µm particle size) was used for the HPLC study. Methanol and acidified water were used as the mobile phase in an isocratic chromatographic separation with a flow rate of 1.0 mL min^{-1}. A 10 µL aliquot of each sample was injected into the HPLC system, and the flow was monitored by a UV detector at 280 nm. An authentic IAA standard was analysed under identical chromatographic conditions, and the retention time used as the standard for the qualitative identification of IAA in the bacterial culture extracts (Patten, C. L., & Glick, B. R. (2002).

Cell-free culture filtrates of four selected isolates of HT-PGPR (HT-1, HT-4, HT-7 and HT-13) were sent for HPLC analysis to Centre for Scientific and Industrial Analytical Facilities (CSAIF), Modern College of Arts, Science and Commerce (Autonomous), Shivajinagar, Pune-411005, Maharashtra, India. The samples were analysed with the IAA analytical method, under use of 10 µL of injection and UV detection at 280 nm. The identification of IAA was carried out by comparing the retention time of peaks obtained from bacterial culture extracts with that of the authentic standard of IAA.

2.4 Effect of Inoculants on *Triticum dicoccum* Growth—pot-Experiment

The four selected halotolerant plant growth-promoting rhizobacterial (HT-PGPR) isolates were formulated as a talc-based bioinoculant. Talc powder was selected as the carrier material because of its high surface area, chemical inertness, moisture-retention capacity, ease of sterilization, low cost, commercial availability, and ability to maintain the viability of beneficial microorganisms during storage and field application (Bashan *et al*, 2014).

Pharmaceutical-grade talc powder (particle size ≤45 µm) was sterilized by autoclaving at 121°C (15 psi) for 30 min on two consecutive days. After cooling under aseptic conditions, 10 g of carboxymethyl cellulose (CMC) per kilogram of talc (1%, w/w) was incorporated as an adhesive to improve bacterial adherence to the carrier and seed surface. Trehalose (20 g kg^{-1} talc; 2%, w/w) was added as an Osmo protectant to enhance bacterial tolerance against desiccation and osmotic stress during storage (Elbein *et al.*, 2003; Bashan *et al.*, 2014).

Each bacterial isolate was grown separately in nutrient broth at 37 ± 2°C for 24 hr under continuous shaking at 180 rpm until culture attained a population density of approximately 1 × 10^8 CFU mL^{-1}. Equal volumes of the four bacterial cultures were mixed to prepare the HT-PGPR consortium. Subsequently, 100 mL of the bacterial consortium suspension (approximately 1 × 10^8 CFU mL^{-1} per isolate) was aseptically mixed with 200 g of the sterile talc carrier mixture, maintaining a 1:2 (v/w) ratio of bacterial suspension to carrier. The formulation was thoroughly mixed using a sterile stainless-steel spatula until a homogeneous distribution of bacterial cells throughout the carrier material was achieved. Similar procedures have been widely adopted for the preparation of talc-based PGPR inoculants.

The moisture content of the final formulation was adjusted to approximately 35% (w/w) using sterile distilled water to facilitate bacterial survival and maintain the physical characteristics of the carrier. The formulated bioinoculant was air-dried under aseptic conditions until the desired moisture level was achieved, packed in sterile low-density polyethylene (LDPE) bags, heat-sealed, and stored at 4 ± 1°C for long-term preservation and 28 ± 2°C for shelf-life evaluation under dark conditions. Viable bacterial counts were determined periodically by the serial dilution and plate count method, and the microbial population was maintained at ≥1 × 10^7CFU g^{-1} throughout the storage period, in accordance with standard recommendations for microbial inoculants.

