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Research Article

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Bio-cellulose seed coating formulations of endophytes to boost growth and yield of wheat: a sustainable approach

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Abstract: Seed coating with beneficial endophytic bacteria and polymers enhances plant growth and stress tolerance, offering a sustainable approach. But bio-cellulose seed coating is in demand because it provides a protective, biodegradable matrix that enhances bacterial survival, adhesion to seeds, and effective colonization of the rhizosphere. This study evaluated ten cellulose-producing endophytes (CPE) for plant growth–promoting traits and abiotic stress tolerance, followed by their encapsulation in a cellulose-based seed coating for wheat growth. Among the isolates, Acetobacter tropicalis (CPE1), Pseudomonas sp. (CPE3), Bacillus amyloliquefaciens (CPE4), Pseudomonas aeruginosa (CPE7), and Enterococcus sp. (CPE9) exhibited superior characteristics and were selected for further study. CPE3 showed the highest indole-3-acetic acid production (304 μg mL^{-1}) and mineral solubilization indices (PSI 325, KSI 321, ZnSI 212). Scanning electron microscopy revealed that dip-coated seeds were uniformly covered with bio-cellulose, ensuring firm bacterial attachment, whereas spray-coated seeds showed an uneven coating layer. Under sterile conditions, bio-cellulose-coated seeds showed improvement 60%–90% in seed-germination, 60%–70% in a seedling-vigor-length index, and 80%–95% in a seedling-weight-vigor-index as compared to untreated control. Wire-house and field trials also confirmed improved seed-germination (85%–92%), morphological traits (60%–80%), physiological traits (50%–85%), and yield (70%–85%) as compared to untreated control seeds. Biochemical analysis indicated enhanced chlorophyll-a, carotenoids, soluble sugars, and protein contents, along with reduced chlorophyll-b and proline levels. Bacterial viability within the cellulose matrix remained stable (10^5–10^7 CFU g^{-1}) for up to 120d under ambient storage. In conclusion, cellulose-based seed coating represents a promising formulation for maintaining survival of beneficial microbes and improving crop performance, providing a sustainable approach for agricultural systems.

Key words: Bio-cellulose; Seed coating; Endophytes; Plant growth promotion; Wheat growth

1 Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally, providing a major source of calories and protein for more than 60% of the world's population (Dargiri et al., 2025). However, wheat production is increasingly constrained by climate variability, soil degradation, and the intensive use of synthetic agrochemical inputs. These challenges have accelerated the search for sustainable agricultural strategies that can maintain or enhance crop productivity while reducing environmental impacts. Approaches integrating microbial biotechnology with biodegradable materials have gained considerable attention (Maslennikova et al., 2023; Saadaoui et al., 2022). Among the emerging sustainable technologies, Plant growth promoting (PGP) endophytes and cellulose-based seed coating systems have shown substantial potential for improving wheat establishment, growth, and yield (Ascheri et al., 2024; Lastochkina et al., 2023; Zvinavashe et al., 2021). PGP endophytes are beneficial microorganisms that inhabit internal plant tissues and enhance plant development through mechanisms such as biological nitrogen fixation, phosphate solubilization, phytohormone production, and increased tolerance to biotic and abiotic stresses. Earlier studies have proved that inoculation of wheat with endophytic bacteria, including *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Pseudomonas fluorescens*, significantly improves

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germination rates, root system architecture, and stress resilience (Abid et al., 2024; Kangsopa & Atnaseo, 2022).

Bacterial cellulose (BC), a biopolymer produced by cellulose-synthesizing bacteria such as *Komagataeibacter xylinus*, has recently emerged as a promising carrier material for agricultural applications. BC is characterized by high purity, biodegradability, excellent mechanical strength, and superior water-holding capacity (Muhammad et al., 2024). Its unique nanofibrillar and porous structure enables effective encapsulation of microbial inoculants, protecting them from desiccation and environmental stresses while facilitating their controlled release during seed germination (Jayani et al., 2020). Moreover, BC-based seed coatings can be engineered to incorporate nutrients or bioactive compounds, thereby enhancing their functional performance (Kangsopa & Atnaseo, 2022).

Recent advances in BC synthesis and seed coating technologies have enabled their large-scale production and field application in agriculture. Improvements in fermentation processes and genetic optimization of cellulose-producing bacteria have resulted in higher BC yields and improved film uniformity and consistency (Korbecka-Glinka et al., 2022; Muhammad et al., 2024). Studies have shown that cellulose-based seed coatings containing microbial consortia enhance seedling vigor, regulate phytohormone signaling, and modulate stress-responsive gene expression, leading to improved crop performance under field conditions. Similarly, polymer-based seed coating technologies, including hydrogels and controlled-release systems, have been reported to improve water retention and nutrient use efficiency (Korbecka-Glinka et al., 2022; Pirzada et al., 2020). For instance, polymer-coated urea fertilizers have enhanced wheat yield and nitrogen recovery efficiency while reducing environmental losses (Sherif & MR Hedia, 2022). In addition, microencapsulation of microbial inoculants using polymer emulsions has been shown to significantly increase bacterial survival and wheat seed germination rates (Gong et al., 2024).

Despite these advances, the combined application of BC–based seed coating and PGP endophytes in wheat production systems remains insufficiently explored, particularly with respect to their interactive effects on microbial colonization, plant physiology, and yield under both laboratory and field conditions. This research aims to overcome that deficiency by investigating the effects of bio-cellulose seed coatings infused with certain endophytes on wheat germination, growth metrics, and yield. The novelty of this study lies in the development of a bio-cellulose seed coating system that functions simultaneously as a protective carrier and a sustained delivery system for endophytic bacteria.

2 Material and methods

2.1 Collection of cellulose producing endophytes (CPE)

Ten cellulose producing bacterial isolates (designated CPE1–CPE10) were obtained from the Department of Microbiology & Molecular Genetics, The Women University Multan, Pakistan. These bacteria were isolated from the internal tissues of various fruits and vegetables and identified by 16S rRNA gene sequencing. The accession numbers of selected strains were provided. The efficiency of cellulose production was evaluated following the method described by (Abol-Fotouh et al., 2020). Briefly, the selected bacterial strains were cultured on Hestrin–Schramm (HS) medium under sterile conditions and incubated statically at 30 °C for seven days. Cellulose production efficiency was then calculated using the formula given in Eq. (1)

$$BC\ production\ efficiency\ (\%) = \left(\frac{m}{v}\right) \Big/ (Si - Sf) \times 100 \quad (1)$$

where ‘m’ represents production of BC, ‘V’ the volume of media (L), ‘Si’ and ‘Sf’ the initial and final substrate concentrations.

2.2 Screening of CPE for plant growth promoting (PGP) traits

PGP traits of the bacterial isolates were evaluated using standard qualitative and quantitative assays.

Indole-3-acetic acid (IAA) production was determined using Salkowski's reagent. Cultures were grown in LB broth supplemented with 0.1% L-tryptophan at 30 °C for 48 h (120 r/min), centrifuged (10,000 r/min, 10 min), and the supernatant was mixed (1:1) with Salkowski's reagent. After incubation in the dark for 60 min, IAA production was indicated by pink coloration and quantified spectrophotometrically at 530 nm using a synthetic IAA standard curve (Glickmann & Dessaux, 1995). Hydrogen cyanide (HCN) production was assessed on L-agar supplemented with glycine using picric acid impregnated filter paper. A yellow to orange colour change indicated positive HCN production (Bakker & Schippers, 1987). Ammonia production was evaluated by culturing isolates in peptone water, followed by reaction of the supernatant with Nessler's reagent in the ratio 1: 0.5. Development of a reddish-brown colour indicated ammonia synthesis. The colour intensity was measured using a spectrophotometer at 450 nm (Dye, 1962). Siderophore production was assessed on chrome azurol S (CAS) agar, where a blue to orange colour change indicated positive activity and the diameter of the zone of siderophore production was measured in mm (Schwyn & Neilands, 1987). 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was qualitatively assessed on Dworkin and Foster (DF) minimal salt agar (Dworkin & Foster, 1958) with and without ACC after incubation at 30 °C for four days; growth on ACC-amended medium indicated ACC deaminase activity (Penrose & Glick, 2003). Nitrogen fixation potential was evaluated on nitrogen-free Jensen's medium incubated at 30 °C for 7d, with halo zone formation around colonies indicating nitrogen-fixing ability (Ji et al., 2023).

2.3 Mineral solubilization activity

The phosphate-solubilizing capability of certain bacterial strains was assessed using National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999). Isolates were spot-inoculated, shielded with aluminium foil and incubated for 48 h at 30 °C in darkness. Appearance of a halo-zone around bacteria was considered positive. The phosphate solubilization index (PSI) was calculated using a standard formula Eq. (2).

$$PSI = \frac{Diameter\ of\ bacterial\ colony + Diameter\ of\ halo\ zone}{Diameter\ of\ bacterial\ colony} \quad (2)$$

The potassium-solubilizing index (KSI) capability was evaluated by spot-inoculating onto Aleksandrov agar. As a control, strains were inoculated on agar lacking potassium aluminium silicate. Plates were incubated at 30 °C for 7 d, with potassium solubilisation evidenced by a clear halo zone present around the colonies.

