BMC Plant Biology

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Enhancing wheat resilience to salinity: the role of endophytic *Penicillium chrysogenum* as a biological agent for improved crop performance

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Abstract

Salinity stress severely impacts wheat productivity, necessitating effective strategies to enhance crop resilience. This study investigates the potential of *Penicillium chrysogenum* CM022 as a biological agent to alleviate the impact of salinity stress on wheat (*Triticum aestivum* L.). *P. chrysogenum* CM022 improved germination of wheat seeds, particularly under salinity of 150 mM NaCl. Fungal inoculation significantly improved plant growth in terms of root length, plant height, and seedling biomass, even under high salinity conditions. Notably, inoculated plants preserved photosynthetic pigments and reduced oxidative damage, evidenced by lower levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), compared to non-inoculated controls. The inoculated plants also exhibited enhanced proline and soluble sugar contents, which are crucial for osmotic adjustment under stress. Additionally, *P. chrysogenum* CM022 significantly increased the antioxidant capacity of wheat, boosting total phenolic and flavonoid contents, and enhancing antioxidant enzyme activity under high salinity. These findings underscore the potential of *P. chrysogenum* CM022 in improving wheat tolerance to salinity stress through physiological, biochemical, and antioxidant defense mechanisms, supporting its use in sustainable agricultural practices to mitigate the adverse effects of salinity on crop production.

Keywords Endophytic fungi, *Penicillium chrysogenum*, Seed germination, Antioxidant enzymes, Salinity resilience

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most essential cereal crops, serving as a staple food for millions of people and contributing significantly to global food security [1]. However, its productivity is increasingly threatened by various environmental stresses, with soil salinity being one of the most critical challenges [2, 3]. Salinity stress is detrimental to wheat, adversely affecting seedling development and crop productivity by disrupting key physiological and metabolic processes, such as protein synthesis, lipid metabolism, photosynthetic efficiency, water uptake and ionic balance [4].

Salinity stress affects over 6% of the world's arable lands, primarily in the dry and semi-arid regions, posing a significant threat to agricultural sustainability and global food security [5]. To counteract this challenge, various strategies have been employed to enhance crop resilience, including the introduction of salt-tolerant genes, genotype selection, and traditional breeding techniques,

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which focus on cross-breeding and trait selection over multiple generations [6]. While these methods have shown promise, they are often limited by high costs, long breeding cycles, and the complexity of improving quantitative traits. Although advances in transgenic technology have allowed the incorporation of salt-tolerant genes into plant materials [7, 8], challenges such as gene loss, regulatory constraints, and high costs hinder their widespread application [9].

Given these limitations, the use of microbial endophytes has emerged in recent years as a sustainable and cost-effective alternative for mitigating salinity stress in crop plants. Fungal endophytes, that reside within plant tissues, offer significant potential to enhance plant growth and productivity under saline conditions, making them a promising solution to one of the agriculture most pressing challenges [10]. These fungi play a critical role in improving plant growth, enhancing tolerance to both biotic and abiotic stresses, and optimizing physiological and metabolic processes [11–13]. The mechanisms through which these endophytes exert their effects include ion balance regulation, improving nutrient uptake, boosting antioxidant production, and reducing oxidative damage caused by reactive oxygen species (ROS) [14]. Furthermore, fungal endophytes can enhance plant adaptation to extreme environmental conditions, such as salinity, drought, and temperature stress, by producing secondary metabolites and hormone-like compounds [15].

This study aims to explore the untapped potential of the endophyte *Penicillium chrysogenum* as a biological agent for improving wheat resilience to salinity stress. Conventional methods for salinity mitigation in agriculture include application of chemical amendments like gypsum or lime, which help improve soil structure and irrigation management practices such as leaching of excessive salts. Salt-tolerant plant varieties are also commonly used. However, these methods have notable drawbacks. Chemical amendments can be costly and require ongoing application, making them unsustainable in the long term. Soil leaching can waste water, thus worsening the problem of water scarcity. While salt-tolerant varieties can be effective, their development is time-consuming and not always applicable to all salinity conditions. This study aims to explore the untapped potential of endophytic *Penicillium chrysogenum* as a biological agent for improving wheat resilience to salinity stress. By focusing on endophyte inoculation, this research offers a sustainable, eco-friendly alternative that reduces the reliance on costly and water-intensive approaches. By advancing our understanding of fungal endophytes, such as *P. chrysogenum*, as bioinoculants, especially in improving salt tolerance of wheat, this research could contribute to ensuring global food security in an era of climate change.

Materials and methods

Fungal isolation and identification

The halophyte species *Caroxylon imbricatum* (synonym: *Salsola imbricata*) of the Amaranthaceae family, commonly known as "Imbricate saltwort" or "saltwort", was collected from the coastal areas of Qeshm, Hormozgan Province, Iran (latitude: 26°95′N; longitude: 56°28′E). Three pieces of sterilized leaf samples were placed on PDA agar plates, sealed with parafilm, and incubated at 28 ± 1 °C. The leaf pieces were surface-sterilized by immersion in 70% ethanol for 1 min, followed by 2% sodium hypochlorite (NaOCl) for 3 min, and then rinsed three times with sterile distilled water. The sterilized samples were placed on filter paper to remove excess moisture before being transferred to PDA agar plates, and the plates were sealed with parafilm and incubated at 28 ± 1 °C. Non-sterilized tissue samples were also prepared and incubated under similar conditions for comparison. Plates were checked every 3 days for fungal growth. After 4–6 weeks, fungal endophytes were isolated using the "tip of the hypha" method, by transferring a hyphal tip to fresh PDA plates for further growth [16]. For molecular identification, genomic DNA was extracted using the CTAB method Baker and Mullin (1994) [17]. The ITS region was amplified and sequenced with the primers ITS1 (5′-TCCGTTGGTGAACCAGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR products were sequenced using the Sanger method, and the sequences were analyzed with BioEdit software and compared to NCBI database sequences using BLAST for fungal identification. The fungal sequences, led to identification of *Penicillium chrysogenum* strain CM022 (GeneBank Accession MT277118.1 https://www.ncbi.nlm.nih.gov/nuccore/MT277118.1).

Seed disinfection and preparation

Wheat seeds of Sardari variety were sourced from the Dryland Agricultural Research Institute of Iran. The seeds were first rinsed with deionized water and then surface-sterilized by immersion in 70% ethanol for 30 s, followed by 0.1% sodium hypochlorite for 150 s. Subsequently, the seeds were thoroughly rinsed with distilled water and air-dried to minimize the risk of fungal contamination.

Cultivation of the fungal isolate

P. chrysogenum CM022 was cultured on potato dextrose agar (PDA, Merck, Germany) and incubated at 25 ± 1 °C for 7 days. The spores were detached from fungal mycelia by adding 10 ml of 20% (v/v) Tween 80 solution, then transferred to a sterile test tube and vortexed for 2 min. After sequential filtration through a double-layered mesh and dilution, the spore concentration was adjusted to 1×10^6 spores/ml using a hemocytometer [18].

Fungal salinity tolerance assessment

To evaluate fungal salt tolerance, *P. chrysogenum* agar discs, 5 mm in diameter, were inoculated onto PDA plates containing different concentrations of NaCl (0, 40, 60, 80, and 100 mM). Triplicate plates were incubated at 25 ± 1 °C in the dark for 7 days, and diameters of the fungal colonies (mm) were measured to assess growth performance under salinity stress [19].

Experiment I: in vitro wheat experiment

Wheat seeds were first immersed in the fungal suspension (1×10^6 spores/ml) for 6 hours, followed by air-drying on paper towels for 30 min. Subsequently, 30 seeds were placed in 80 mm Petri dishes, lined with filter papers saturated with 0, 50, 100, and 150 mM NaCl; three replicates per treatment. A control group of non-inoculated seeds was included for each NaCl concentration for comparison. The sealed Petri dishes were then incubated at 25 ± 1 °C in the dark under controlled conditions [20].

Germination metrics, including germination percentage (GP), mean germination time (MGT), mean germination rate (R), and germination vigor index (GVI), were calculated following standard procedures [21–23]:

$$GP\ (\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

undefined

$$\text{MGT (Days)} = \frac{\Sigma \text{ Number of seeds newly germinated at time ti} \times \text{Time of observation (ti)}}{\text{Total number of germinated seeds}}$$

$$R\,(Seeds/day) = \frac{\text{Total number of germinated seeds}}{\text{Time period of observation}}$$

$$GVI = (\text{Mean root length} + \text{Mean shoot length}) \times \text{GP}$$

Following the germination period, the shoot and root lengths (SL and RL, respectively) were measured using a caliper. The seedling fresh weight (FW) and dry weight (DW) were recorded with a high-precision scale (accuracy of 0.0001 g). Seedling dry weight (DW) was recorded after drying in an oven at 70 °C for 48 h. Salinity tolerance of wheat was assessed using stress tolerance indices for shoot length (SLSI), root length (RLSI), seedling fresh weight (FWSI), seedling dry weight (DWSI) and final germination percentage (GSI) by expressing the mean value of the variable in salinized plants relative to its value in the non-salinized control.

Experiment II: greenhouse wheat experiment

To enhance endophyte colonization, the seeds were stirred in 1% carboxymethyl cellulase for 6 h. Uninoculated control seeds were soaked in distilled water containing 20% Tween 80 for the same period. After treatment, seeds were sown in autoclaved soil in pots of 30 cm high and 22 cm in diameter [19]. The soil separates consisted of 68% sand, 14% silt, and 18% clay, with a pH of 7.4 and an electrical conductivity of 1.3 dS m^{-1}. The soil nutrient content included total nitrogen (TN) 2.7 g/kg, phosphorus (P) 650 mg/kg, zinc (Zn) 7 mg/kg, manganese (Mn) 15 mg/kg, copper (Cu) 3 mg/kg, and iron (Fe) 12 mg/kg. The organic matter (OM) content was 3.7%.