2.5 Pot Experiment Design

To assess the impact of halotolerant rhizobacteria on *Triticum dicoccum* growth and salt tolerance, a natural pot experiment was carried out. The test crop was *Triticum dicoccum* (Khapli wheat) seeds that were purchased from a nearby market. Ten kg of air-dried dirt were put into earthen pots. The dirt was extensively blended to guaranty consistency after being sieved through a 2 mm mesh screen to remove debris. Prior to sowing, the soil was treated to obtain the desired salinity level using sodium chloride (NaCl) solution. The experiment was performed by preparing the consortium. The Concentration of Consortium was added in pot is 10 ppm ,20 ppm,30ppm 40ppm. The treatments consisted of uninoculated control and inoculation with selected halotolerant rhizobacterial isolates (Khan.*et.al*,2022).

2.6 Treatment Application Methods

Bacterial inoculation followed standardized procedures developed during formulation studies. Seeds were treated with bacterial formulations at a rate of 1 ml/kg seed, using carboxymethylcellulose as an adhesive. For control treatments, seeds were coated with sterile carrier material without bacteria. Additional rhizosphere inoculation was performed one week after seedling emergence by applying consortium (10^8 CFU/mL) near the root zone. Irrigation was managed carefully to maintain consistent soil moisture levels while preventing leaching of salts. A modified Hoagland's nutrient solution adjusted for different salinity treatments was applied weekly to ensure adequate nutrient availability. Soil salinity levels were monitored regularly using portable EC meters, with adjustments made as necessary to maintain target salt concentrations (Nawaz.*et.al*,2020).

2.7 Statistical analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) to determine the effects of different HT-PGPR consortium concentrations on the vegetative growth parameters of *Triticum dicoccum*. Tukey's honestly significant difference (HSD) post-hoc test was used to further assess significant differences between treatment means at a significance threshold of $p < 0.05$. The mean ± standard deviation (SD) was used to express the results.

3. RESULT AND DISCUSSION

3.1 Physicochemical Analysis of Soil Samples

Chemical analysis of the soil sample revealed highly saline conditions characteristic of irrigated agricultural soils in Arjunwad Kolhapur District. The soil exhibited a pH of 8.4 (alkaline) and an EC of 11.3 dS/m — far exceeding the FAO threshold of 4 dS/m for saline soil classification, and firmly placing it in the 'extremely saline' category (>8 dS/m) (Table 1). These extreme conditions provided an ideal selection environment for halotolerant bacterial isolates with robust salinity adaptation mechanisms.

Table 1: Physicochemical characteristics of the soil sample (Argunwad Kolhapur District, Maharashtra)

Parameter	Value	Classification
Weight of Sample	1 kg (composite)	—
Soil pH	8.4	Alkaline
Electrical Conductivity (EC)	11.3 dS/m	Extremely Saline (>8 dS/m)
Salinity Classification (FAO)	Extremely Saline	EC > 8 dS/m threshold
Sampling Period	Pre-monsoon (March–April)	Peak salt accumulation period

EC, electrical conductivity; dS/m, decisiemens per metre. Soil salinity classification is based on EC values. The soil sample was collected during the pre-monsoon period (March–April).

3.2 Cultural, Morphological, Biochemical and Molecular identification of the Isolates

A total of 25 bacterial isolates exhibiting potential plant growth-promoting properties were recovered from the soil samples. These isolates were initially subjected to qualitative and quantitative screening for key PGPR traits, including indole-3-acetic acid (IAA) production, nitrogen-fixation potential, phosphate-solubilization ability, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. The isolates were also evaluated for their antagonistic activity against selected soil-borne phytopathogenic fungi to assess their potential biocontrol activity. Based on their performance in the preliminary screening assays, 15 isolates exhibiting comparatively promising plant growth-promoting characteristics were selected for further characterization. The selected isolates were characterized using morphological, physiological, biochemical, and molecular approaches to determine their taxonomic identity and functional attributes. Among those 15 isolates 4 efficient isolates were identified by16srRNAsequencing and selected for the HPLC analysis of IAA, which is further its used for formation of consortium preparation.