The zinc solubilization index (ZnSI) was evaluated by spot-inoculating bacteria onto basal agar supplemented with 0.2% (0.02g/mL) insoluble ZnO. Controls were established on zinc-free agar. Plates were incubated at 30 °C under static conditions, colonies with a distinct halo indicating Zn solubilization. The ZnSI and KSI were calculated by the same formula used for PSI calculation (Haque et al., 2020).

2.4 Extracellular enzyme production and abiotic stress tolerance

Extracellular enzyme production (protease, pectinase, and chitinase) was evaluated following the method described by (Elbeltagy et al., 2000). Protease activity was determined on skim milk agar by the appearance of clear hydrolysis zones. Pectinase production was assessed on M9 medium supplemented with pectin, indicated by halo formation around colonies. Chitinase activity was evaluated on nutrient agar containing colloidal chitin, with clear zones confirming enzyme production. Drought and salinity tolerance of bacterial isolates (CPE1–CPE10) were assessed in Luria-Bertani (LB) broth supplemented with 30%–40% polyethylene glycol (PEG) 6000 and 5%–20% sodium chloride (NaCl), respectively. Cultures were incubated at 30 °C for 24 h with shaking, and growth was measured at OD_{660} (≥ 0.1 indicating tolerance). Isolates were further evaluated under different temperatures (30 °C, 35 °C, and 45 °C) and pH levels (4.0, 7.0, and 9.0). Uninoculated LB broth served as the control (Ansari et al., 2021).

2.5 Preparation of seed coatings and experimental design

Following screening of the ten CPE strains for PGP traits and abiotic stress tolerance, five promising isolates were selected. Strain identities were confirmed through accession numbers using the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). Selected strains were used for wheat seed coating and evaluation for plant growth ability. Certified wheat seeds (cv. Dilkash-20) were obtained from the Punjab Seed Corporation, Multan, and experiments were conducted at the Dept. of Microbiology & Molecular Genetics, The Women University Multan, Pakistan. Healthy seeds were surface-sterilized with 4% sodium hypochlorite for 30 min and rinsed thoroughly with sterile distilled water. Each selected strain was cultured in nutrient broth at 30 °C (150 r/min) for 48 h, followed by centrifugation (3000 r/min, 20 min). A 2% (0.02g/mL) BC slurry was prepared following the method of (Jong et al., 2024) with some modification. The BC slurry was stirred for 24 h with a magnetic stirrer then sonicated for 90 min at a frequency of 20 kHz to ensure homogeneity, followed by sterilization. Bacterial pellets were suspended in sterile BC to obtain 1×10^8 CFU/mL. Seed coating was performed using dip and spray coating methods. Coated seeds were air-dried aseptically overnight. Seeds treated with sterile BC slurry without bacteria served as controls (Saadaoui et al., 2022). To determine the effects of seed coatings, two types of treatments with five different bacteria and the control were used. The experimental design and seed treatment combinations are summarized in Table (1). All the experiments were performed in duplicate.

Table 1 Experimental design and seed coatings combinations used in dip and spray coating assays

Treatment type	Dip coating T1	Spray coating T2
CPE treatments	CPE1; CPE3; CPE4; CPE7; CPE9	CPE1; CPE3; CPE4; CPE7; CPE9
Control	Cellulose coating without bacteria	Cellulose coating without bacteria

Footnotes: CPE= Cellulose producing endophytes; T1 and T2= Dip and spray coating of microbial cellulose

2.6 Characterization of bio-cellulose coated seed surface

Scanning electron microscopy (SEM, AURIGA, Germany) was conducted at 5000X magnification and 5.00 kV to acquire surface images of both coated and uncoated seeds. High-resolution images were obtained by affixing samples to conductive carbon tape on specialized microscopy tables, which were subsequently coated with a conductive gold layer using a Q150R ES vacuum sputtering apparatus (Quorum Technologies, Lewes, UK).

2.7 Laboratory, wire-house and field trials

The effects of seed coatings were evaluated under laboratory, wire-house, and field conditions. For laboratory germination assays, eight seeds per treatment (dip and spray-coated) were placed on moistened filter paper in Petri dishes and incubated at 28 °C in darkness. Germination was recorded after 7 d and continued for 15 d. Seed germination percentage (SGP), seedling length vigor index (SLVI), and seedling weight vigor index (SWVI) were calculated as described by (Kerbab et al., 2021).

$$SGP = {(\text{Number of seeds germinated})}/{(\text{Total number of seeds})} \times 100 \quad (3)$$

$$SLVI = Length(cm) \times Percentage\ of\ germination \quad (4)$$

$$SWVI = Seedling\ dry\ weight(mg) \times Percentage\ of\ germination \quad (5)$$

A pot experiment was conducted under controlled laboratory conditions for two weeks using sterilized soil

(200 g soil /pot), with five seeds sown per treatment. Pots were maintained at 28 ± 2 °C with a 16-h photoperiod and irrigated with sterile distilled water. Seed germination and vigor were assessed using the same parameters as described above. To validate coating efficacy under natural conditions, wire-house and field trials were conducted from November 2023 to April 2024 using unsterilized soil. In the wire-house trial, pots were filled with 5 kg soil (5kg soil /pot) and sown with 15 seeds per treatment. Growth parameters, including shoot and root length and biomass, were recorded during plant development, while yield traits (spike length, number of spikes per spikelet, and 1000-grain weight) were measured at harvest. The field trial was conducted using a randomized complete block design (RCBD) with two replicates per treatment. Soil physicochemical properties were analysed prior to sowing. Standard agronomic practices were followed throughout the growing season. Growth, physiological, biochemical, and yield parameters were recorded at plant maturity and at harvest.

2.8 Plant biochemical analysis

Leaf biochemical parameters were analysed using standard spectrophotometric methods. Chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a*+*b*), and carotenoids were quantified following (Kerbab et al., 2021). Fresh leaf tissue (0.5 g) was extracted in 80% acetone then centrifuged, and absorbance was recorded at 663 nm, 645 nm, and 470 nm for pigment estimation.

$$Chla = 12.70\,A663 - 2.69A645 \quad (7)$$

$$Chlb = 22.90\,A645 - 4.68\,A663 \quad (8)$$

$$Total\ Chlorophyll\ (a+b) = 20.21\,A645 + 8.02\,A663 \quad (9)$$

$$Carotenoids = \frac{1000\,A470 - 1.9\,Chla - 63.14\,Chlb}{214} \quad (10)$$

Total soluble proteins were determined from frozen seedling tissue using the Folin–Ciocalteu method, with absorbance measured at 750 nm. Total sugars were extracted with 80% ethanol and quantified using the phenol–sulfuric acid method at 490 nm against a glucose standard (Ghosh et al., 2019). Proline content was estimated from fresh leaf tissue using the ninhydrin method, with absorbance measured at 520 nm and quantified using a proline standard (Cherif-Silini et al., 2019).

2.9 CPE root rhizoplane colonization

Root rhizoplane colonization by the bacterial strain was analysed using SEM. Inoculated plants were carefully uprooted and their roots gently washed with sterile saline solution to remove loosely adhered soil particles while retaining firmly attached bacterial cells. Root segments from the elongation and root hair zones were fixed in 2.5% (v/v) glutaraldehyde to preserve cellular structures, followed by post-fixation in 2% osmium tetroxide for 120 min to enhance membrane contrast. The samples were dehydrated through a graded ethanol series, sputter-coated with gold using an automated coating system for 3 min, and examined under a scanning electron microscope (AURIGA, Germany) at various magnifications to visualize bacterial colonization. Bacterial colonization was confirmed by the presence of attached bacterial cells and microcolonies on the root epidermis and root hairs, and the observations were compared with uninoculated control roots (Ansari et al., 2021).

2.10 Assessment of bacterial viability in coated seeds during storage

To assess the impact of shelf life on bacterial viability in coated seeds, 1-g samples were maintained at ambient temperature for durations of 0 d, 30 d, 60 d, and 120 d, followed by suspension in 10 mL of sterile distilled water. Bacterial counts were performed in duplicate on HS medium at a temperature of 30 °C for 48 h, with results expressed as CFU/g of seeds (Saadaoui et al., 2022).

2.11 Statistical analysis

Results are expressed as the mean and standard deviation. Data were analysed using ANOVA, and significant differences were subsequently assessed with Tukey's HSD test ($p<0.05$). Statistical analyses were performed using IBM SPSS Statistics version 22, while graphical representations were generated using Origin 2025b.

3 Results

3.1 Cellulose production efficiency of selected strains

The evaluation of cellulose production efficiency revealed clear strain-dependent differences among the CPE isolates (Fig. 1). CPE4 showed the highest efficiency of cellulose production, reaching about 63%, followed by CPE9 61% and CPE1 57%. In contrast, CPE2 showed the lowest efficiency, at about 44%, indicating a markedly reduced capacity for cellulose production compared with the other strains. The remaining isolates (CPE3, CPE5, CPE6, CPE7, and CPE8) showed intermediate efficiencies, generally ranging from 48% to 55%. Overall, these findings demonstrate substantial variability in cellulose production efficiency among the evaluated CPE strains.