After 20 days of growth, the seedlings were sprayed with 10 ml of the fungal spore suspension (10^6 spores/ml) to ensure proper establishment of the endophyte. Salinity treatments (0, 50, 100, and 150 mM NaCl) were then applied by irrigation every 7 days for 60 days [19]. To confirm the presence of inoculated endophytes in wheat plants, the root and leaf samples were surface-sterilized by immersion in 70% ethanol for 1 min, followed by 2% sodium hypochlorite (NaOCl) for 3 min. Afterward, the samples were rinsed three times with sterile distilled water to remove any residual chemicals. The sterilized samples were then cultured on PDA medium to isolate the endophyte. After incubation, fungal growth was observed and isolates were identified morphologically. Molecular verification was also performed by PCR using ITS primers to confirm fungal endophytes, and the sequences were compared with GenBank data. Control plants were processed in the same manner to check for contamination. PCR products were verified for purity using universal primers [24].

The experiment was conducted with three replicates, and the plants were harvested for analysis after 60 days of salinity treatment. Plant height, leaf length and leaf width (LL and LW), and root length (RL) were measured using a ruler. Fresh weight (FW) and dry weight (DW) of leaves were determined using a digital balance with an accuracy of 0.0001 g.

Quantification of photosynthetic pigments and relative water content (RWC)

The chlorophylls and carotenoids were extracted in 80% acetone following the procedure of Arnon (1949) [25]. The plant tissue was homogenized in 80% acetone and centrifuged at 4,000 rpm for 10 min at 4 °C. The supernatant was collected and used for spectrophotometric measurements. Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (ChT), and carotenoids were quantified using a spectrophotometer (CECIL-2501, England) at wavelengths of 663, 645 and 470 nm. Pigment concentrations were calculated using the following equations of Arnon (1949):

$$Chl\ a\ (mg\ g-1\ FW) = 12.7 \times\ A663\ - 2.69 \times\ A64$$

$$Chl\ b\ (mg\ g-1\ FW) = 22.9 \times\ A645\ - 4.68 \times\ A6635$$

$$ChT\ (mg\ g-1\ FW) = 20.2\ \times\ A645 + 8.02\ \times\ A663$$

$$Carotenoids\ (mg\ g-1\ FW) = (1000\ \times\ A470\ -1.82\ \times\ Chl\ a\ -85.02\ \times\ Chl\ b)\ /\ 198$$

RWC was determined by measuring leaf fresh weight (FW), fully turgid fresh weight (TW) after soaking in distilled water for 24 h in the dark, and dry weight (DW) post 48-hour oven drying at 72 °C [26] RWC was calculated using the formula:

$$RWC\ =\ \frac{(\mathrm{FW}-\mathrm{DW})\ \times\ 100}{(\mathrm{TW}-\mathrm{Dw})}$$

Quantification of hydrogen peroxide and malondialdehyde

Approximately 0.2 g of fresh leaf tissue was homogenized with 2 ml of 0.1% trichloroacetic acid (TCA). The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. After centrifugation, 250 µl of the supernatant was mixed with 250 µl of 50 mM phosphate buffer (pH 7.0) and 500 µl of 1 M potassium iodide (KI) reagent. The mixture was incubated in the dark for 1 h. The absorbance of the resulting solution was measured at 390 nm using a spectrophotometer. Hydrogen peroxide (H_2O_2) content was calculated using an extinction coefficient of 0.28 mM^{-1} cm^{-1}.

Malondialdehyde (MDA) content was determined according to the procedure described by Hodges et al. (1999) [27]. Fresh leaf samples (0.5 g) were ground in 5 ml of 1% trichloroacetic acid, after centrifugation (10 min at 10,000 rpm), 500 µl of the extract were added to 2 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid and heated at 95 °C for 30 min. The cooled mixture was centrifuged at 10,000 rpm for 10 min. Absorbance of the samples was read at a wavelength of 532 nm. Using MDA extinction coefficient of 0.155 mM^{-1} cm^{-1}, the malondialdehyde (MDA) concentration was calculated as µmol MDA g^{-1} leaf fresh weight.

Quantification of osmolytes (proline and soluble sugars)

Ninhydrin reagent was prepared by dissolving 1.25 g ninhydrin in a mixture of 30 ml glacial acetic acid and 20 ml phosphoric acid with gentle warming. The reagent was stored at 4 °C for 24 h. Fresh leaf tissue (0.5 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid, and filtered through Whatman filter paper. An aliquot of 2 ml of the filtrate was reacted with 2 ml glacial acetic acid and 2 ml of the acid-ninhydrin reagent. The mixture was heated at 100 °C for 1 h, cooled in an ice bath to halt the reaction, vortexed for 15–20 s, and warmed to room temperature [28]. Absorbance of the reaction mixture was measured at 520 nm. To calculate proline content, a standard curve was generated using proline concentrations of 0–0.5 mM.

Total soluble sugars were extracted by homogenizing about 0.5 g of fresh leaf tissue in 10 ml of 80% ethanol and incubating at 80 °C for 15 min in a water bath. The extract was then filtered through Whatman filter paper, and the filtrate was centrifuged at 4,000 rpm for 10 min at 4 °C. Total soluble sugars were quantified using the anthrone method Yemm and Willis, (1954) [29]. Anthrone reagent was prepared by dissolving 150 mg anthrone in 100 ml of 72% sulfuric acid. The reaction mixture, containing 50 µl of the alcoholic extract and 950 µl deionized water, was mixed with 2000 µl of the anthrone reagent in an ice bath; the mixture was incubated at 100 °C for 3 min, and cooled to room temperature. Absorbance of the samples was measured at 625 nm, and quantification of total soluble sugars was performed with reference to a standard curve of glucose in the range of 0–0.5 mg/ml [29].

Assay of phenolic and flavonoid contents

Methanolic extract was prepared by macerating 0.5 g fresh leaves in 3 ml of 85% methanol for 24 h at room temperature (approximately 25 °C), followed by centrifugation at 13,000 rpm for 10 min. Total phenolic content, total flavonoid content and antioxidant activity of the extract were determined using the methods described by Singleton and Rossi (1965) [30] with minor modifications. The total phenolic content was determined using the Folin-Ciocalteu method. Briefly, 100 µl of the methanolic leaf extract was mixed with 2.9 ml distilled water and 100 µl of Folin-Ciocalteu reagent. After 5 min, 300 µl of 20% sodium carbonate was added, and the mixture was incubated at room temperature for 30 min. The absorbance of the reaction mixture was then measured at 750 nm using a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (µg $GAEg^{-1}$ FW), based on a standard curve of gallic acid in the range of 0–0.5 mg/ml [30].

Flavonoid concentration was determined in the methanolic extract using the aluminum chloride colorimetric assay. A mixture of 200 µl of the methanolic extract, 600 µl of 85% methanol, 40 µl of 10% aluminum chloride, 40 µl of 1 M potassium acetate and 1120 µl distilled water was incubated in darkness for 30 min. Absorbance of the reaction mixture was read at 415 nm and flavonoid concentration was expressed as mg quercetin equivalents (QE) per g leaf fresh weight with reference to a standard curve of quercetin in the range of 0–0.5 mg/ml [31].

The antioxidant activity of the methanolic leaf extract was monitored by assay of the DPPH scavenging activity [30]. The reaction mixture, containing 30 µl of the methanolic extract and 1170 µl of 0.1 mM methanolic DPPH, was incubated in darkness for 30 min at room temperature, and absorbance was measured at 517 nm.

The Radical scavenging activity (RSA) was calculated using the following equation:

$$\mathrm{RSA} = \left(\frac{\mathrm{Ac} - \mathrm{As}}{\mathrm{Ac}}\right) \times 100$$

Where A_c is the absorbance of the DPPH solution without leaf extract and A_s, is the absorbance of the DPPH solution with leaf extract.

Assay of antioxidant enzyme activities

Leaf extract was prepared by homogenizing fresh leaf tissue (0.5 g) in 5 ml of 50 mM potassium phosphate buffer (pH 7.8) using a mortar and pestle. The homogenate was then centrifuged at 13,000 rpm for 15 min at 4 °C, and the supernatant used for enzyme assays.

Catalase (CAT) activity was determined following the procedure of Aebi (1984) [32], by measuring the decrease in absorbance at 240 nm due to H_2O_2 decomposition. CAT activity was expressed as μmol H_2O_2 g^{-1} FW min^{-1}. The content of hydrogen peroxide (H_2O_2) was calculated by applying an extinction coefficient of 0.28 mM^{-1} cm^{-1} for accurate quantification.

Peroxidase (POD) activity was measured according to the method of Kar and Mishra (1976) [33] using guaiacol as a substrate. The increase in absorbance at 470 nm was monitored. POD activity was expressed as the increase in absorbance (ΔA_{470} mg^{-1} protein min^{-1}).

Polyphenol oxidase (PPO) activity was measured using the method of Ghorbanli [34], by monitoring the oxidation of catechol at 420 nm. PPO activity was expressed as the change in absorbance (ΔA_{420} mg^{-1} protein min^{-1}).

Ascorbate peroxidase (APX) activity was determined following Nakano and Asada (1981) [35], by measuring the reduction of H_2O_2 at 290 nm. APX activity was expressed as μmol H_2O_2 g^{-1} FW min^{-1}. The calculation was based on a standard curve for H_2O_2.

For all enzyme assays, protein concentration was determined using the Bradford method [36], with a standard curve prepared using bovine serum albumin (BSA).

Statistical analysis

All the experiments were conducted using a completely randomized design (CRD) with three replicates. The response of *P. chrysogenum* to salinity stress was analyzed using one-way ANOVA, while the interaction of salinity stress and *P. chrysogenum* on wheat germination and vegetative growth were assessed using two-way ANOVA. Prior to ANOVA, germination percentage (GP) of wheat seeds was arcsine transformed to ensure normality. Data analysis was performed using SAS 9.4 software, with mean comparisons conducted using Duncan's Multiple Range Test at a $p < 0.05$. Principal component analysis (PCA), hierarchical cluster analysis, and Pearson correlation analysis were conducted using R software (www.r-project.org).