The selected HT-PGPR isolates exhibited distinct cultural, morphological, and biochemical characteristics. HT-1 produced cream-colored, circular, raised colonies and showed a Gram-positive, rod-shaped morphology, with positive reactions for catalase, oxidase, starch hydrolysis, glucose utilization, amylase production, nitrate reduction, and sucrose utilization, and was identified as *Bacillus paralicheniformis (*KY694465). HT-4 formed cream-colored, round colonies and was Gram-positive with rod-

shaped cells; it showed positive reactions for catalase, citrate utilization, methyl red (MR), glucose utilization, and nitrate reduction, and was identified as *Bacillus nitratireducens (*KJ812430). HT-7 exhibited yellow, circular colonies and was Gram-positive, with rod-to-coccoid-shaped cells, and showed positive reactions for catalase, citrate utilization, glucose utilization, and nitrate reduction; based on these characteristics, it was identified as *Ornithinimicrobium panacihumi(*NR_165752.1). In contrast, HT-13 produced cream-colored, mucoid colonies and was Gram-negative with rod-shaped cells, showing positive reactions for catalase, citrate utilization, methyl red, glucose utilization, and nitrate reduction, and was identified as *Enterobacter kobei* (MH729052.1). Overall, the four elite isolates displayed distinct phenotypic and biochemical profiles, supporting their preliminary identification prior to molecular confirmation through 16S rRNA gene sequencing.

Fig 1: Phylogenetic tree showing the relationships among selected halotolerant bacterial isolates (HT-1, HT-4, HT-7, and HT-13).

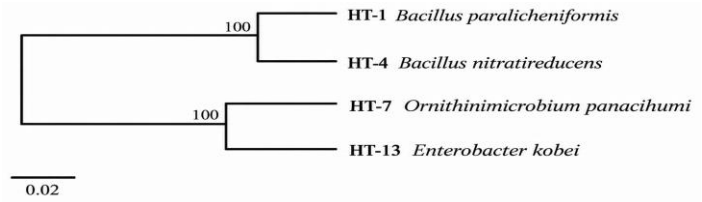


Fig 1: Bootstrap supporting value are shown at nodes (%). The scale bar is "0.02 substitutions per nucleotide site. Isolates HT-1 and HT-4 were clustered into two different groups (HT-1 and HT-4); and (HT-7 and HT-13), with 100% bootstrap support in each group.

Following physicochemical analysis of the collected saline soil samples and gene sequencing the most efficient strains exhibiting multiple PGPR traits were subsequently selected for further investigation. Their IAA-producing ability was subjected to instrumental confirmation using high-performance liquid chromatography (HPLC). HPLC analysis was performed to verify IAA production in the selected efficient isolates by comparing the chromatographic profiles and retention times of bacterial culture extracts with those of an authentic IAA standard.