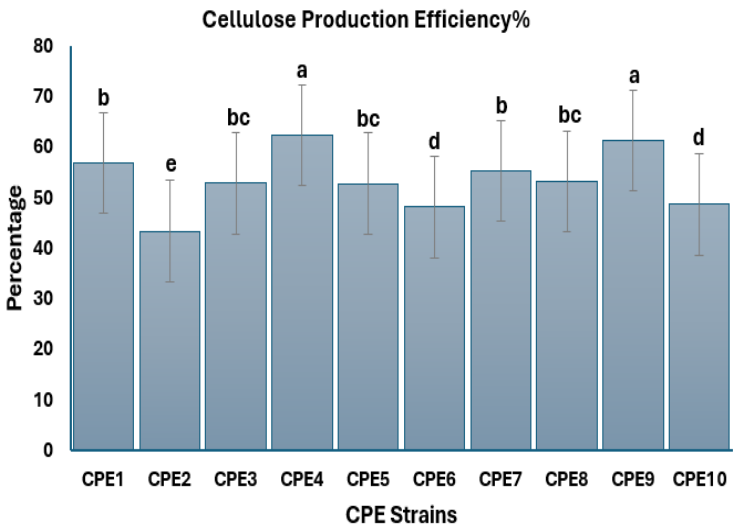


Fig. 1 Cellulose production efficiency of cellulose producing endophytes (*CPE)* after 7 d of incubation at 30 °C. Data were represented as means of three replicates (*n*=3). Standard error bars that do not share a same letter were considered significantly different (*P*≤0.05).

3.2 CPE bacteria exhibit PGP traits

Selected strains showed variability in PGP abilities (Table 2). IAA production was detected in most of the isolates, except for CPE2, CPE8, and CPE10. Among the positive strains, CPE3 produced the most IAA (304 μg/mL), followed by CPE4 (205 μg/mL), whereas CPE6 produced the least (98 μg/mL). HCN production was strongly expressed by CPE3 and CPE4 but was not observed in CPE6 and CPE10. Quantitative analysis of ammonia production revealed significant variation among strains, with CPE1 producing the highest amount (4.0 μg/mL) and CPE9 the lowest (1.9 μg/mL). Siderophore production also varied markedly: CPE1 generated the

largest siderophore halo (5.5 mm in diameter), while no siderophore activity was detected in CPE2. ACC deaminase activity was prominent in CPE3, CPE4, and CPE7 but absent in CPE2, CPE5, and CPE10. Nitrogen fixation capacity differed significantly across the strains, with CPE9 showing the highest activity (3.8 mm), whereas CPE8 lacked detectable nitrogen fixation.

Mineral solubilization assays further highlighted the functional diversity of the isolates. CPE3 exhibited the highest phosphate and potassium solubilization indexes (325 PSI and 312 KSI, respectively), while the zinc solubilization index was highest in CPE9 (245 ZSI). Enzymatic activity profiling revealed that protease and pectinase activities were notably higher in CPE4. Chitinase activity was absent in CPE2 and CPE6 but was detected in all remaining strains. Overall, the observed heterogeneity in PGP traits among the CPE isolates underscores the importance of strain-specific evaluation and supports the selection of multifunctional strains for the development of effective seed coating formulations and biofertilizer applications.

Table 2 Plant growth promoting traits, mineral solubilization and extracellular enzyme production activities of cellulose producing endophytes (CPE)

PGP Traits	CPE1	CPE2	CPE3	CPE4	CPE5	CPE6	CPE7	CPE 8	CPE9	CPE10
Auxin Production(μg/mL)	193.5±0.53	NR	304.8±0.32	205.2±0.26	157.9±0.10	98.0±0.23	160.5±0.12	NR	180.0±30	NR
Hydrogen Cyanide Production	++	++	+++	+++	+	NR	++	+	++	NR
Ammonia Production(μg/mL)	4.0±0.21	2.44±0.13	3.56±0.11	3.89±0.41	NR	1.9±0.12	2.87±0.31	NR	1.9±0.11	3.5±0.21
Siderophore Production (mm)	4.5±0.41	NR	4.1±0.22	1.8±0.15	3.5±0.11	4.3±0.23	2.3±0.13	3.4±0.55	4.0±0.52	3.9±0.21
ACC deaminase Production	++	NR	+++	+++	NR	+	+++	+	++	NR
Nitrogen Fixation (mm)	1.8±0.23	2.0±0.11	2.9±0.15	1.2±0.34	2.5±0.25	2.3±0.12	3.3±0.45	NR	3.8±0.23	1.7±11
Phosphate Solubilization (PSI)	270±0.46	130±0.13	325±0.23	140±0.12	221±0.11	321±0.37	189±0.45	NR	102±0.56	141±0.20
Potassium Solubilization (KSI)	125±0.14	NR	312±0.22	198±0.03	115±0.15	NR	102±0.31	NR	87±0.24	301±0.19
Zinc Solubilization (ZSI)	110±0.22	NR	221±0.32	69±0.26	18±0.33	NR	50±0.02	NR	245±0.13	134±0.21
Protease Production	++	+	++	+++	NR	NR	+	++	++	+
Pectinase Production	+++	++	+	++	+	++	+	++	+++	NR
Chitinase Production	++	NR	+++	+++	+	NR	+	+	++	+++

Selected strains	S	–	S	S	–	–	S	–	S	–

Footnotes: All values are the means of 3 replicates ± standard error; 'NR' means negative result; 'S' means selected strains; -show no activity; +, ++, +++ show intensity of activity low, moderate, high respectively.

3.4 Abiotic stress tolerance capacity of CPE

Among the ten bacterial isolates, CPE5 showed the highest tolerance to drought stress, maintaining optical density (OD) values of 0.70, 0.54, and 0.47 under 30%, 35%, and 40% polyethylene glycol (PEG) induced osmotic stress. CPE3 and CPE1 also showed strong osmotic stress tolerance, whereas CPE8 and CPE2 showed the lowest growth at 40% PEG, indicating weaker drought resistance. Note that all isolates retained measurable growth even at the highest PEG concentration, confirming their capacity to withstand severe drought-like conditions. Under salinity stress, strains CPE1, CPE3, and CPE5 exhibited the greatest salt tolerance, maintaining OD values above 0.30 at 20% NaCl. In contrast, CPE6 and CPE2 showed pronounced growth inhibition, particularly at higher salinity levels. Although increasing NaCl concentrations resulted in a general decline in bacterial growth, the superior performance of these strains highlights their potential adaptability to saline environments. Temperature tolerance assays revealed optimal growth at 30 °C across all isolates, with CPE1 recording the highest OD value (0.87). Growth declined at elevated temperatures, but CPE7, CPE10, and CPE8 showed better survival at 45 °C, suggesting enhanced thermotolerance. In contrast, CPE5, CPE3, and CPE9 were the most sensitive to heat stress. With respect to pH tolerance, all isolates exhibited maximum growth under acidic conditions (pH 4), with CPE1 showing the highest OD value (0.97). Moderate growth was observed at neutral (pH 7), where CPE1 again demonstrated superior performance (OD = 1.03). In contrast, bacterial growth was markedly reduced under alkaline conditions (pH 9), with only CPE5, CPE1, and a few other isolates maintaining minimal growth (OD = 0.10), indicating limited tolerance to alkaline stress.

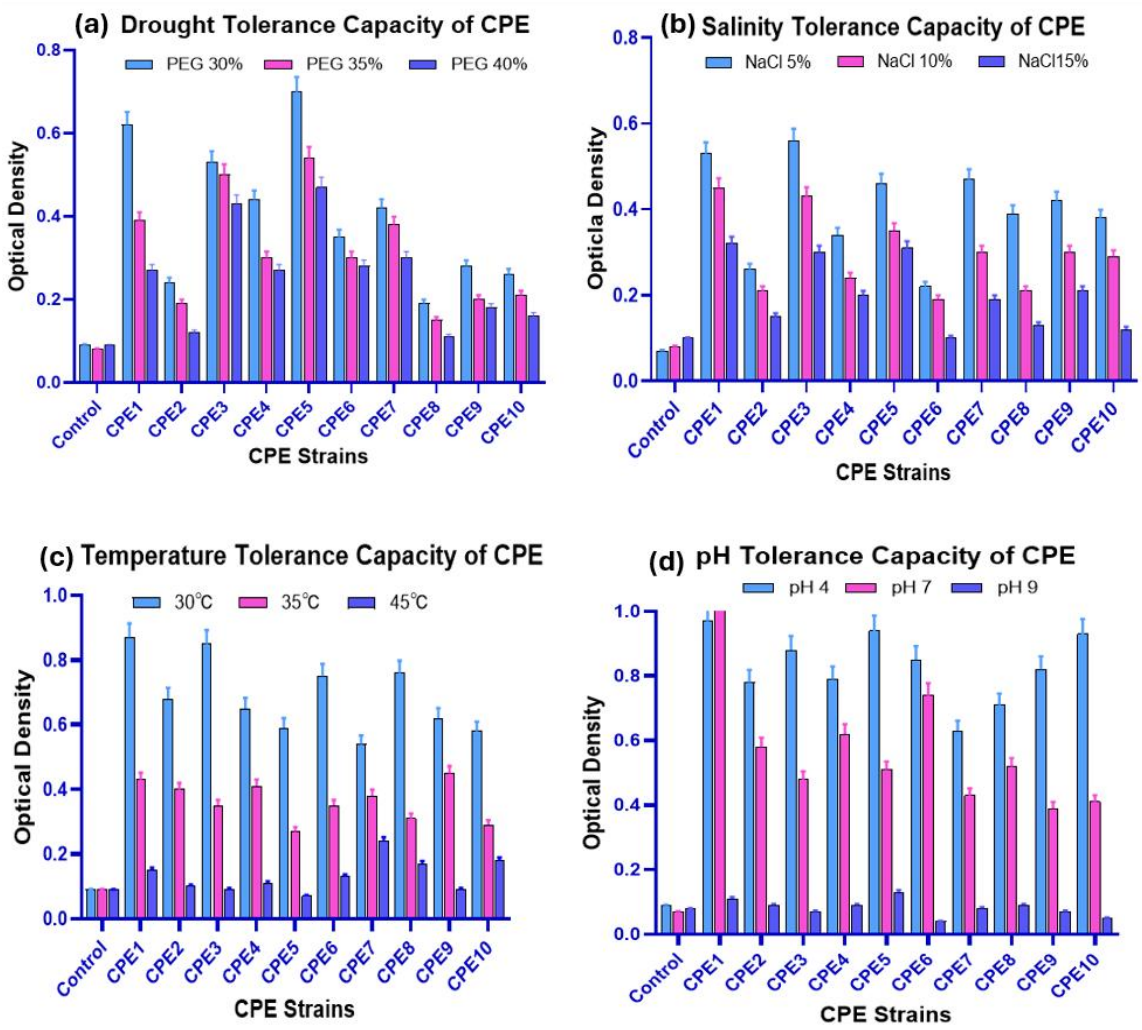
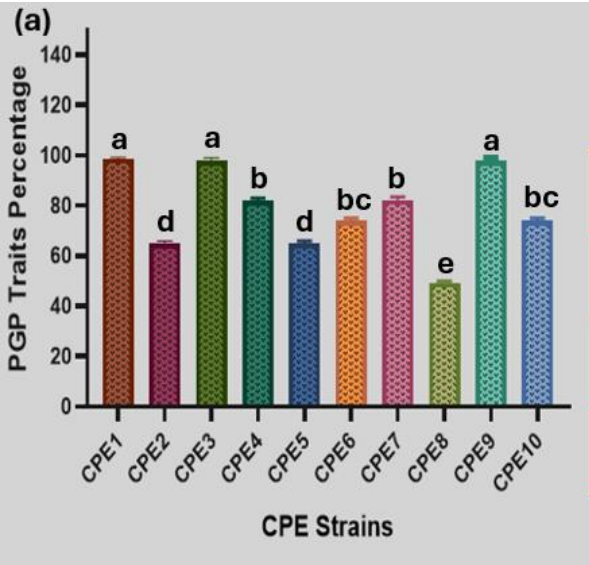