Results and discussion

Salinity tolerance of *P. chrysogenum* CM022

Increasing salinity in the culture medium progressively reduced colony diameter of *P. chrysogenum* CM022. However, the fungus can tolerate salinity levels up to 100 mM NaCl with appreciable growth (Fig. 1). This is consistent with previous studies showing that salinity disrupts cellular processes such as nutrient uptake, enzyme activity, and osmotic balance in fungi, leading to reduced growth rates [4]. Despite this reduction, the ability of *P. chrysogenum* CM022 to tolerate salinity levels up to 100

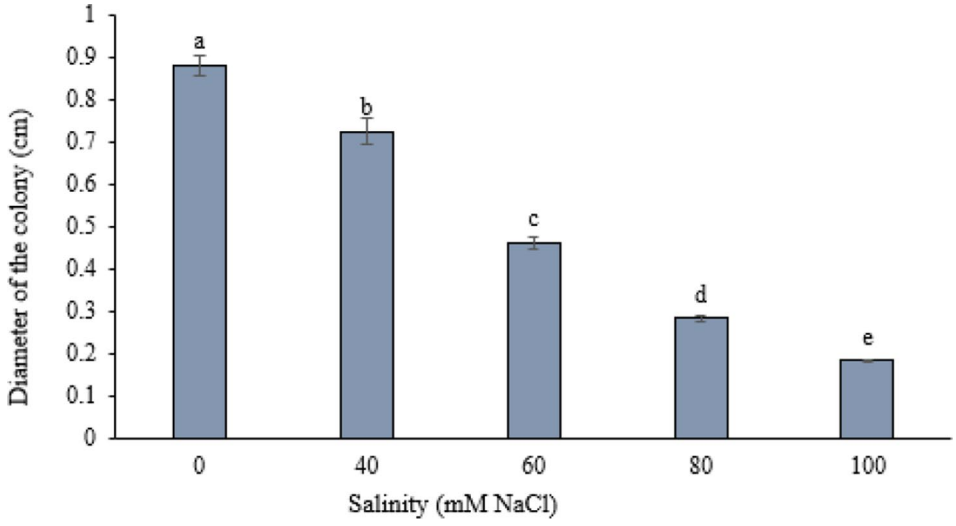

Fig. 1 Salinity tolerance of *Penicillium chrysogenum* CM022 across 0, 40, 60, 80 and 100 mM NaCl in PDA medium. Each column represents the mean of three replicates ± SD. Similar letters indicate non-significant differences at $P^{<}0.05$

mM NaCl highlights its potential as a halotolerant endophyte. Such resilience suggests the presence of adaptive mechanisms, such as activating antioxidant defense systems to counteract the harmful effects of ROS produced under salinity stress [31, 37]. Our findings align with those of Galeano et al. (2023) [37], who reported similar salinity tolerance in *Penicillium chrysogenum* strain 34-P isolated from saline environments, showing reduced colony diameter but sustained growth under high salinity conditions. Similarly, Adedayo and Babalola (2023) [38] found that *Aspergillus* species could maintain viability and even promote plant growth under salinity stress, emphasizing the role of fungal endophytes in supporting plant adaptation to saline environments.

Experiment I: wheat germination and seedling growth under salt stress

Seed germination under in vitro conditions

Inoculation of wheat seeds with *P. chrysogenum* CM022 improved germination percentage (GP) and alleviated the impact of salinity on seed germination. Maximal GP (100%) was observed in seeds inoculated with *P. chrysogenum* CM022 both in absence of salinity stress and at 50 mM NaCl, with non-significant reduction at higher salinities; this is in contrast to the lowered GP and the progressive salinity-induced reduction in non-inoculated seeds (Fig. 2a). These findings are consistent with previous studies that highlighted the beneficial role of fungal endophytes in mitigating the adverse effects of salinity stress on seed germination [39, 40].

The mean germination time (MGT) of inoculated seeds was significantly lowered by about 24% compared to non-inoculated seeds across all salt concentrations (Fig. 2b). This reduction in MGT indicates that the endophyte can accelerate wheat seed germination under salinity stress, which is consistent with studies showing that fungal endophytes can enhance seedling vigor and germination speed under stress conditions [41, 42]. Moreover, the mean germination rate (MGR) was significantly higher in seeds inoculated with *P. chrysogenum* CM022 compared to non-inoculated seeds (Fig. 2c). These findings align with previous research showing that fungal endophytes enhance germination dynamics under stress by improving water uptake, nutrient availability, and enzymatic activity [4, 43]. The accelerated germination of endophyte-treated seeds may result from the alleviation of osmotic stress, enhanced ion homeostasis, and modulation of oxidative stress, facilitating faster metabolic activation and embryo development [44].

Morphological traits and germination Vigor index (GVI)

The improvement in plant height and root length of wheat by inoculation with *P. chrysogenum* CM022 was especially pronounced under stress conditions and

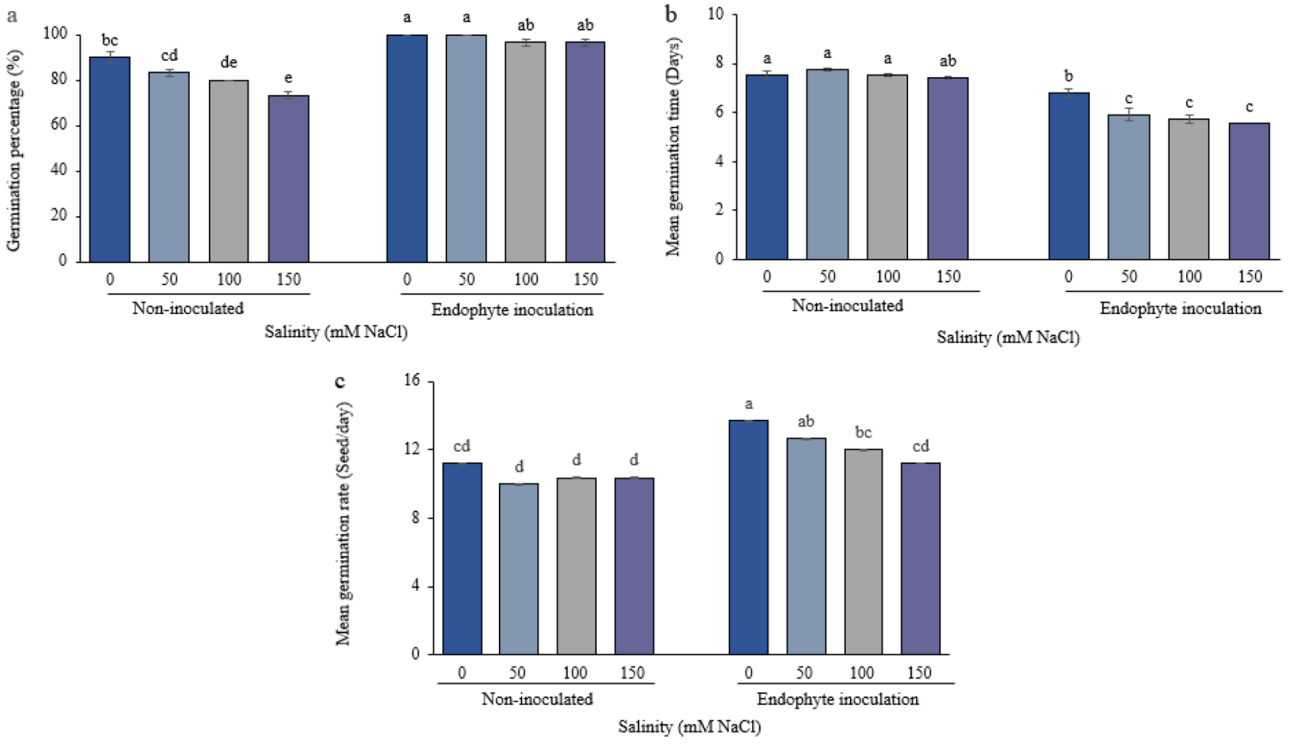

Fig. 2 Effect of *Penicillium chrysogenum* CM022 inoculation on response of wheat germination to salinity of 0, 50, 100, and 150 mM NaCl in terms of (**a**) final germination percentage (**b**) mean germination time (**c**) mean germination rate. Each column represents the mean of three replicates ± SD. Means with the same letter are not significantly different at $P^{<}0.05$

amounted to 57.5% and 169%, respectively under 150 mM NaCl compared to non-inoculated plants (Table 1). Likewise, seedling fresh weight (FW) and dry weight (DW) were enhanced by *P. chrysogenum* CM022 inoculation, particularly under 150 mM NaCl stress, with increases of 400% and 31%, respectively above non-inoculated seedlings (Table 1). These results emphasize the beneficial effects of fungal inoculation, particularly under stress, and align with previous studies reporting positive impacts of endophytes on plant growth under salinity stress [45, 46]. For example, Khan et al. (2022) [36] observed significant increases in root and shoot growth of *Triticum aestivum* inoculated with *Aspergillus* species under 100 mM NaCl stress. Similarly, Sreevidya et al. (2015) [47] found that inoculation with *Penicillium* species enhanced root development and biomass accumulation in *Cicer arietinum* under salt stress, likely due to phytohormone production and improved water uptake. The endophyte likely supports seedling establishment by enhancing stress tolerance mechanisms [4].

The highest GVI was observed in seeds inoculated with *P. chrysogenum* CM022 without salt stress (Table 1). Pandya et al. (2015) [48] reported that inoculation with *Penicillium* species significantly enhanced the GVI of *Vigna radiata* seedlings under normal conditions, likely through the production of plant growth regulators like gibberellins, which promote early seedling growth. Similarly, Estévez et al. (2020) [49] found that *Trichoderma asperellum* T34 increased the GVI of *Zea mays* seedlings by improving root development and enhancing nutrient and water uptake, which are crucial for seedling establishment [49].