3.3 HPLC confirmation of IAA production by selected halotolerant bacterial isolates

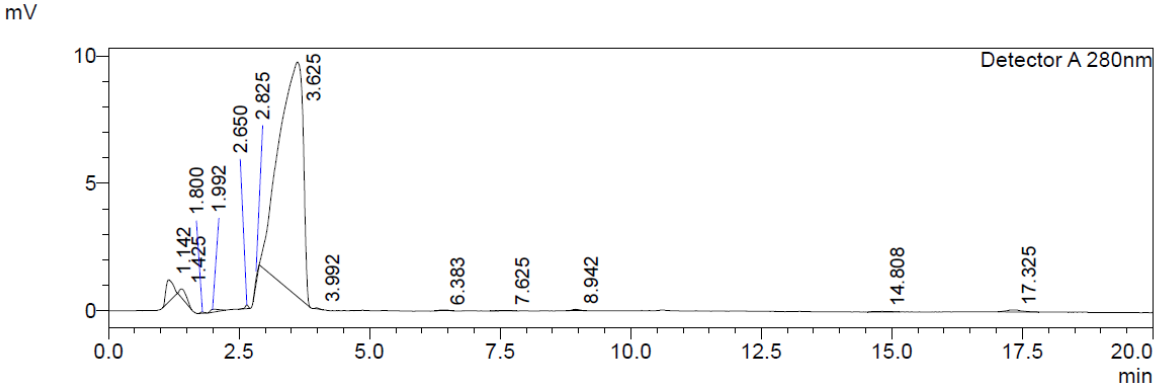


Fig 2: Representative HPLC chromatograms of the IAA standard and bacterial culture extracts Peak 7 at RT 3.625 min corresponds to authentic IAA standard (RT:3.618 min), constituting 94.07% of total peak area, confirming high-purity IAA production by isolate HT-1.

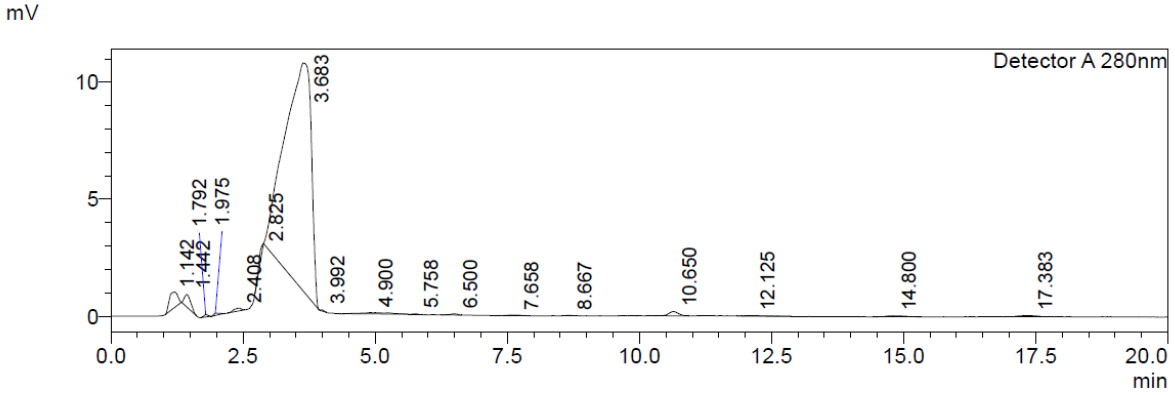


Fig 3: Representative HPLC chromatograms of the IAA standard and bacterial culture extracts Peak 7 at RT 3.683 min corresponds to authentic IAA standard (RT:3.647 min), constituting 92.93% of total peak area, confirming IAA production by isolate HT-4.

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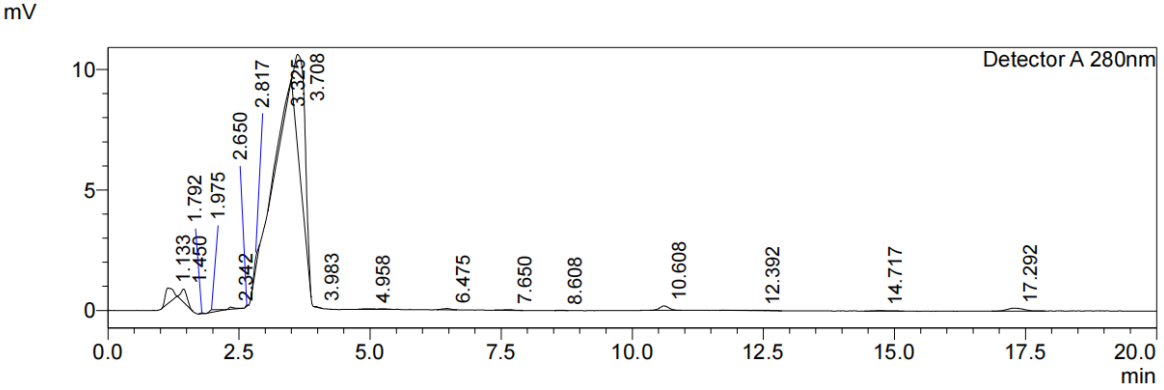


Fig 4: Representative HPLC chromatograms of the IAA standard and bacterial culture extracts Peak 9 at RT 3.708 min corresponds to authentic IAA standard (RT:3.619 min), constituting 67.84% of total peak area. The more complex chromatographic profile of HT-7 with additional peaks (notably at RT 3.325, 4.958 min) suggests the concurrent production of related indolic metabolites alongside IAA.