Fig. 2 Abiotic stress tolerance capacity of selected cellulose producing endophytes (CPE): (a) drought polyethylene glycol (PEG 30%–40%), (b) salinity (NaCl 5%–15%), (c) temperature (30°C–45°C), and (d) pH (4–9). The data were expressed as mean±standard deviation (SD), (*n*=3).

3.5 Selection of significant CPE for seed coating

The top five strains CPE1, CPE3, CPE4, CPE7, and CPE9 exhibited the most diverse and highest levels of PGP traits, including auxin production, nutrient solubilization, nitrogen fixation, and activity of stress-alleviating enzymes (Fig 3a). These strains also demonstrated significant performance under abiotic stress conditions. Their combined capabilities make them strong candidates for the development of effective biofertilizers and seed coating formulations for enhancing plant growth and nutrition. The identification of all the selected bacterial strains was confirmed by using accession numbers from the NCBI database (https://www.ncbi.nlm.nih.gov/) (Fig. 3b).



(b)

Bacteria code	Identified bacterial name on base of 16S rRNA gene analysis	Sequence similarity %	Accession number
CPE1	*Acetobacter tropicalis*	98.93%	PV017707
CPE3	*Pseudomonas sp.*	99.66%	PV017900
CPE4	*Bacillus amyloliquefaciens*	97.77%	PV017902
CPE7	*Pseudomonas aeruginosa*	99.75%	PV018011
CPE9	*Enterococcus sp.*	98.71%	PV017722

Fig. 3 (a) Percentage of plant growth promoting (PGP) traits exhibited in CPE strains; The data are expressed as mean±standard deviation (SD), (*n*=3). Standard bars that do not share a same letter were considered significantly different (*P*≤0.05). (b) NCBI accession numbers of identified CPE strains.

3.6 Surface characterization of seed coatings with cellulose embedded CPE

SEM images show the surface morphology of wheat seeds coated with endophytic bacteria embedded in BC, using two application methods, dip-coating (Fig. 4 a–f) and spray-coating (Fig. 4 g–l). Control seeds had smooth surfaces devoid of visible microbial attachment, while coated seeds exhibited distinct structural modifications. The dip-coated seeds exhibited a uniform and continuous film of BC on the seed surface, effectively embedding and protecting the bacterial cells, thereby demonstrating successful immobilization. In contrast, spray-coated seeds exhibited irregular and patchy deposition of BC, with bacterial aggregates concentrated in specific regions rather than uniformly distributed. Both coating techniques effectively entrapped endophytes within the cellulose matrix, but dip-coating yielded denser and more homogeneous coating, whereas spray-coating resulted in a thinner and more heterogeneous coating.

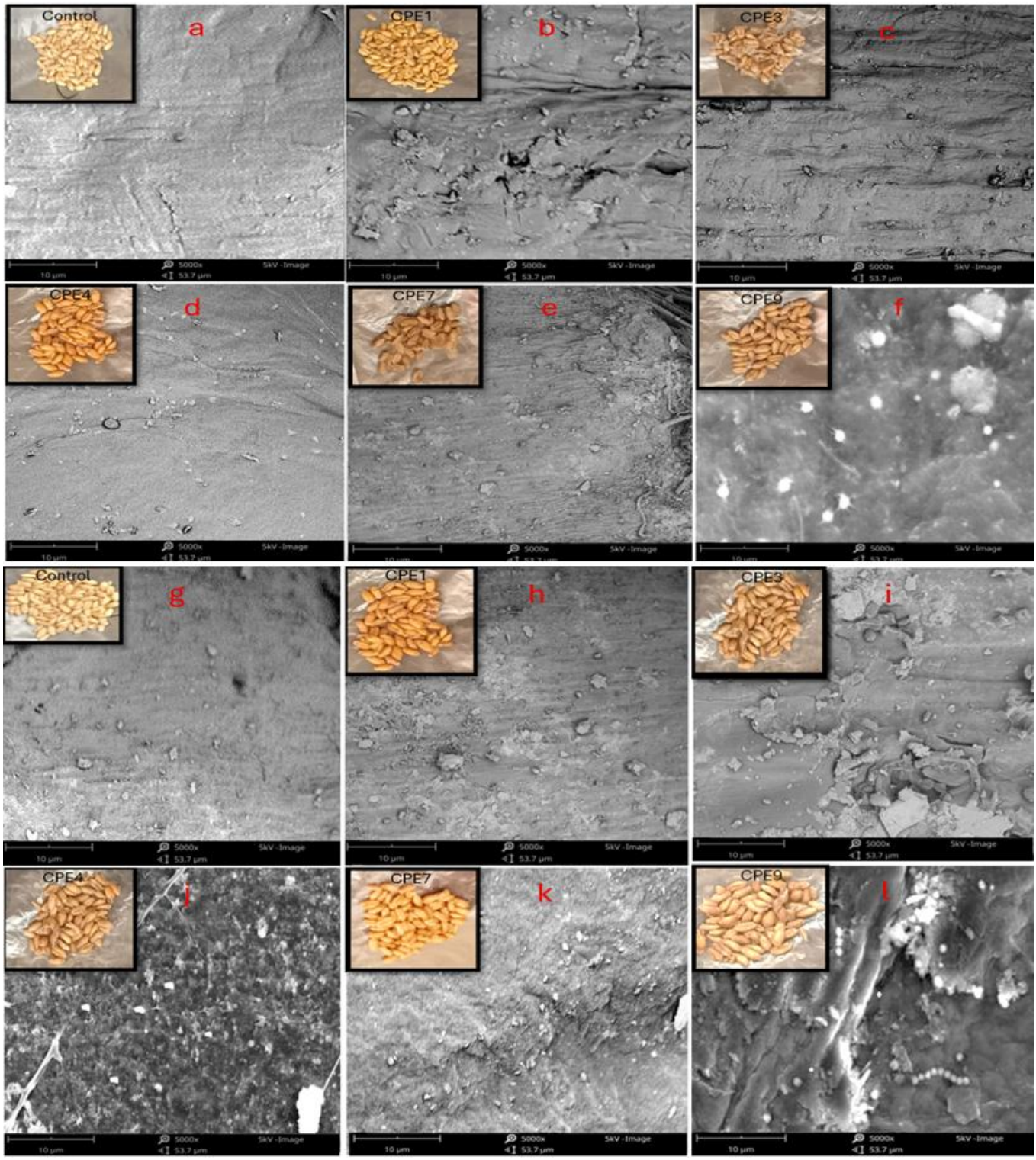


Fig. 4 SEM images of dip and spray coated seeds: (a) Control dip coated seeds without bacteria; (b-f) dip coated seed surface with bio-cellulose embedded endophytes; (g) Control spray coated seeds without bacteria (H-l) spray coated seed surface with bio-cellulose embedded endophytes.