Salinity tolerance indices: in vitro

The salinity tolerance index is employed to quantify plant resilience to saline conditions, a critical assessment in agriculture for selecting salt-tolerant plant species and cultivars [50]. Salinity stress leads to a progressive decrease in plant growth, with reductions in both SLSI and RLSI as salinity levels increased. This decrease reflects the negative impact of salinity on plant development. However, inoculation with *P. chrysogenum* CM022 alleviated these detrimental effects, leading to significant improvements in both shoot and root lengths compared to non-inoculated plants. For example, at 150 mM NaCl, the stress tolerance indices based on shoot length (plant height) (SLSI) and root length (RLSI) of inoculated plants were 47.8% and 152.7%, respectively higher than those of non-inoculated plants (Fig. 3a, b). Similarly, the fresh weight-salinity tolerance index (FWSI) and dry weight-salinity tolerance index (DWSI) were enhanced with fungal inoculation by 62% and 136%, respectively under 150 mM NaCl stress (Fig. 3c, d). Inoculation of wheat seeds with *P. chrysogenum* CM022 improved the germination percentage index (GPI) and reduced the effect of salinity on seed germination compared to non-salinity stress (Fig. 3e). These findings highlight the ability of *P. chrysogenum* CM022 inoculation to improve seedling growth and biomass accumulation of wheat under salinity stress, suggesting its potentiality as a biostimulant for enhancing stress tolerance in saline environments.

Principal component analysis and correlations of germination data

A significant positive correlation was observed between GP, root length (R), and seedling fresh weight (FW) (Fig. 4a). The PCA analysis (Fig. 4b) further highlighted the beneficial role of *P. chrysogenum* CM022 inoculation in enhancing seed germination and seedling growth under salinity stress. Under 100 mM NaCl, inoculation with *P. chrysogenum* CM022 positively correlated with GP, R, and seedling FW, demonstrating its ability to improve these parameters under high salinity stress. Similarly, under low salinity (50 mM NaCl), inoculation showed a positive association with GVI, plant height, and root length, emphasizing its beneficial effects in both low

Table 1 Effect of the endophytic fungus *P. chrysogenum* CM022 in alleviation of salt stress on morphological traits of wheat seedlings under in vitro conditions. Each value is the mean of three replicates ± se

Fungal inoculum and salinity (mM NaCl)	Plant height (cm)	Root length (cm)	Seedling FW (g/seedling)	Seedling DW (g/seedling)	GVI (%)
Non-inoculated					
0	6.10 ± 0.21^{bc}	3.01 ± 0.15^{b}	0.122 ± 0.007^{cd}	0.0395 ± 0.00^{b}	891 ± 0.027^{c}
50	2.44 ± 0.30^{d}	2.20 ± 0.13^{c}	0.105 ± 0.001^{d}	0.0272 ± 0.000^{d}	417 ± 0.032^{e}
100	1.53 ± 0.10^{de}	1.05 ± 0.12^{d}	0.097 ± 0.000^{d}	0.0374 ± 0.000^{c}	223 ± 0.027^{g}
150	1.13 ± 0.13^{e}	0.39 ± 0.014^{e}	0.027 ± 0.001^{e}	0.0531 ± 0.000^{ab}	126 ± 0.054^{h}
Endophyte inoculation					
0	9.28 ± 0.70^{a}	6.78 ± 0.67^{a}	0.305 ± 0.012^{a}	0.0397 ± 0.001^{b}	1498 ± 0.072^{a}
50	7.07 ± 0.41^{b}	6.17 ± 0.51^{a}	0.293 ± 0.017^{a}	0.0399 ± 0.001^{b}	1324 ± 0.054^{b}
100	5.06 ± 0.20^{c}	3.03 ± 0.31^{b}	0.223 ± 0.0007^{b}	0.0386 ± 0.000^{bc}	809 ± 0.027^{d}
150	1.78 ± 0.15^{de}	1.05 ± 0.05^{d}	0.135 ± 0.0005^{c}	0.0696 ± 0.017^{a}	273 ± 0.072^{f}

GVI: Germination vigor index. Similar letters indicate non-significant differences at P ˂0.05

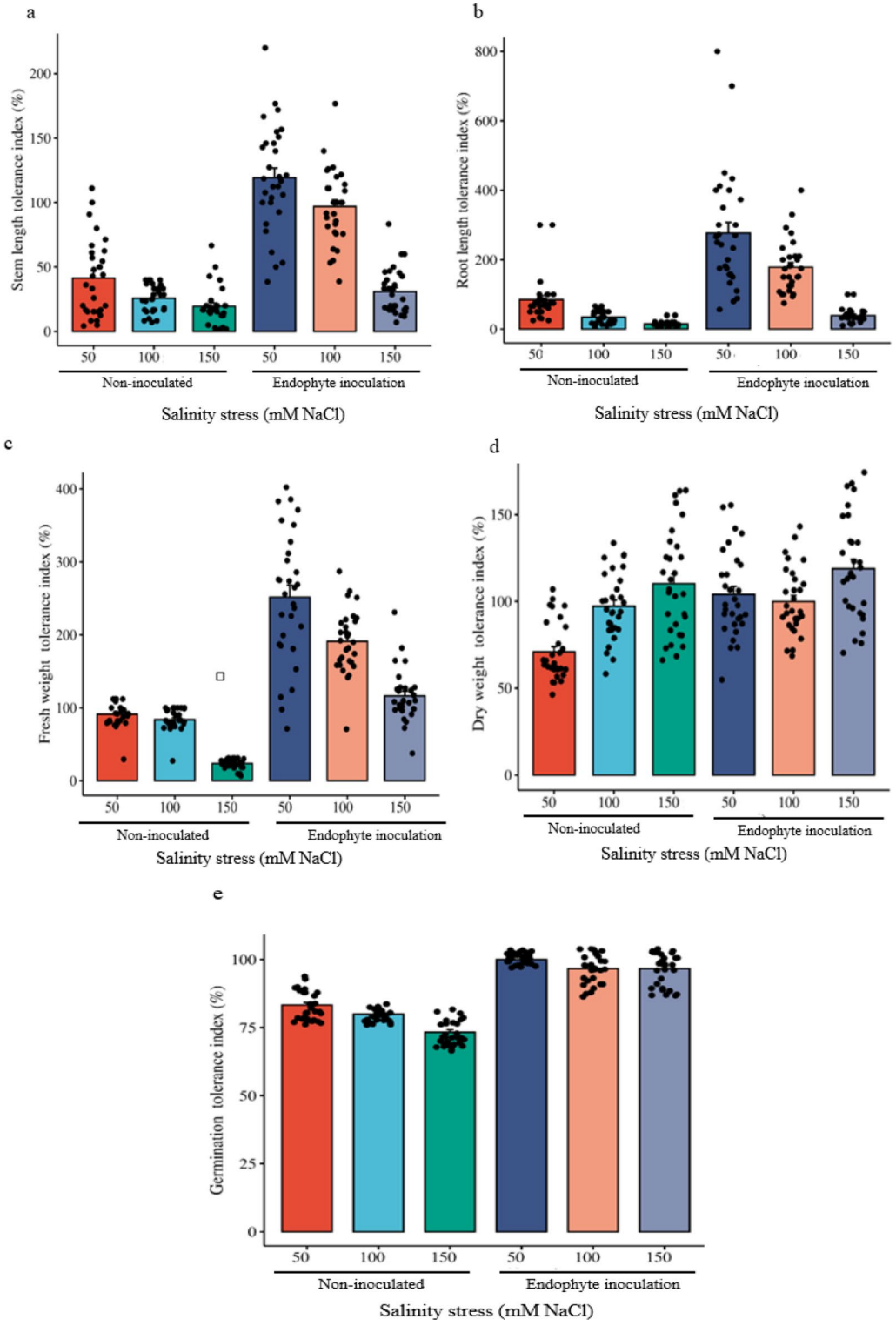

Fig. 3 Effect of *P. chrysogenum* CM022 inoculation on salinity tolerance indices of wheat seedlings: (**a**) stem length tolerance index, (**b**) root length tolerance index, (**c**) fresh weight stress tolerance index, (**d**) dry weight stress tolerance index and (**e**) germination tolerance index