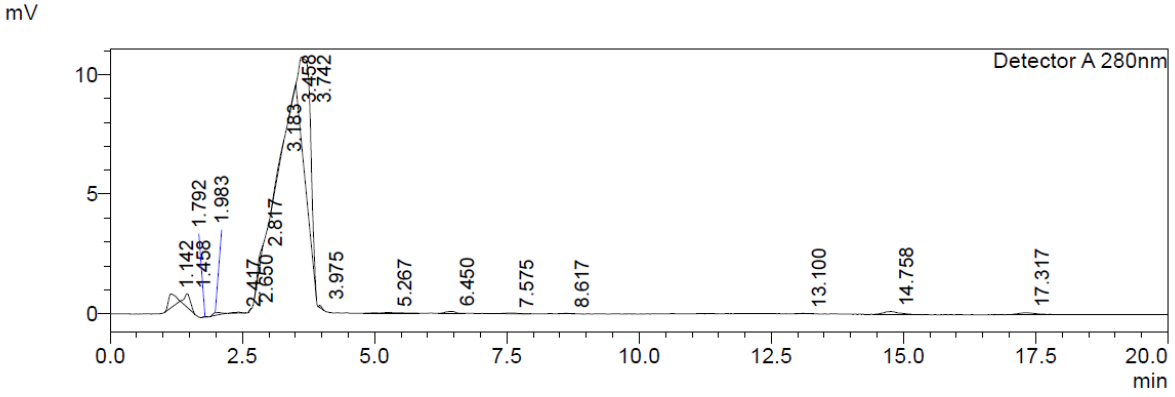


Fig 5: Representative HPLC chromatograms of the IAA standard and bacterial culture extracts Peak 10 at RT 3.742 min corresponds to authentic IAA standard (RT:3.742 min), constituting 76.29% of total peak area, confirming IAA production by isolate HT-13.

Table 2: Comparative HPLC Summary of IAA Production by Elite HT-PGPR Isolates:

Isolate	Sample ID	IAA Peak RT (min)	Std. RT (min)	IAA Peak Area	Total Area	IAA Purity (%)	Total Peaks
HT-1	1	3.625	3.618	290938	309303	94.07	13
HT-4	2	3.683	3.647	336773	362346	92.93	17
HT-7	3	3.708	3.619	65208	96118	67.84	18
HT-13	4	3.742	3.742	74640	97844	76.29	18

Table: The HT-Halotolerant PGPR ,RT, retention time; IAA, indole-3-acetic acid. IAA purity (%) = (IAA peak area / total peak area) × 100. Peak identity was assessed by comparing sample RT with the authentic IAA standard.

The IAA-producing ability of the four elite HT-PGPR isolates (HT-1, HT-4, HT-7, and HT-13) was further evaluated by HPLC. The chromatographic profiles of all four isolates exhibited distinct peaks corresponding to the retention time of the authentic IAA standard, providing instrumental evidence for IAA production by the selected isolates.

Overall, the close correspondence between the retention times of the sample peaks and the authentic IAA standard supported the presence of IAA in the culture extracts of all four elite HT-PGPR isolates. Based on the IAA-associated peak area, HT-4 exhibited the strongest chromatographic response, followed by HT-1, HT-13, and HT-7. The relatively high peak-area percentages observed for HT-1 and HT-4 further indicated that the corresponding IAA-associated peaks represented major components of their chromatographic profiles (Table 2). These HPLC findings provided additional instrumental support for the preliminary screening results and confirmed the IAA-producing potential of the selected elite HT-PGPR isolates.