3.7 Effects of seed coatings on seed growth in lab (Petri dish plate and pot trial)

Fig. 5a–f shows the effects of CPE treatments on seed germination and seedling vigor index in Petri-dish and pot assays after two weeks under laboratory conditions. In the Petri-dish assay, dip-coated seeds had a control germination rate of 60%, while treatments CPE3, CPE9, and CPE1 reached 80%, 79%, and 75%. Spray-coated seeds had a control rate of 70%, with CPE3 and CPE9 achieving 90%, an increase of 29% over the control (Fig. 5a). Seedling vigor, measured as the Seedling Length Vigor Index (SLVI) and Seedling Weight Vigor Index (SWVI), also improved. For dip-coated seeds, the SLVI control value was 800, and CPE3, CPE4, and CPE9 increased to 1350, 1400, and 1350, corresponding to 69%–75% increases. SWVI control was 45, with CPE3, CPE4, and CPE9 reaching 83, 80, and 70, representing increases of 56%–84%. For spray-coated seeds, SLVI control was 1100, and treatments CPE3, CPE4, and CPE9 reached 1650, 1600, and 1450 (32%–50% increase). SWVI control was 55, with CPE3, CPE4, and CPE9 increasing to 75, 95, and 85 units (36%–73% increase) (Fig. 5b, c). In the pot assay, dip-coated seeds had a control germination rate of 65%, with CPE3, CPE4, and CPE9 increasing to 75%, 78%, and 80%. Spray-coated seeds had a control of 70%, and CPE3 and CPE9 reached 90% (20% increase) (Fig. 5d). SLVI and SWVI showed similar trends. dip-coated control values were 800 (SLVI) and 45 (SWVI), increasing by 30%–75% and 35%–85%, respectively. Spray-coated control values were 1100 (SLVI) and 55 units (SWVI), with increases of 32%–50% (SLVI) and 36%–73% (SWVI) in treatments CPE3, CPE4, and CPE9 (Fig. 5e, f). Overall, both dip and spray coatings significantly enhanced seed germination and seedling vigor compared to the control, demonstrating consistent and effective improvement in early seedling growth.

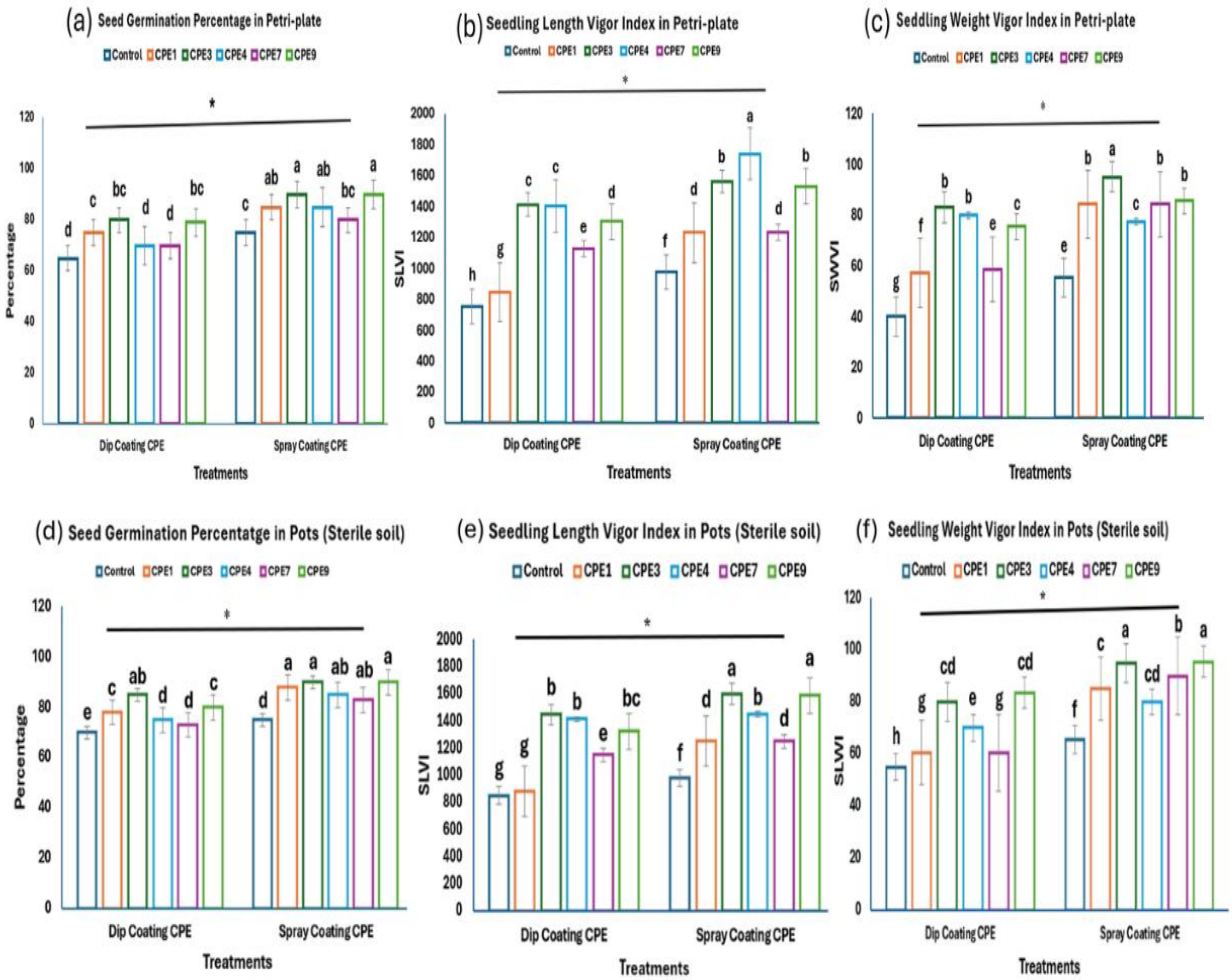


Fig. 5 Effects of seed coatings on seed growth in petri-plates and pots for two weeks in the lab; Fig. (a-c) shows the Petri-dish assay results while Fig. (d-f) shows the pot assay results. Data represent means of replicate treatments (*n*=10). Standard bars that do not share a same letter were considered significantly different (*P*≤0.05).

3.8 Effects of seed coatings on wheat growth and yield under natural conditions (wire-house and field trials (non-sterile soil)

Across both wire-house and field experiments, untreated control plants consistently exhibited lower growth performance compared with bio-cellulose treated plants (Tabel 3-4). Under wire-house conditions, control values for dip and spray coating ranged from 60–70.5% for seed germination, 28.2–30.9 cm for root length, 42.9–49.5 cm for shoot length, 12.0–15.8 mg for root weight, and 20.8–24.0 mg for shoot weight. Relative to these control values, dip-coated CPE treatments increased seed germination by up to 31.2%, root length by 72.0%, shoot length by 36.8%, root weight by 95%, and shoot weight by 44.2%, while spray coating resulted in comparable improvements, particularly in root length (up to 61.8%) and root biomass (up to 59.5%). Similarly, under field conditions, control plants recorded seed germination of 70.5–73.3%, root length of 23.1–35.0 cm, shoot length of 49.6–60.5 cm, root weight of 18.0–22.9 mg, and shoot weight of 24.7–35.3 mg for dip and spray methods. When compared with these controls, dip-coated CPE treatments enhanced shoot length and biomass accumulation, with shoot and root weights increasing by up to 61.9% and 60.0%, respectively. Spray coating under field conditions further improved root and shoot growth, with root length increasing by as much as 33.3% and shoot weight by over 23%.

In the wire-house pot trial and field conditions, untreated control plants showed comparatively lower spike length, number of spikelets per spike, grains per spike, and 1000-grain weight under both dip and spray coating methods. Dip-coated CPE treatments increased spike length by 60%–95%, while spray coating enhanced spike length by 40%–54%. Similarly, the number of spikelets and grains per spike, as well as 1000-grain weight, were consistently higher in CPE-treated plants, with maximum relative increases of 71%–95% in spikelets per spike, 50%–59% in grains per spike, and 30%–40% in 1000-grain weight, recorded at higher CPE concentrations (CPE3, CPE4, and CPE9). Spray coating generally resulted in superior improvements compared with dip coating, particularly for grain number and grain weight. A similar response pattern was observed under field conditions. In dip-coated treatments, spike length increased by 46%–82% and grains per spike by 17%–50%. Spray-coated CPE treatments produced the highest yield responses, with grains per spike and 1000-grain weight increasing by 32%–49% and 30–33%, respectively, at higher CPE concentrations. Overall, both experiments demonstrated that CPE application significantly improved wheat yield attributes, with spray coating and higher CPE concentrations showing the most pronounced effects.