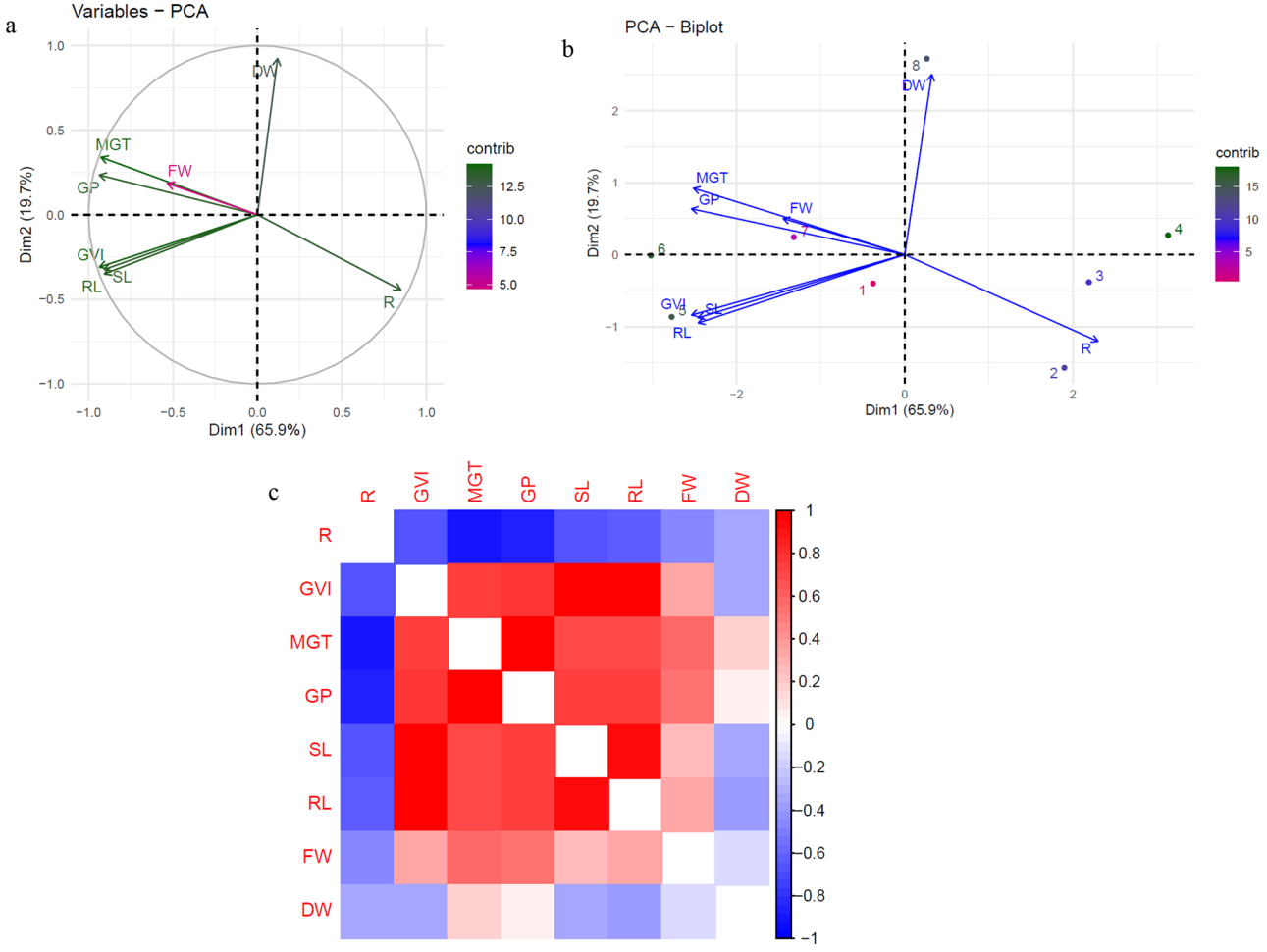

Fig. 4 (**a**) PCA of growth and germination traits of wheat, (**b**) Relationship analysis of wheat growth and germination traits under control and stress conditions, (**c**) Heatmap of Pearson correlation coefficients. Clustering distance is shown on the y-axis, with red and blue indicating positive and negative correlations, respectively. R: mean germination rate, GVI: germination vigor index, MGT: mean germination time, GP: germination percentage, SL: stem length, RL: root length, FW: fresh weight, DW: dry weight

and high salinity conditions. These results underscore the potential of *P. chrysogenum* CM022 as a strategy to improve seed germination, seedling growth, and vigor indices in salinity-stressed wheat plants.

Experiment II: wheat growth and biochemical traits under greenhouse conditions

Wheat growth

Salinity stress generally reduced wheat growth, but fungal inoculation improved it, particularly under the impact of salinity. The greatest improvements were observed in plant height and leaf width (LW) at 50 mM NaCl, in LL and seedling FW at 100 mM NaCl, and in RL and seedling DW at 150 mM NaCl. Specifically, at 150 mM NaCl, inoculation with *P. chrysogenum* CM022 increased plant height, LL, LW, RL, FW, and DW by 16.6%, 19%, 52%, 116%, 82.4% and 87.7%, respectively (Table 2). Fungal inoculation can enhance plant growth through various mechanisms, such as improving nutrient availability and modulating stress responses [51–53]. In this study, *P. chrysogenum* CM022 alleviated some of the detrimental effects of salinity stress, leading to improved root length (RL), plant height, and dry weight (DW) in inoculated wheat plants. Significant improvements were particularly evident at high salinity levels, such as 150 mM NaCl, highlighting the fungal ability to mitigate salinity-induced stress and enhance resource allocation for better growth and survival. This finding aligns with Oljira et al. (2020) [54], who reported improved root and shoot growth and biomass accumulation in *Triticum aestivum* under salinity stress following inoculation with *Trichoderma* spp. Similarly, Manjunatha et al. (2022) [19] demonstrated that *Chaetomium globosum* promoted root and shoot growth of wheat under NaCl stress.

Table 2 Effect of the endophytic fungus *P. chrysogenum* CM022 in alleviation of salt stress on morphological traits of wheat seedlings under *greenhouse* conditions. Each value is the mean of three replicates ± sd

Fungal inoculum and salinity (mM NaCl)	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Seedling FW (g/seedling)	Seedling DW (g/seedling)
Non-inoculated						
0	13.53 ± 0.60^{bc}	37.33 ± 2.30^{cd}	0.50 ± 0.047^{bc}	11.83 ± 0.19^{e}	0.332 ± 0.008^{cd}	0.0470 ± 0.002^{c}
50	12.00 ± 0.47^{c}	36.33 ± 0.27^{cd}	0.36 ± 0.027^{c}	11.47 ± 0.21^{e}	0.224 ± 0.002^{f}	0.0432 ± 0.0005^{c}
100	13.66 ± 0.72^{b}	34.00 ± 1.24^{d}	0.43 ± 0.027^{c}	12.26 ± 0.31^{e}	0.260 ± 0.008^{ef}	0.0469 ± 0.0005^{c}
150	14.00 ± 0.27^{bc}	34.33 ± 1.18^{d}	0.50 ± 0.000^{bc}	12.83 ± 0.11^{e}	0.301 ± 0.001^{de}	0.0519 ± 0.0008^{bc}
Endophyte inoculation						
0	14.66 ± 0.35^{b}	39.33 ± 1.15^{bcd}	0.66 ± 0.072^{ab}	14.49 ± 0.64^{d}	0.417 ± 0.010^{b}	0.0752 ± 0.010^{ab}
50	16.33 ± 0.27^{a}	45.00 ± 0.000^{ab}	0.70 ± 0.027^{a}	19.09 ± 0.19^{c}	0.241 ± 0.012^{ef}	0.0581 ± 0.001^{bc}
100	17.10 ± 0.47^{a}	48.00 ± 0.82^{a}	0.73 ± 0.027^{a}	22.40 ± 0.47^{b}	0.526 ± 0.029^{a}	0.0715 ± 0.012^{b}
150	16.33 ± 0.27^{a}	40.86 ± 2.80^{bc}	0.76 ± 0.072^{a}	27.77 ± 0.77^{a}	0.549 ± 0.042^{a}	0.0974 ± 0.006^{a}

Similar letters indicate non-significant differences at P ˂0.05

Table 3 Effect of the endophytic fungus *P. chrysogenum* CM022 in alleviation of salt stress on photosynthetic pigments and relative water content of wheat seedlings under greenhouse conditions. Each value is the mean of three replicates ± sd

Fungal inoculum and salinity (mM NaCl)	Chl a (mg /g FW)	Chl b (mg /g FW)	Chl T (mg /g FW)	Chl a/Chl b ratio	Carotenoids (mg /g FW)	RWC (%)
Non-inoculated						
0	1.48 ± 0.025^{c}	0.80 ± 0.021^{de}	2.34 ± 0.038^{c}	1.72 ± 0.048^{bc}	2.28 ± 0.069^{b}	51.28 ± 0.64^{c}
50	1.45 ± 0.014^{c}	0.81 ± 0.068^{e}	2.26 ± 0.021^{c}	1.79 ± 0.044^{b}	1.54 ± 0.025^{e}	27.69 ± 1.98^{d}
100	1.28 ± 0.030^{d}	0.76 ± 0.005^{e}	2.04 ± 0.046^{d}	1.68 ± 0.046^{c}	1.37 ± 0.014^{ef}	27.04 ± 0.63^{d}
150	1.14 ± 0.027^{d}	0.66 ± 0.15^{f}	1.80 ± 0.041^{c}	1.72 ± 0.043^{bc}	1.18 ± 0.024^{f}	24.71 ± 0.72^{d}
Endophyte inoculation						
0	2.44 ± 0.020^{a}	1.61 ± 0.042^{a}	4.05 ± 0.030^{a}	1.51 ± 0.016^{d}	3.35 ± 0.11^{a}	81.61 ± 2.39^{a}
50	1.75 ± 0.033^{b}	0.91 ± 0.010^{c}	2.66 ± 0.010^{bc}	1.92 ± 0.014^{a}	2.28 ± 0.037^{b}	65.16 ± 0.99^{b}
100	1.62 ± 0.007^{bc}	1.16 ± 0.053^{b}	2.78 ± 0.049^{b}	1.39 ± 0.016^{b}	2.75 ± 0.051^{c}	66.24 ± 1.48^{b}
150	1.55 ± 0.094^{c}	0.86 ± 0.007^{cd}	2.35 ± 0.14^{c}	1.93 ± 0.062^{a}	2.50 ± 0.10^{d}	25.82 ± 1.51^{d}

Similar letters indicate non-significant differences at P ˂0.05

Plant physiology

Photosynthetic pigments

The effect of salinity stress on photosynthetic pigments varied between inoculated and non-inoculated plants. In non-inoculated wheat plants, salinity stress led to a significant reduction in Chl a and Chl b contents, particularly at high salinity levels (100 and 150 mM NaCl). While inoculated plants exhibited overall higher pigment content under both control and stress conditions, the relative reductions in Chl a and Chl b due to salinity were more pronounced compared to non-inoculated plants. Increasing salinity beyond 50 mM up to 150 mM NaCl reduced Chl a and Chl b by 21.4% and 18.5% in non-inoculated plants; whereas in inoculated plants, the reductions were progressive and amounted to 36.5% and 46.6%, respectively across the whole range of salinity. Despite the relatively severe decline in pigment content of inoculated plants, the concentrations of Chl a and Chl b remained higher in inoculated plants than in non-inoculated ones under stress, highlighting the protective role of *P. chrysogenum* CM022. A contrasting trend was observed in carotenoid content, where salinity stress led to a greater reduction in non-inoculated plants than in inoculated ones. Increasing salinity from 0 to 150 mM NaCl resulted in 48.2% reduction in carotenoid content of non-inoculated plants, whereas the reduction was only 25.4% in inoculated plants. The higher carotenoid retention in inoculated plants under stress suggests a crucial role of fungal inoculation in enhancing stress tolerance [55]. Compared to non-inoculated plants, *P. chrysogenum* CM022 inoculation increased the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl T), and carotenoids by 36%, 30.3%, 30.6%, and 112%, respectively, under 150 mM NaCl stress (Table 3). This aligns with Akhtar et al. (2020) [56], who found that inoculation with *Aspergillus terreus* significantly increased chlorophyll and carotenoid contents in *Solanum lycopersicum* under salt stress. Similarly, *Penicillium pinophilum* inoculation mitigated salinity-induced reductions in photosynthetic pigments in *Lactuca sativa*, thereby improving growth and stress tolerance [55].