Table 3: Effect of Consortia on Vegetative Characters of Wheat (*Triticum dicoccum*).

Treatment of Consortia in ppm	Vegetative Characters of Wheat plant		
	Root length (cm)	Shoot length (cm)	Plant height (cm)
Control	1.03 ± 0.06	1.67 ± 0.15	2.23 ± 0.25
10 ppm	2.13 ± 0.15	3.13 ± 0.12	4.20 ± 0.20

20 ppm	3.63 ± 0.15	5.23 ± 0.25	6.77 ± 0.25
30 ppm	5.17 ± 0.21	7.40 ± 0.36	9.43 ± 0.45
40 ppm	6.63 ± 0.12	9.63 ± 0.60	11.87 ± 0.40

Table: The Values are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3). Control represents the uninoculated treatment. Treatment concentrations were 10, 20, 30, and 40 ppm of the PGPR consortium.

Table 4.: Statistical analysis of vegetative growth parameters of *Triticum dicoccum*

Growth parameter	F-value	Degrees of freedom	p-value
Root length	714.50	4, 10	3.08×10^{-12}
Shoot length	259.28	4, 10	4.73×10^{-10}
Plant height	423.08	4, 10	4.18×10^{-11}

Table: Data were analysed using one-way analysis of variance (ANOVA) to determine differences among treatments. F-values indicate the ratio of between-treatment variance to within-treatment variance. Degrees of freedom are presented as between-group and within-group degrees of freedom, respectively. Differences were considered statistically significant at $p < 0.05$.

Table 5: Tukey's HSD test for vegetative growth parameters of *Triticum dicoccum*

Treatment	Root length (cm)	Shoot length (cm)	Plant height (cm)
Control	1.03 ± 0.06^{a}	1.67 ± 0.15^{a}	2.23 ± 0.25^{a}
10 ppm	2.13 ± 0.15^{b}	3.13 ± 0.12^{b}	4.20 ± 0.20^{b}
20 ppm	3.63 ± 0.15^{c}	5.23 ± 0.25^{c}	6.77 ± 0.25^{c}
30 ppm	5.17 ± 0.21^{d}	7.40 ± 0.36^{d}	9.43 ± 0.45^{d}
40 ppm	6.63 ± 0.12^{e}	9.63 ± 0.60^{e}	11.87 ± 0.40^{e}

Values are given as mean ± standard deviation (SD) (n=3, independent replicates). Means in the same column with different superscript letters are significantly different ($p<0.05$) as determined by Tukey's honestly significant difference (HSD) test after a one-way analysis of variance (ANOVA).

The application of the HT-PGPR consortium had a pronounced positive effect on the vegetative growth characteristics of *Triticum dicoccum* compared with the untreated control. Root length, shoot length, and plant height increased progressively with increasing consortium concentration from 10 to 40 ppm (Table 3).

The uninoculated control exhibited the lowest growth, with mean root length, shoot length, and plant height of 1.03 ± 0.06 cm, 1.67 ± 0.15 cm, and 2.23 ± 0.25 cm, respectively. In contrast, the application of the consortium at 40 ppm resulted in the highest values, with root length reaching 6.63 ± 0.12 cm, shoot length 9.63 ± 0.60 cm, and plant height 11.87 ± 0.40 cm. The intermediate treatments (10, 20, and 30 ppm) also showed progressive improvements in these growth characteristics. These observations indicate that the 40-ppm treatment was the most effective concentration among those evaluated under the experimental conditions.