Table 3 Effects of seed coatings on root and shoot length and dry biomass under wire house and field conditions(non-sterile soil)

Exp.	Treatments	Dip Coating CPE					Spray Coating CPE				
	Plant Traits	S.G (%)	R.L (cm)	S.L (cm)	R.W (mg)	S.W (mg)	S.G (%)	R.L (cm)	S.L (cm)	R.W (mg)	S.W (mg)
Wire-house Pot Trial	Control	70.5 d	28.2 c	42.9 d	12.0 cd	20.8 c	70 d	30.9 c	49.5 c	15.8 c	24.0 b
	CPE1	85.3 c	40.6 c	50.6 c	18.7 bc	25.9 b	85.5 c	45.5 b	50.4 c	20.5 b	24.5 b
	CPE3	90.0 a	48.1 a	55.4 b	22.0 ab	29.0 a	90.0 a	49.2 a	59.3 a	25.2 a	32.8 a
	CPE4	92.5 ab	46.5 ab	55.8 b	22.5 ab	29.5 a	95.5 a	49.2 a	55.2 b	22.1 b	27.2 ab
	CPE7	83 c	43.9 b	50.4 c	18.0 bc	25.0 b	85.8 c	43.1 b	53.0 b	22.0 ab	25.7 b
	CPE9	92.0 ab	48.5 a	58.7 a	24.0 a	30.0 a	95.0 a	50.0 a	59.0 a	25.0 a	32.0 a

Exp.	Treatments	Dip Coating CPE					Spray Coating CPE				
	Plant Traits	S.G (%)	R.L (cm)	S.L (cm)	R.W (mg)	S.W (mg)	S.G (%)	R.L (cm)	S.L (cm)	R.W (mg)	S.W (mg)
Field Trial	Control	73.3 d	23.1 d	49.6 e	18.0 e	24.7 f	70.5 cd	35.0 c	60.5 c	22.9 cd	35.3 c
	CPE1	85.0 c	48.6 bc	54.3 d	24.3 cd	26.3 de	90.2 b	49.8 bc	65.3 b	27.0 bc	40.6 ab
	CPE3	92.0 ab	50.3 bc	58.4 c	25.0 c	30.9 d	95.8 a	54.2 b	69.6 a	30.9 ab	43.0 a
	CPE4	90.5 b	50.0 bc	60.8 c	25.0 c	35.0 c	95.0 a	59.9 a	70.1 a	32.1 a	43.5 a
	CPE7	87.4 bc	45.3 c	50.4 d	22.9 cd	27.2 de	85.8 c	47.5 bc	65.3 b	25.0 c	40.0 ab
	CPE9	90.6 b	52.0 b	65.7 b	28.8 bc	40.0 ab	95.0 a	60.0 a	69.1 a	32.0 a	43.5 a

Footnotes: Symbols represent: CPE (Cellulose Producing Endophytes); Exp. (Experiment); S.G (Seed Germination); R.L. (Root Length); S.L (Shoot Length); R.W(Root weight); S.W(Shoot Weight). Data represented as means of replicate treatments (n=10). Standard bars that don't share a letter are significantly different ($P \leq 0.05$).

Table 4 Effects of seed coatings on dry biomass of root and shoot under wire house and field condition

Exp.	Treatments	Dip Coating CPE				Spray Coating CPE			
	Plant Traits	Spk.L (cm)	No. Spk/Spkl	No. G/Spkl	W.1000 G (g)	Spk.L (cm)	No. Spk/Spkl	No G/Spkl	W.1000 G (g)
Wire-house Pot Trial	Control	4.5 ^{bc}	6.2 ^{cd}	25.0 ^{d}	33.5 ^{cd}	6.8 ^{bc}	9.5 ^{bc}	22.0 ^{cd}	30.0 ^{c}
	CPE1	7.2 ^{ab}	10.6 ^{bc}	28.5 ^{c}	34.2 ^{c}	7.5 ^{ab}	10.5 ^{bc}	28.5 ^{c}	34.5 ^{c}
	CPE3	9.0 ^{a}	12.5 ^{ab}	32.9 ^{ab}	40.0 ^{ab}	10.0 ^{a}	13.0 ^{a}	35.0 ^{a}	42.0 ^{a}
	CPE4	9.2 ^{a}	12.5 ^{ab}	32.9 ^{ab}	40.0 ^{ab}	9.5 ^{a}	13.0 ^{a}	35.0 ^{a}	42.0 ^{a}
	CPE7	7.5 ^{ab}	10.6 ^{bc}	28.0 ^{c}	34.2 ^{c}	8.0 ^{ab}	10.0 ^{bc}	28.5 ^{c}	34.5 ^{c}
	CPE9	9.0 ^{a}	12.5 ^{ab}	32.7 ^{ab}	40.0 ^{ab}	10.5 ^{a}	13.3 ^{a}	34.9 ^{a}	41.9 ^{a}

Exp.	Treatments	Dip Coating CPE				Spray Coating CPE			
	Plant Traits	Spk.L (cm)	No. Spk/Spkl	No G/Spkl	W.1000 G (g)	Spk.L (cm)	No. Spk/Spkl	No G/Spkl	W.1000 G (g)
Field Trial	Control	5.5 ^{ab}	10.0 ^{cd}	30.0 ^{ef}	35.0 ^{f}	8.0 ^{ab}	12.0 ^{c}	37.0 ^{e}	38.0 ^{de}
	CPE1	8.9 ^{a}	11.5 ^{cd}	40.0 ^{d}	33.7 ^{fg}	9.2 ^{a}	13.0 ^{bc}	41.5 ^{cd}	40.0 ^{cd}
	CPE3	9.5 ^{a}	13.9 ^{bc}	43.0 ^{bc}	41.9 ^{c}	10.5 ^{a}	14.5 ^{ab}	45.8 ^{b}	42.2 ^{c}
	CPE4	9.5 ^{a}	13.5 ^{bc}	43.0 ^{bc}	41.9 ^{c}	10.0 ^{a}	14.0 ^{ab}	45.2 ^{b}	42.9 ^{c}
	CPE7	8.0 ^{ab}	10.5	35.1 ^{ef}	33.7 ^{fg}	8.5 ^{ab}	11.6 ^{c}	40.0 ^{c}	39.7 ^{cd}
	CPE9	10.0 ^{a}	14.0 ^{a}	45.0 ^{b}	48.5 ^{ab}	10.5 ^{a}	15.5 ^{a}	49.0 ^{a}	50.5 ^{a}

Footnotes: Symbols represent: CPE (Cellulose Producing Endophytes); Exp. (Experiment); Spk.L (Spike Length); No. Spk/Spkl (No of Spikes Per Spikelet); No. G/Spkl (No of Grains Per Spikelet); W.1000 G (Weight of 1000 Grains). Data represented as means of replicate treatments (n=10). Standard bars that don't share a letter are significantly different ($P \leq 0.05$).

3.9 Effects of seed coatings on plant biochemicals

In both trials (wire-house and field), the heatmap (Fig. A & B) shows hierarchical clustering of different treatments based on multiple biochemical parameters, i.e. chlorophyll a (Chl a), chlorophyll b (Chl b), proline (Prol), total sugar content (Total S), carotenoids (car), and total protein (Total Pro). The colour gradient ranges from blue (low values) to red (high values). Spray coated and dip coated seeds treated with CPE 9, CPE3 or CPE4 exhibited elevated concentrations of chlorophylls, proline, and total sugars, signifying superior physiological activity compared to the control. Both coating techniques had no effect on carotenoids and total

protein. The clustering indicates that spray applications were more efficacious than dip treatments in enhancing essential biochemical characteristics, while both coating methods showed significant improvements compared to the control.

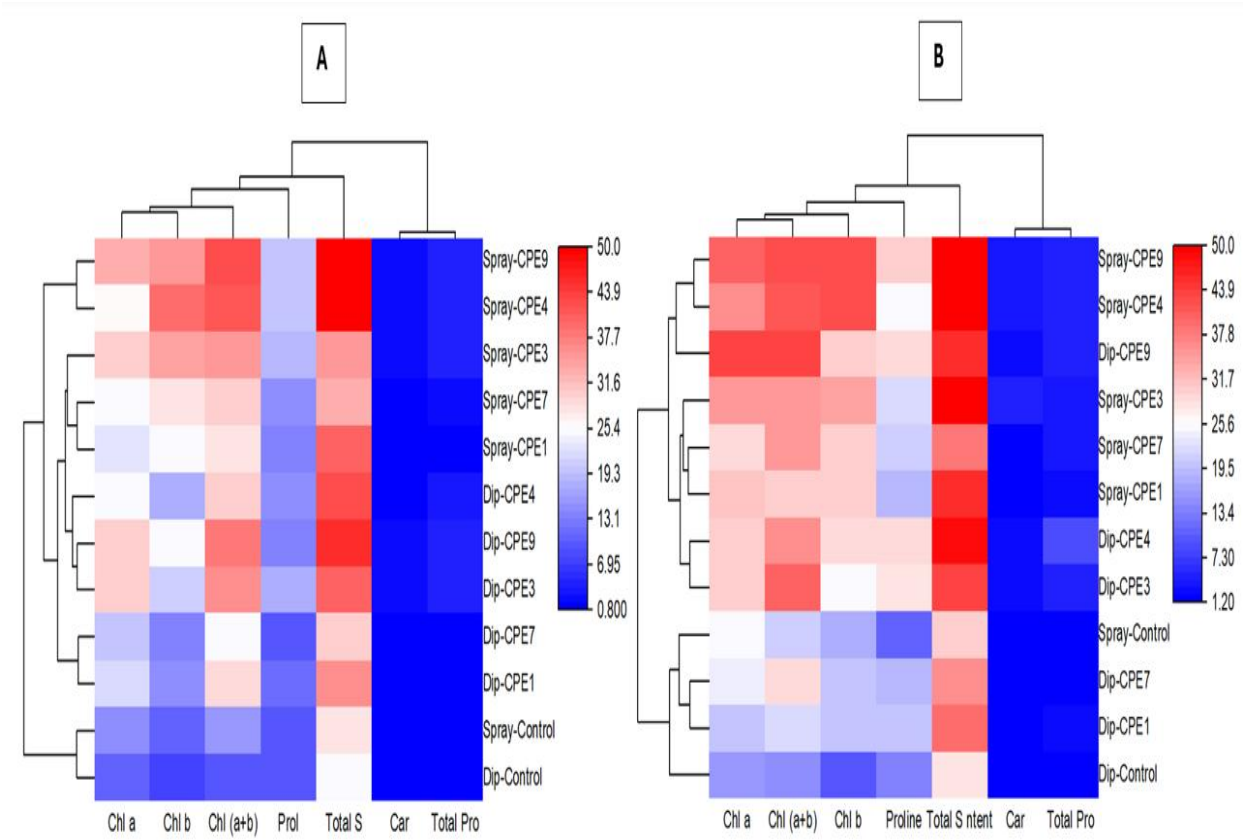


Fig. 6 Effects of seed coatings on plant biochemicals; Fig. (A) shows the wire-house pot trial results while Fig. (B) shows the field trial results. Heatmap visualization of biochemical parameters including chlorophyll a (Chl a), chlorophyll b (Chl b), proline (Prol), total sugar content (Total S), carotenoids (Car), and total protein (Total Pro) across different treatment groups. Colour gradient represents relative values, ranging from blue (low) to red (high).

