

## EFFECTS OF *PLEUROTUS PULMONARIUS* FERMENTATION BROTH ON THE PHYSIOLOGY OF WHEAT DURING SEED GERMINATION AND SEEDLING GROWTH UNDER SALT STRESS

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### Abstract

This research investigated the influence of differing concentrations of *Pleurotus pulmonarius* fermentation broth (PP-FB) on the germination and seedling growth of wheat under salt stress conditions. Six treatment groups were established: sterile water (CK0), 0.8% NaCl (CK1), 2.0% PP-FB + 0.8% NaCl (F1), 1.0% PP-FB + 0.8% NaCl (F2), 0.5% PP-FB + 0.8% NaCl (F3) and 0.25% PP-FB + 0.8% NaCl (F4). Seeds of the wheat cultivar 'Jimai 22' were soaked for 12 hours, and parameters related to the germination period and seedling stage were measured. The results indicated that, in response to salt stress, the inclusion of 1.0% PP-FB resulted in improvements in wheat seed performance under salt stress conditions, with increases of 5.29% in germination rate, 47.37% in germination potential, and 44.28% in germination index compared to the salt stress control CK1. Compared to CK1, the root length, shoot length, dry weight and fresh weight of wheat seedlings showed increases of 93.98%, 106.35%, 87.23% and 87.40%, respectively. Furthermore, the malondialdehyde (MDA) content in wheat seedlings decreased by 45.29% compared to CK1, while the contents of proline, soluble sugar, and soluble protein increased by 55.29%, 31.27% and 7.71%, respectively. Superoxide dismutase (SOD) activity increased by 6.33%. The levels of Chlorophyll a (Chl a), Chlorophyll b (Chl b), total Chlorophyll [Chl (a+b)] and Carotenoids (Car) were enhanced by 7.56%, 7.69%, 7.62% and 42.47%, respectively, in comparison to CK1. As a result, under salt stress, PP-FB can encourage wheat seed germination and seedling growth.

**Key words:** *Pleurotus pulmonarius* fermentation broth; Wheat, Salt stress; Growth promotion

### Introduction

Soil salinization, characterized by high ion concentration, high osmotic potential, and strong alkalinity, severely threatens agricultural production and food security, representing a major abiotic stress in global agriculture (Kumar *et al.*, 2020). Excessive salinity in the rhizosphere can adversely impact various physiological and biochemical processes in crops, resulting in a substantial decrease in yield (Azeem *et al.*, 2019). Salt stress inhibits seed sprouting and seedling development, which reduce photosynthetic efficiency and stress resistance in crops (Zhang *et al.*, 2021). Concurrently, salt stress causes crop seedlings to produce reactive oxygen species (ROS), which can damage cellular structures, encourage the peroxidation of membrane lipids, and have a detrimental effect on seed germination (Khan *et al.*, 2025). Therefore, enhancing seed germination capacity under salt stress is crucial for robust seedling growth.

It's reported that microbial fermentation broths can promote seed germination and seedling growth under salt stress. The addition of fermentation broth from the endophytic fungus Su100 significantly enhanced salt tolerance in maize (Li *et al.*, 2024). *Klebsiella oxytoca* Rs-5, which produces the plant hormone indole-3-acetic acid (IAA), significantly promoted cotton seedling growth under salt stress (Liu *et al.*, 2013). Exopolysaccharides produced by the endophytic

bacterium *Pantoea alhagi* NX-11 in fermentation broth demonstrated significant mitigation of salt stress-induced toxicity in rice seedlings (Sun *et al.*, 2019).

Wheat (*Triticum aestivum* L.), a globally predominant staple crop, serves as a vital source of dietary energy and protein (Shen *et al.*, 2025). However, its productivity is notably constrained by soil salinity, with NaCl concentrations around 0.8% representing moderately saline conditions that markedly suppress seed germination and early seedling development. These initial growth phases are particularly critical for wheat establishment, with the seedling stage exhibiting heightened susceptibility to salt stress (Gong *et al.*, 2023). Consequently, salinity adversely affects both yield potential and grain quality (De Santis *et al.*, 2021).