Relative water content (RWC)

Inoculation with *P. chrysogenum* CM022 increased relative water content (RWC) of wheat leaves, and the effect was more pronounced under mild to moderate salinity

(50 and 100 mM NaCl) with an average increase of 140% above non-inoculated plants than either absence of stress (only 59% increase) or 150 mM NaCl (with almost no benefit). (Table 3). The salinity-induced lowering in RWC of leaves was less severe in inoculated relative to non-inoculated plants and amounted to 51.8% and 68.4%, respectively across the whole range of salinity. The relative water content (RWC) of wheat leaves dropped to about 25% in both inoculated and non-inoculated plants under 150 mM NaCl stress. Endophytic fungi can enhance antioxidant activity, thereby protecting plants from oxidative damage and indirectly improving leaf water status [57]. For example, an endophyte isolate of *Yarrowia lipolytica* was found to enhance RWC in *Zea mays* under salinity stress [58].

Oxidative stress markers

H_2O_2 levels in wheat leaves were progressively increased with increasing salinity particularly in the non-inoculated plants, reflecting enhanced oxidative damage. Inoculation with *P. chrysogenum* CM022 effectively mitigated this effect, particularly under the impact of salinity, where the reduction in H_2O_2 content amounted to 77% below non-inoculated plants at 150 mM NaCl (Fig. 5a). Bashyal et al. (2021) [59] observed that *Trichoderma harzianum* inoculation reduced H_2O_2 content in wheat leaves, enhancing the plant's oxidative stress response. Similarly, Ismail et al. (2020) [60] reported a significant reduction in H_2O_2 levels in *Helianthus annuus* and *Glycine max* when inoculated with *Rhizopus oryzae*, highlighting the role of endophytes in mitigating oxidative stress.

MDA content of leaves in both inoculated and non-inoculated plants exhibited a transient reduction at 50 mM NaCl, followed by an increase to a level comparable to that of control upon further increase in salinity up to 150 mM NaCl. Interestingly, the lowest MDA content observed at moderate salinity (50 mM NaCl) suggests a possible acclimation response, where plants activate protective mechanisms to limit lipid peroxidation, and inoculation with *P. chrysogenum* CM022 resulted in lower MDA levels across all salinity treatments compared to non-inoculated plants, further highlighting the potential role of the endophyte in mitigating oxidative stress. At 100 and 150 mM NaCl, MDA levels in inoculated plants were decreased by 79% and 41.7%, respectively below non-inoculated plants, indicating the endophyte's role in mitigating oxidative damage on cell membranes under salinity stress (Fig. 5b). Endophytes help reduce lipid peroxidation and MDA levels in membranes, likely through the production of secondary metabolites like flavonoids,

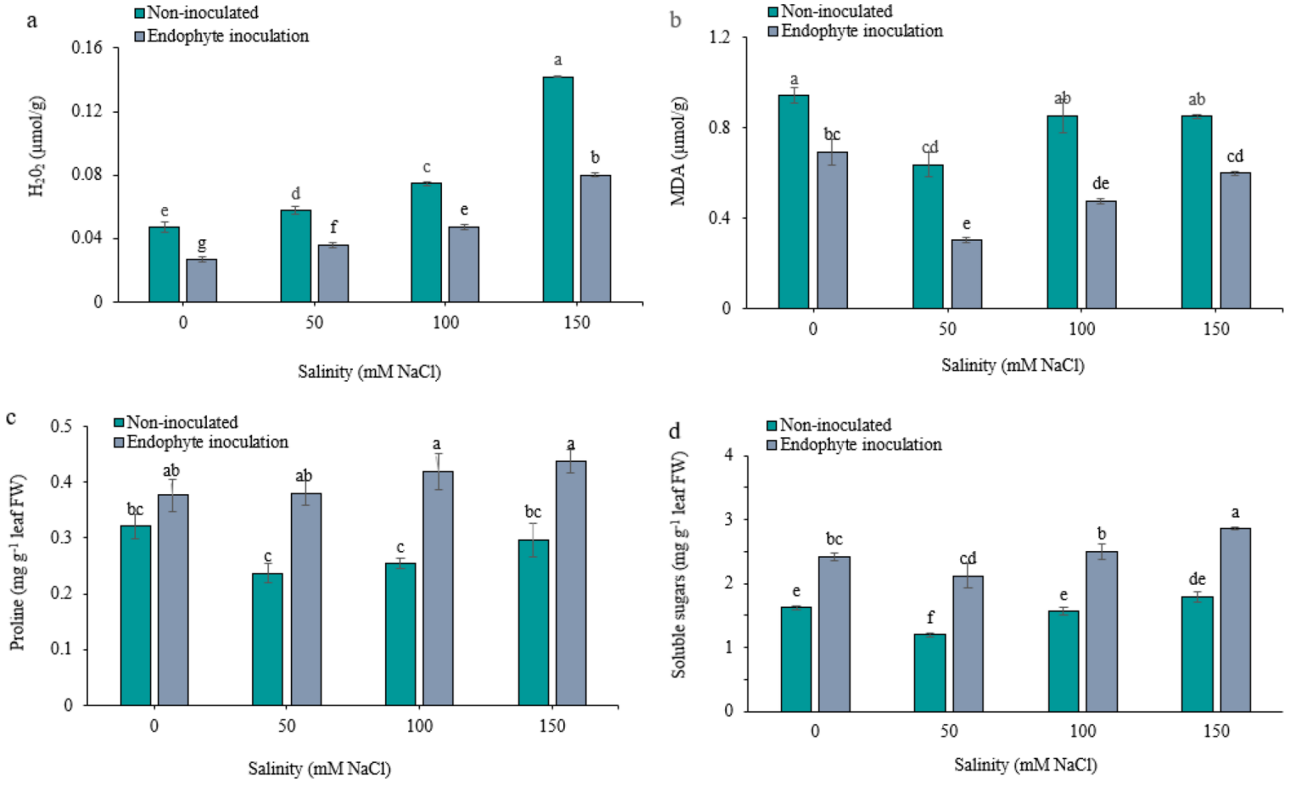

Fig. 5 Effect of wheat inoculation with *P. chrysogenum* CM022 on plant performance under the impact of salinity stress. (**a**) hydrogen peroxide, (**b**) malondialdehyde, (**c**) proline and (**d**) soluble sugars. Each column represents the mean of 3 replicates ± SD. Similar letters indicate non-significant differences at $P<0.05$

phenolics, and anthocyanins, which possess antioxidant properties and lower ROS levels under salinity stress [61]. Studies have shown that salinity stress increases MDA levels in plant leaves, but inoculation with mycorrhizae and specific fungal endophytes, such as *Penicillium funiculosum*, *Fusarium oxysporum* (in corn), and *P. chrysogenum* (in wheat), can alleviate oxidative damage caused by salinity stress [37, 62].

Proline and soluble sugars

Salinity stress mildly increased proline content of wheat leaves, and the increase was particularly evident in inoculated plants. Therefore, inoculation with *P. chrysogenum* CM022 can significantly enhance the plant's ability to cope with salinity stress, leading, for example, to 36% increase in proline content above non-inoculated plants receiving 150 mM NaCl (Fig. 5c). Proline acts as an osmoprotectant, stabilizing cellular structures and scavenging ROS under stress conditions [63]. Salinity stress increased soluble sugar content in wheat leaves, with the most notable improvement in inoculated plants. Inoculation with *P. chrysogenum* CM022 enhanced soluble sugar levels at all salinity levels (Fig. 5d), suggesting their role in maintaining osmotic balance and providing energy for stress tolerance. This finding supports previous studies on endophytes enhancing osmolyte accumulation under salinity stress. For example, Shahzad et al. (2017) [64] found that *Bacillus subtilis*-inoculated wheat plants showed higher proline and soluble sugar levels, improving stress tolerance. Similarly, Kaboosi et al. (2020) [65] reported reduced oxidative damage markers (e.g., H_2O_2 and MDA) due to endophyte-induced upregulation of osmoprotectants and antioxidant enzymes. The increase in proline and soluble sugar levels with endophyte inoculation may result from the modulation of stress-responsive genes, enhancing the synthesis of these compounds [66]. Furthermore, endophyte-host interactions might regulate ion transporters to maintain ion homeostasis, reducing NaCl toxicity [67].