Table 4 shows that the differences among the treatments were statistically significant (one-way analysis of variance (ANOVA)). The analysis indicated significant treatment effects on root length ($F = 714.50$, $p = 3.08 \times 10^{-12}$), shoot length ($F = 259.28$, $p = 4.73 \times 10^{-10}$), and plant height ($F = 423.08$, $p = 4.18 \times 10^{-11}$). The differences in vegetative growth between the tested consortium treatments were found to be statistically significant as all the p-values were below 0.05. Individual treatment means were then compared using Tukey's honestly significant difference (HSD) test (Table 5). The treatment means are indicated by different superscript letters, and the letters in each growth parameter are significantly different at $p < 0.05$. The control and each of the consortium-treated groups were separated into different significance groups, indicating that their mean growth measurements differed significantly under the assumptions of the analysis. The 40-ppm treatment recorded the highest values for all three parameters, followed by the 30, 20, and 10 ppm treatments, respectively.

Overall, the results demonstrate that application of the HT-PGPR consortium was associated with significant improvements in the vegetative growth of *Triticum dicoccum*, with the greatest response observed at 40 ppm. The findings support further evaluation of this consortium under field conditions to determine its potential for improving wheat growth in salt-affected soils.

The enhanced root and shoot development may be associated with the combined plant growth-promoting activities of the constituent HT-PGPR, including phytohormone production, nutrient mobilization, nitrogen fixation, and improved plant adaptation to saline soil conditions.

4. CONCLUSION

The present study showed that the indigenous halotolerant plant growth promoting rhizobacteria (HT-PGPR) isolated from the salt affected agricultural soil of Argunwad, Kolhapur District, Maharashtra has the potential to enhance the growth of Triticum dicoccum under saline condition. A total of 25 bacterial strains were isolated in which 15 were halotolerant and four of these strains (HT-1, HT-4, HT-7, and HT-13) were selected for their plant growth promotion activities. In all the isolates, the production of indole-3-acetic acid (IAA), production of siderophores, nitrogen fixation, production of phosphate solubilizing and activity of ACC deaminase showed significant beneficial characteristics. Furthermore, all four selected isolates were discovered to be capable of producing IAA by HPLC analysis (Table 2). *Bacillus paralicheniformis* HT-1, *Bacillus nitratireducens* HT-4, *Ornithinimicrobium panacihumi* HT-7 and *Enterobacter kobei* HT-13 respectively based on phenotypic characterization and 16S rRNA gene sequencing. Of these, HT-7 was most tolerant to NaCl (up to 20%) and maintained its growth and plant growth promoting activity under these conditions.

A talc-based consortium containing the selected HT-PGPR strains was successfully formulated and evaluated for its effect on *T. dicoccum* growth under saline conditions. The pot experiment demonstrated a clear positive response to consortium application. Root length, shoot length, and plant height progressively increased with increasing consortium concentration, with the from 10 to 40 ppm treatment producing the highest values.

The maximum growth response was recorded at 40 ppm, with root length, shoot length, and plant height reaching 6.63 ± 0.12 cm, 9.63 ± 0.60 cm, and 11.87 ± 0.40 cm, respectively, compared with 1.03 ± 0.06 cm, 1.67 ± 0.15 cm, and 2.23 ± 0.25 cm in the uninoculated control.
Results of one-way analysis of variance (ANOVA) showed significant differences between treatments for the three growth parameters ($p < 0.05$). When comparing individual treatment means the Tukey's honestly significant difference (HSD) test was used.

Overall, the findings indicate that the developed HT-PGPR consortium can substantially enhance early vegetative growth of *T. dicoccum* under salt-affected conditions. The results highlight the value of native halotolerant rhizobacteria as promising candidates for the development of sustainable microbial bioinoculants for salt-affected agriculture. However, further validation through

replicated field experiments, quantitative assessment of yield and yield-attributing characters, and molecular investigation of the mechanisms underlying salt tolerance and plant growth promotion will be necessary before large-scale agricultural application. The present study therefore provides a useful foundation for developing indigenous HT-PGPR-based bioformulations for improving crop establishment and productivity in saline soils.

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