3.9 Effects of seed coatings on root rhizoplane colonization

Scanning electron microscopy (SEM) revealed distinct differences in root colonization between dip coated seeds (Fig. 7a-f) and spray coated seeds (Fig. 7g-l). In the untreated control, root surfaces appeared smooth with no visible microbial adherence. In contrast, all CPE treatments exhibited variable degrees of microbial attachment and colonization along the root epidermis and rhizoplane (green arrows indicate bacterial attachment to roots). More extensive colonization was evident in CPE1 and CPE3, where dense microbial aggregates occupied root grooves and intercellular spaces, indicating stronger root microbe interactions. CPE4 and CPE7 showed initial colonization with small clusters of microbial cells adhering to root surfaces in both treatments. Strong bacterial colonization was observed under CPE9, where extensive biofilm-like structures and cross-linked microbial networks covered the root surface, suggesting enhanced adhesion and establishment of microbial communities. These results demonstrate that the bio-cellulose seed coating with CPE application effectively promoted microbial root colonization patterns compared with the control.

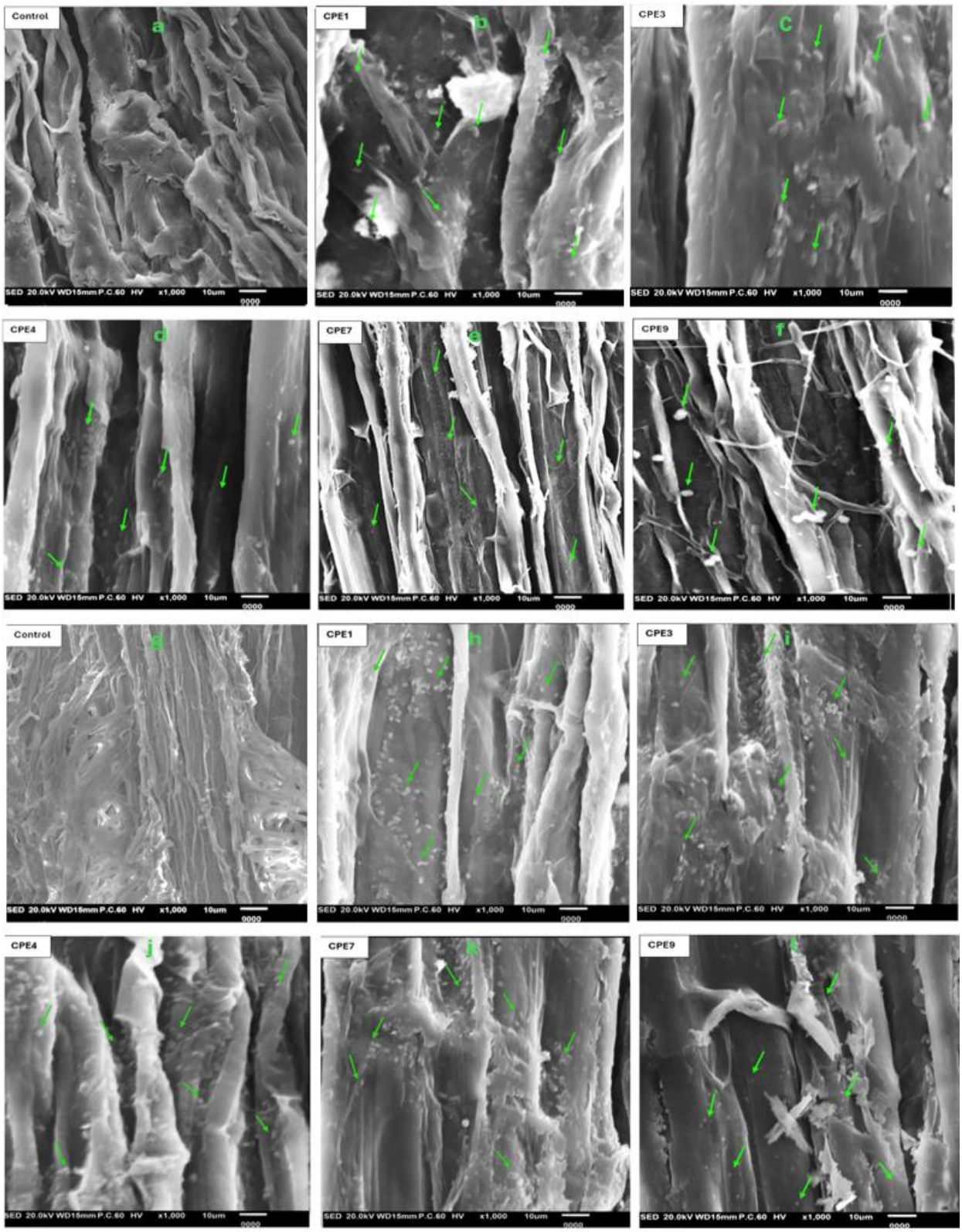


Fig. 7 Effects of CPE bacteria seed coatings on root colonization: (a-f) dip coated seeds root colonization; (g-l) spray coated seeds root colonization.The green arrows pointed towards bacterial attachment with root.

3.10 Effects of storage on bio-cellulose embedded endophytes in seed coatings

The survival rate of bacteria in seed coatings was evaluated over a 120-day storage period, comparing dip coating and spray coating techniques across five distinct bio-cellulose embedded bacteria (CPE1, CPE3, CPE4, CPE7 and CPE9). Initially, bacterial populations (10^7 to 10^8 log CFU/g of seeds) were consistently maintained. A decline in bacterial survival was observed in both methods over time, but the reduction was more significant in dip-coated seeds, especially those coated with strain CPE7 5.1×10^8 CFU/g. Overall the survival rate was high for CPE3, CPE4 and CPE9 bacteria. At 120 d, the spray-coated seeds exhibited consistently higher viable bacterial counts than the dip-coated seeds, indicating that spray coating enhances the long-term survival of beneficial bacteria (Fig. 8). The observed superiority was likely attributable to the reduced exposure time of bacteria to liquid media during spray application. This reduction minimizes osmotic and desiccation stress, enabling the formation of more uniform and thinner coatings that enhance oxygen diffusion and expedite drying.

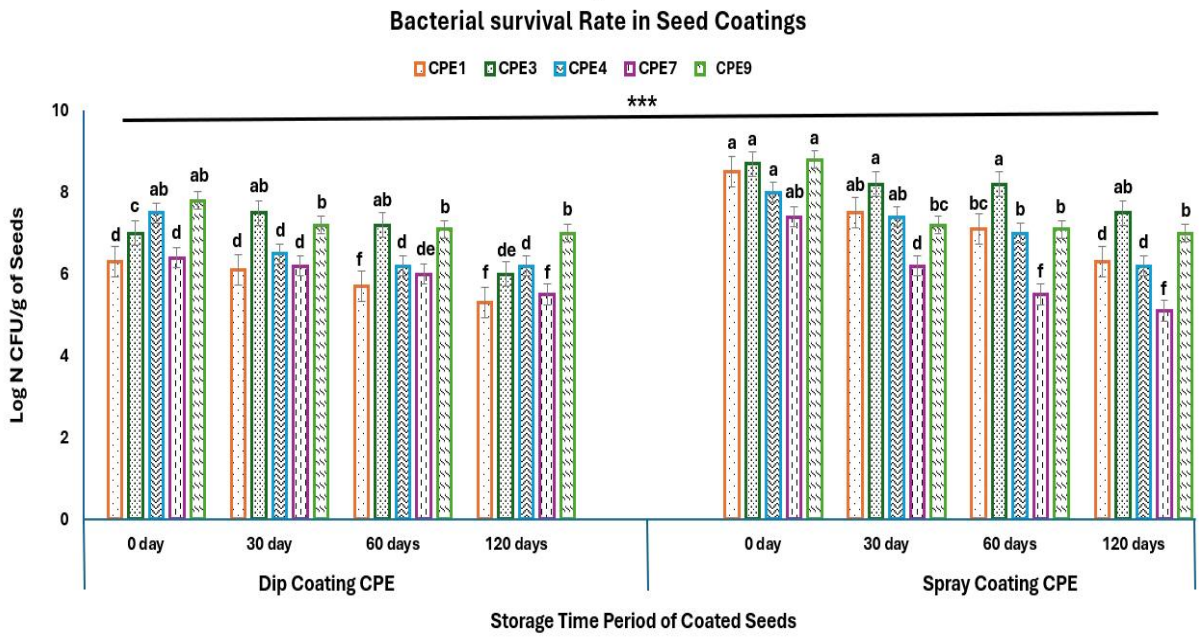


Fig. 8 Effect of storage at ambient temperature on the survival of bio-cellulose–embedded endophytes in seed coatings after 120 d. Data are presented as mean±standard error (*n*=6). Bars labelled with different lettersindicate statistically significant differences (*P*≤0.05).