Phenolics, flavonoids and anthocyanin contents

Salinity stress induced changes in secondary metabolite production and antioxidant capacity in wheat plants. In both non-inoculated and inoculated plants, salinity caused a significant increase in phenolic and flavonoid contents and antioxidant activity with different extents depending on the type of antioxidant. Increasing salinity from 0 to 150 mM NaCl increased phenolic content of leaves by 38.6 and 64.2% in non-inoculated and inoculated plants, respectively. In turn, inoculation with *P. chrysogenum* CM022 increased phenolic content by 20.6% and 42.8% at 0 mM and 150 mM NaCl,

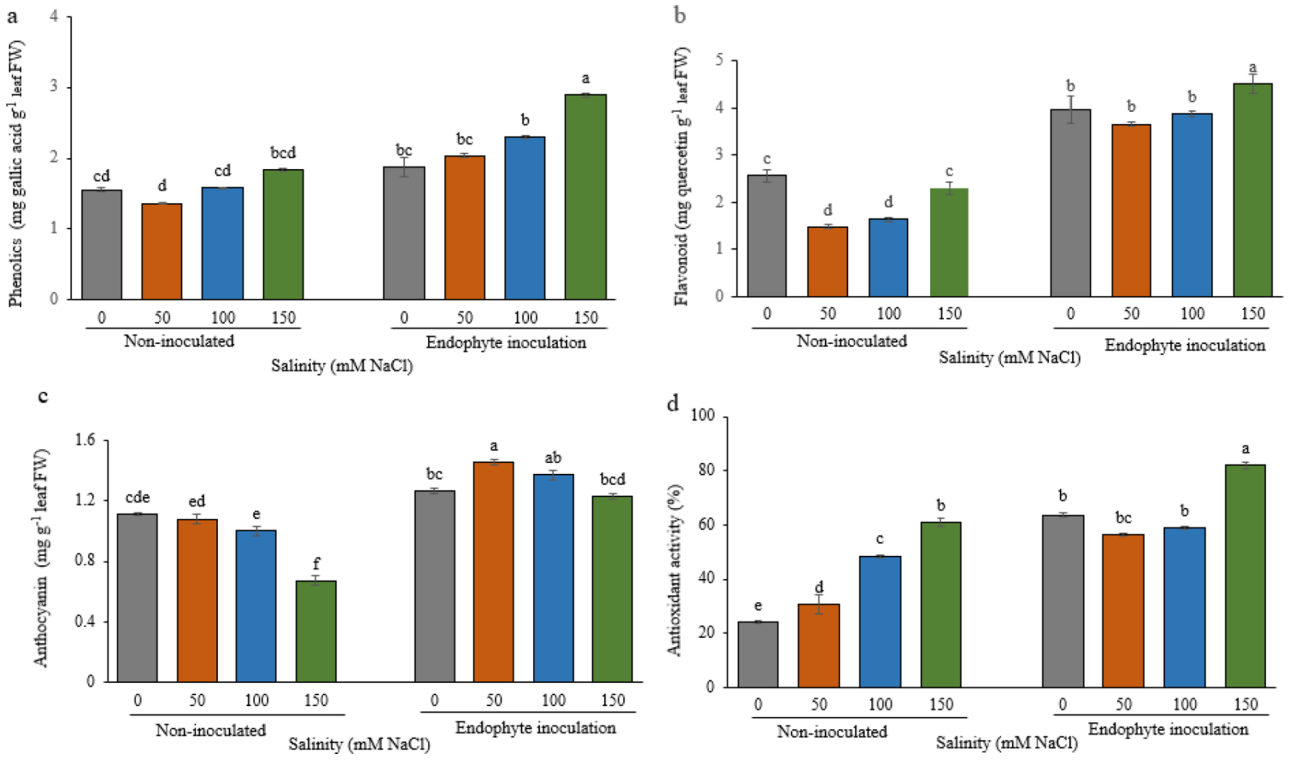

Fig. 6 Effect of *P. chrysogenum* CM022 inoculation on wheat non-enzymatic antioxidant activity under the impact of salinity stress. (**a**) phenolics, (**b**) flavonoids, (**c**) anthocyanin and (**d**) antioxidant activity. Each column represents the mean of 3 replicates ± SD. Similar letters indicate non-significant differences at $P^{<}0.05$

respectively. Under 150 mM NaCl stress, flavonoid content was increased by 111.8% in inoculated plants compared to non-inoculated plants (Fig. 6a, b). In this study, 150 mM NaCl induced an appreciable decrease in anthocyanin content of wheat leaves. But, whereas the decrease was significant and progressive with the increase in salinity in non-inoculated plants, it was mild and occurred post a transient increase at low salinity in inoculated plants (Fig. 6c). High levels of anthocyanins can act as antioxidants, protecting cells from oxidative damage caused by ROS under salt stress [68]. Fungal inoculation enhances plant stress resistance, partly through the production of secondary metabolites that help mitigate oxidative stress [69].

This increase in anthocyanin synthesis is part of a broader plant strategy to cope with saline environments [70]. Anthocyanins serve as antioxidants, aid in light absorption, and protect against UV radiation, supporting the plant's response to abiotic stress [71]. The antioxidant capacity, measured by DPPH scavenging activity, improved with fungal inoculation. The most significant increase in DPPH activity occurred in the absence of salinity (Fig. 6d), but the endophyte also provided protective effects under salinity stress, emphasizing its beneficial role in both conditions. The increased phenolic and flavonoid content in inoculated plants suggests that endophytes activate the phenylpropanoid pathway, enhancing these antioxidant metabolites in plant tissues [71]. Phenolics and flavonoids not only scavenge ROS but also stabilize membranes, protecting against salinity stress [14]. Our findings align with Ait Bessai et al. (2023) [72], who showed increased phenolic accumulation and antioxidant activity in wheat plants inoculated with *Bacillus megaterium* under salinity stress. Additionally, the elevated DPPH-scavenging activity observed here, either by salinity or endophyte inoculation, mirrors Kaboosi et al. (2022) [65], where *Piriformospora indica*-inoculated tomato plants showed reduced oxidative damage due to enhanced antioxidant enzyme activities and secondary metabolite production. This increased antioxidant capacity may also result from endophyte modulation of stress-related gene expression, boosting the synthesis of phenolics and flavonoids [73].

Antioxidant enzyme activities

With the exception of CAT in inoculated plants and APX in non-inoculated plants, salinity induced a marked increase in antioxidant enzyme activity, particularly in inoculated plants. Thus, inoculation with *P. chrysogenum* CM022 further enhanced enzyme activities, leading to 34.9%, 37.4%, 53.9%, and 35.8% increases in CAT, PPO, POD, and APX activities, respectively, compared to

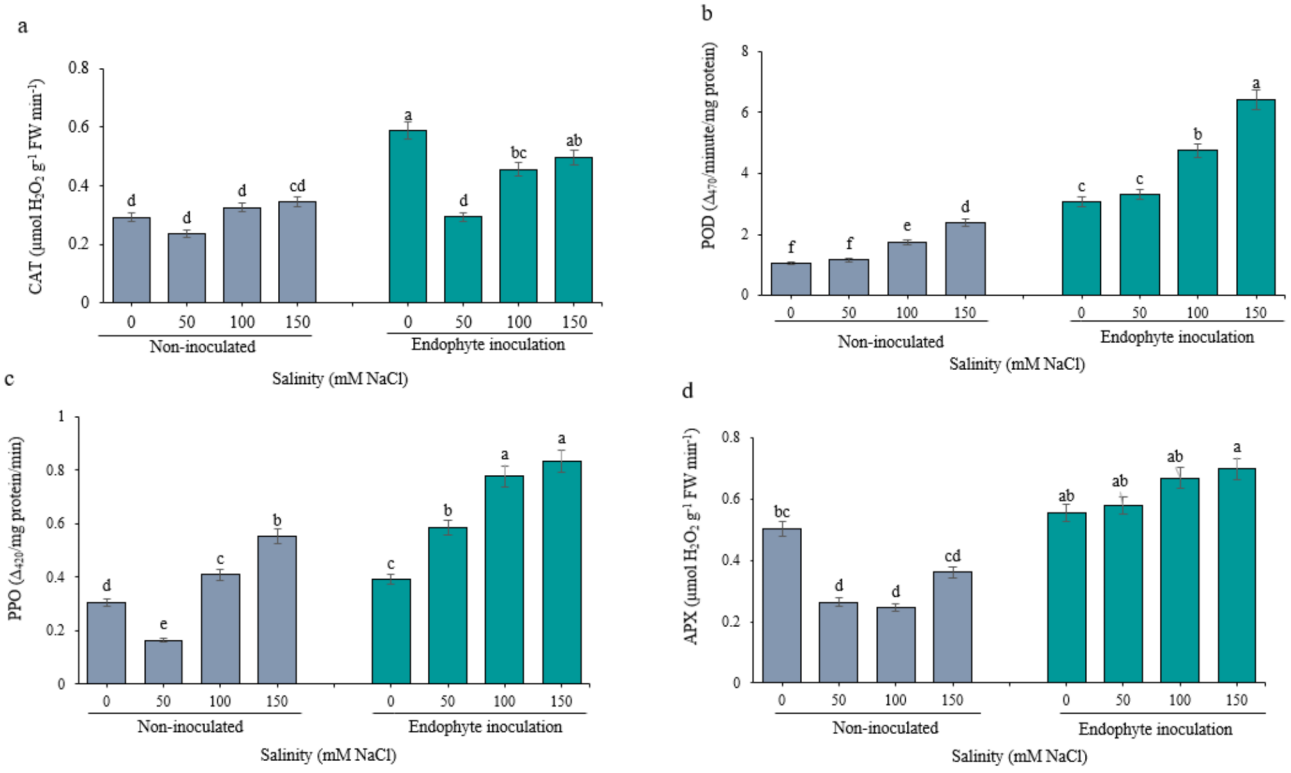

Fig. 7 Effect of *P. chrysogenum* CM022 inoculation on the activity of (**a**) catalase, (**b**) peroxidase, (**c**) polyphenol oxidase, and (**d**) ascorbate peroxidase in wheat leaves under the impact of salinity stress. Each column represents the mean of 3 replicates ± SD. Similar letters indicate non-significant differences at $P<0.05$

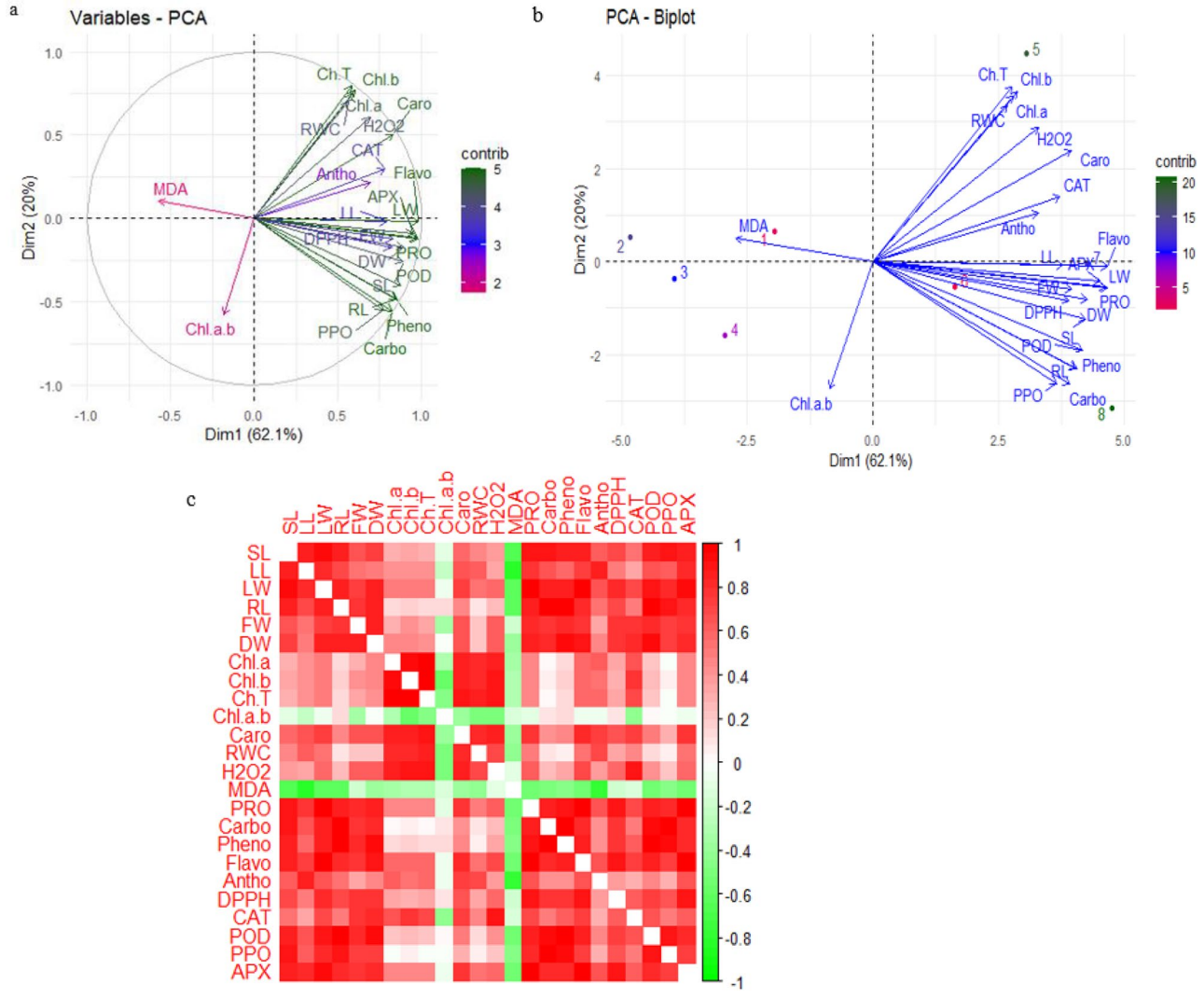

Fig. 8 (**a**) PCA of growth, physiological and biochemical traits of wheat, (**b**) Analysis of wheat growth and performance in relation to endophyte inoculation across stress levels, (**c**) Heatmap of Pearson correlation coefficients. The height axis displays the distance among clusters. The heatmap's y-axis represents clustering distance, with color intensity reflecting the strength of correlation. SL: Stem length, LL: Leaf length, LW: Leaf width, RL: Root length, FW: Fresh weight, DW: Dry weight, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Chl T: Chlorophyll Total, Chl a.b: Chlorophyll a/ Chlorophyll b ratio, RWC: Relative water content of leaf, H_2O_2: Hydrogen peroxide, MDA: Malondialdehyde, PRO: Proline, Carbo: Carbohydrates, Pheno: Phenolics, Flavo: Flavonoids, Antho: Anthocyanin, DPPH: Antioxidant activity, CAT: Catalase, POD: Peroxidase, PPO: Polyphenol oxidase, APX: Ascorbate peroxidase

non-inoculated plants under the same stress at a salinity level of 150 mM NaCl.

This suggests that fungal inoculation plays a critical role in enhancing antioxidant defense against oxidative stress (Fig. 7). The enzymes involved in scavenging reactive oxygen species (ROS) during salinity stress help mitigate oxidative damage and maintain cellular homeostasis. The increase in antioxidant enzyme activities observed with endophyte inoculation can be attributed to the ability of endophytes to modulate the plant's stress response. Catalase (CAT) decomposes hydrogen peroxide (H_2O_2) into water and oxygen, preventing oxidative damage [74]. Peroxidase (POD) and polyphenol oxidase (PPO) catalyze the oxidation of phenolics to quinones, potentially consuming ROS, while ascorbate peroxidase (APX) reduces H_2O_2 into water, further alleviating ROS levels [36]. These enzymatic activities collectively ensure efficient ROS detoxification. Similar results were reported by Shahzad et al. (2017) [64], who found higher CAT and APX activities in wheat plants inoculated with *Bacillus amyloliquefaciens* RWL-1 and *Fusarium oxysporum* under salinity stress, leading to reduced oxidative stress markers. Kaboosi et al. (2022) [65] also observed enhanced POD and APX activities in endophyte-inoculated plants, along with lower lipid peroxidation and improved stress tolerance. These effects are likely mediated by the upregulation of antioxidant enzyme-encoding genes and the production of signaling molecules, such as

phytohormones and secondary metabolites, which prime the plant's defense system [75]. The activation of enzymatic antioxidants works synergistically with the accumulation of non-enzymatic antioxidants like phenolics, anthocyanins, and flavonoids, contributing to a robust ROS-scavenging network [76].

Principal component analysis and correlations

Principal component analysis (PCA) of growth, physiological, and biochemical data revealed that PC1 and PC2 together accounted for 82.1% of the variation, with PC1 explaining 62.1% and PC2 20% (Fig. 8a). The chlorophyll a/b ratio showed a significant negative correlation with MDA content. Under 100 mM NaCl stress, endophyte inoculation positively correlated with leaf length (LL), leaf weight (LW), APX activity, and flavonoid content. At 150 mM NaCl stress, antioxidant capacity (DPPH scavenging activity), soluble sugars, phenolics, proline, fresh weight (FW), dry weight (DW), plant height, root length (RL), and POD and PPO activities exhibited strong positive correlations (Fig. 8b). Notably, non-inoculated plants showed a negative correlation between growth performance and MDA content, indicating oxidative damage. Positive correlations between plant growth (height, LW, RL) and antioxidants (APX, POD, PPO), along with osmoprotectants like proline, soluble sugars, and flavonoids, demonstrate the role of these compounds in enhancing stress tolerance (Fig. 8c).

These findings suggest that endophyte inoculation supports the coordinated improvement of growth and physiological responses, particularly under salt stress. The PCA results highlight the interconnection of osmoprotectants and antioxidants in mitigating stress-induced damage and supporting plant growth.

Conclusion

This study manifests the utility of *P. chrysogenum* CM022 as a salinity mitigating bioinoculant for wheat under salinity stress. Under stress, *P. chrysogenum* CM022 inoculation markedly enhanced seed germination and biomass production and altered morphological characteristics of wheat. *P. chrysogenum* CM022 inoculation enhanced stress adaptation mechanisms through improved osmotic control (by accumulation of proline and soluble sugars) and enhanced antioxidant activity through accumulation of phenolics and flavonoids. Through lowering H_2O_2 and MDA levels and increasing the activity of antioxidant enzymes like CAT, POD, PPO and APX, *P. chrysogenum* CM022 successfully reduced the oxidative damage to wheat tissues. Together, these physiological and biochemical reactions can enhance the plant's resistance to salinity stress, evidenced by better salt tolerance indices of root and shoot growth. The results highlight the role of *P. chrysogenum* CM022 inoculation as a promising effective and environmentally benign way to deal with salinity issues in wheat farming. To further confirm its use in sustainable agriculture, future studies should concentrate on field tests and the underlying molecular mechanisms.

Abbreviations

ROS	Reactive Oxygen Species
PDA	Potato Dextrose Agar
GP	Germination Percentage
MGT	Mean Germination Time
R	Germination Rate
GVI	Germination Vigor Index
SLSI	Shoot Length-based Stress Tolerance Index
RLSI	Root Length-based Stress Tolerance Index
FWSI	Fresh Weight-based Stress Tolerance Index
DWSI	Dry Weight-based Stress Tolerance Index
GSI	Germination Stress Index
MDA	Malondialdehyde
POD	Peroxidase
CAT	Catalase
APX	Ascorbate Peroxidase
PPO	Polyphenol Oxidase
BSA	Bovine Serum Albumin
CRD	Completely Randomized Design
PCA	Principal Component Analysis

Acknowledgements

We would like to express our sincere gratitude for the financial support provided by the Iran National Science Foundation (INSF) and the Agricultural Research, Education, and Extension Organization (AREEO).

Author contributions

SAD: formal analysis, investigation, writing—original draft. SN: funding acquisition, resources, project administration, review & editing. MKN: review & editing.

Funding

Financial funding by Iran National Science Foundation (INSF) and Agricultural Research, Education and Extension Organization (AREEO) with project code no. 4020315 has been provided.

Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 2 December 2024 / Accepted: 12 March 2025
Published online: 19 March 2025

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