4 Discussion

Coating seeds with plant beneficial microorganisms incorporated with polymers (gelatine, starch, cellulose), fungicides and herbicides are a technique often used in modern agriculture systems to protect seeds and enhance plant growth. It enables reductions in agrochemical inputs and increases nutrient absorption and stress tolerance (Ajayi et al., 2024; Gong et al., 2024). Endophytes, the beneficial microorganisms residing within plant tissues, can enhance plant development under natural and environmentally adverse conditions. They enhance plant nutrient absorption, improve soil health, optimise plant nutrition, and activate plant defence mechanisms(Hazarika et al., 2021; Santoyo et al., 2016). In this study we showed that bio-cellulose seed coatings infused with PGP endophytes represent a viable approach to improving crop performance and reducing reliance on agrochemicals. Cellulose is a natural polymer that forms a film which tightly coats the surface of

seeds. Another special property of cellulose is its quick absorption and release of active compounds (Khorasani & Shojaosadati, 2016). For these reasons, it is used as a binding agent in seed coatings. It is reported as being a good binding agent for beneficial microbes. In this study, five bacterial isolates were selected and identified as *Acetobacter tropicalis* (CPE1), *Pseudomonas* sp. (CPE3), *Bacillus amyloliquefaciens* (CPE4), *Pseudomonas aeruginosa* (CPE7), and *Enterococcus* sp. (CPE9). Our results showed strain-dependent variability in both BC production efficiency and PGP functionality among the evaluated CPE isolates, underscoring the importance of targeted strain selection for agricultural applications. Differences in cellulose yield observed among the strains reflect intrinsic metabolic and enzymatic heterogeneity, a phenomenon widely reported in cellulose-producing bacteria, including members of *Komagataeibacter* and *Bacillus* (Rezaei et al., 2025). In particular, strains CPE4, CPE9, and CPE1 exhibited superior cellulose synthesis compared with CPE2. In addition to cellulose production, there was clear functional diversity among the isolates in PGP traits. All isolates exhibited various PGP traits that are advantageous for the growth of wheat plants. Auxin production by beneficial bacteria is crucial for PGP microorganisms, as it serves as one of the key phytohormones that regulate growth and various developmental processes in plants (Egamberdieva et al., 2017). In this study, the CPE3 strain showed the highest production of IAA at 304 μg mL^{-1}, while the CPE6 strain had the lowest at 98 μg mL^{-1}. The production of IAA is influenced by various pathways, leading to variability in the quantity of IAA produced among different isolates (Moliszewska & Nabrdalik, 2020). (Khianngam et al., 2023) reported that two bacteria, *Enterobacter hormaechei* and *Bacillus aryabhattai*, produced 246.00 and 195.55 μg/mL IAA with L-tryptophane, which is consistent with our results. HCN production activity was strongly expressed by CPE3 and CPE4 but not observed in CPE6 and CPE10. The production of ammonia by endophytes has the potential to act as a source for the fixation of atmospheric nitrogen, thereby stimulating plant growth and enhancing plant defence mechanisms. The quantitative analysis of ammonia production indicated that CPE1 yielded the highest concentration at 4.0 μg mL^{-1}, while CPE9 yielded the lowest at 1.9 μg mL^{-1}. Previous studies provide evidence supporting this, as nearly all isolates produced ammonia, with *Bacillus* sp. yielding a higher amount of ammonia (Kang et al., 2020; Khianngam et al., 2023). Siderophore production by plant-associated bacteria facilitates plant growth through the scavenging of iron from the environment, addressing the issue of low iron bioavailability (Ahmed & Holmström, 2014). The endophytic isolates demonstrated significant siderophore production under iron-limiting conditions in this study. CPE1 generated the largest siderophore halo zone (5.5 mm in diameter), while no siderophore activity was detected in CPE2. Previous studies reported *Bacillus* sp. *Pseudomonas* sp., *Acinetobacter* sp., and *Ochrobactrum* sp. as producers of siderophores (Ferreira et al., 2019; Ferreira et al., 2018). ACC deaminase activity was prominent in CPE3, CPE4, and CPE7 but absent in CPE2, CPE5, and CPE10. Nitrogen fixation capacity differed significantly across the strains. Mineral solubilization and enzymatic activity assays further highlighted the functional diversity of selected isolates. Previous studies indicated that numerous beneficial bacteria associated with plants produce ACC deaminase, extracellular enzymes, and facilitate mineral solubilisation through indirect mechanisms for plant growth promotion (Gupta et al., 2015). The production of hydrolytic enzymes by endophytes is significant for the colonisation of plant roots and the movement of bacteria into the interior of plants, thereby facilitating plant growth (Choubane et al., 2016; Khianngam et al., 2023). Abiotic stress drought, salinity, and high temperature directly influence soil properties and negatively impact plant growth and yield. Studies show that endophytes can be used to reduce abiotic stress tolerance (Abo Nouh et al., 2024; Bokhari et al., 2019; Kaur & Karnwal, 2023). All selected strains in this study showed stress tolerance and can be used to support plant growth under stress conditions.

Lab, wire house and field trials were conducted to evaluate the potential of bio cellulose seed coating for promoting wheat growth. Results showed that the use of cellulose-embedded endophytes markedly enhanced wheat growth, biochemical characteristics, and yield. Under controlled conditions, bio-cellulose-coated seeds demonstrated seed-germination rate (60%–90%) and seedling-vigor-length (60%–70%) and seedling-weight-vigor indexes (80%–95%) while control seed germination rate was 50%-60%, seedling-vigor-length 30%-40% and weight-vigor 40% -50%. The results align with the findings of (Kthiri et al., 2020). The seed coating with *Trianum-P* and *S.INAT* exhibited the highest seed germination rates, ranging from

85% to 90%. In contrast, S.IO1 and S.IO2 demonstrated the lowest germination rates at 66% and 68%, respectively. Additionally, these treatments enhanced the seedling vigour index compared to the control group.

Our wire-house and field trials further confirmed improved seed-germination (85%–92%), morphological traits (60%–80%), physiological traits (50%–85%), and yield (70–85%) compared to controls. The findings align with research indicating that wheat seeds coated with microcapsules containing beneficial bacteria increased wheat growth and yield (Saadaoui et al., 2022). Plants exhibited notable enhancements in chlorophyll content, total protein, total sugar, and carotenoid levels. Additionally, plants that received sufficient irrigation in conjunction with PGP endophyte treatments produced the highest grain yields. The yield of wheat is significantly affected by traits like 1000-grain weight, which showed notable enhancement through the application of beneficial microorganisms (Saadaoui et al., 2022).

Effective production of bioinoculants necessitates the development of formulations that sustain high cell densities and ensure long-term microbial viability during storage (Gautam & Gautam, 2021). In this study we evaluated the shelf life of bio-cellulose embedded CPE-coated wheat seeds over 120 d at ambient conditions. Initial bacterial populations were greater than 10^7 CFU/g for dip coatings and surpassed 10^8 CFU/g for spray coatings. Cell viability was relatively stable over the initial 30 d, followed by a modest decline after 60 d, ultimately reducing to between 10^5 and 10^6 CFU/g for both treatment groups. The observed trends align with earlier research indicating that co-cultures of *Pantoea ananatis* and *Pseudomonas fluorescens* sustained stable viability near 10^6 CFU over a storage period of 55 to 70 d (Anwar et al., 2019). *Bacillus amyloliquefaciens* and *B. pumilus* maintained viability for up to nine months in bio-formulations using carriers like sawdust, rice husks, and talcum powder, achieving cell densities of about 7.0 log10 CFU/mL (Chakraborty et al., 2013). The results validate the robust survival capacity of the microbial strains over prolonged storage durations, as corroborated by the recent study by Ji et al. (2023).

5 Conclusions

This research showed that bio-cellulose seed coatings containing PGP endophytes significantly improved seed germination, growth, and yield. The superior performance of three strains CPE3, CPE4 and CPE9 was significant in all trial experiments. While spray coating methods provided superior microbial delivery and enhanced plant performance relative to dip coating methods, compared to controls, both methods showed significant positive results. The coatings enhanced endophyte cell viability and optimized the seed microenvironment. Bio-cellulose-based coatings provide a sustainable formulation to enhance crop productivity and reduce dependence on agrochemical inputs.

Data availability statement

The data used to support the findings of this study are included within the article and its supplementary materials. No additional datasets were generated or analysed during the current study.

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Author contributions

Shumaila Batool conducted the experiments, performed data collection and formal analysis, and prepared the original draft of the manuscript. Atia Iqbal supervised the research work, reviewed and edited the manuscript, and approved the final version for submission.

Compliance with ethics guidelines

Shumaila Batool and Atia Iqbal declare that they have no conflicts of interest. This study did not involve any human participants or animals. All experiments were conducted in accordance with relevant institutional, national, and international guidelines.

Declaration on the use of generative AI tools

The authors declare that no generative AI tools were used in the creation of this manuscript. All content, analysis, and writing were performed solely by the authors.

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Supplementary information
No