*Pleurotus pulmonarius* (*P. pulmonarius*) is an edible and medicinal fungus whose fermentation broth contains various active substances such as polysaccharides and phenolics. Currently, the impact of *Pleurotus pulmonarius* fermentation broth (PP-FB) on wheat seed sprouting and seedling growth under saline stress conditions has not yet been studied. In order to provide a theoretical basis for improving wheat salt tolerance, this study investigated the impacts and underlying physiological processes of PP-FB on wheat seed germination and seedling growth under salt stress conditions.

## Materials and Methods

**Materials:** Wheat seeds 'Jimai 22' were purchased by Hebei Letu Seed Industry Co, Ltd. *P. pulmonarius* 'Xinxiu 169' was obtained from Anhui Science and Technology University.

## Experimental Methods

**Culture media:** Seed culture medium and Liquid fermentation medium: 200 g potato, 20 g glucose, 1.5 g yeast extract, 1.5 g MgSO<sub>4</sub>, 3 g KH<sub>2</sub>PO<sub>4</sub>, 0.1 g vitamin B<sub>1</sub>, 1000 mL distilled water, natural pH.

**Preparation of *P. pulmonarius* seed culture:** *P. pulmonarius* was inoculated onto PDA solid medium and incubated at 25°C for 7 d. After three rounds of activation, it was transferred to the seed culture medium and incubated at 25°C, 165 rpm for 7 days to obtain the *P. pulmonarius* seed culture.

**Liquid fermentation of *P. pulmonarius*:** A 250 mL conical flask was sterilized containing 150 mL of liquid fermentation medium, inoculated with 10% (v / v) *P. pulmonarius* seed culture, and incubated at 25°C, 165 rpm for 7 d.

**Processing of *P. pulmonarius* fermentation broth and analysis of main components:** After fermentation, the broth was filtered under vacuum to collect mycelia. The filtrate constituted the PP-FB. The broth was concentrated to 1 / 3 of its original volume, freeze-dried, and weighed. Soluble protein content was determined using the Coomassie Brilliant Blue method (Deng *et al.*, 2024). Polysaccharide content was determined using the phenol-sulfuric acid method (Baeva *et al.*, 2019). Soluble reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller *et al.*, 2021). Total phenolic content was determined using the Folin-Ciocalteu method (Xu *et al.*, 2019). Flavonoid content was detected according to the Al(NO<sub>3</sub>)<sub>3</sub> method (Staszowska-Karkut *et al.*, 2023).

$$\text{Germination potential} = \frac{\text{Number of seeds germinated on day 3}}{\text{Total seeds}} \times 100\% \quad (\text{Al-Ani } \textit{et al.}, 1985)$$

$$\text{Germination rate} = \frac{\text{Number of seeds germinated on day 7}}{\text{Total seeds}} \times 100\% \quad (\text{Lai } \textit{et al.}, 2019).$$

$$\text{Germination index (GI)} = \sum (\text{Gt} / \text{Dt}),$$

where Gt is number of germinations within 't' days, and Dt is the corresponding germination days (Liang *et al.*, 2020).

**Radicle and plumule length:** Measured using a vernier caliper.

**Seedling fresh weight per plant:** Fresh weight measured after blotting surface moisture.

**Seedling dry weight per plant:** Dry weight measured after oven-drying at 50°C to constant weight.

## Seedling stage indicators

**Photosynthetic indicators:** Fresh leaves from 14-day-old seedlings were used to determine chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll [Chl (a+b)] and carotenoid (Car) contents using spectrophotometry (Sikorska-Zimny *et al.*, 2021).

## Wheat seed germination experiment

**Wheat seed pretreatment:** Uniform, plump wheat seeds were selected and subjected to surface sterilization by immersion in 75% ethanol for 10 minutes, followed by three rinses with sterile water.

**Seed soaking treatments:** Six treatments were established: sterile water (CK0), 0.8% NaCl (CK1), 2.0% PP-FB + 0.8% NaCl (F1), 1.0% PP-FB + 0.8% NaCl (F2), 0.5% PP-FB + 0.8% NaCl (F3) and 0.25% PP-FB + 0.8% NaCl (F4). Sterilized seeds were soaked in the different treatment solutions in the dark for 12 h, washed three times with sterile water and gently blotted dry. Thirty seeds were uniformly distributed across two layers of sterile germination paper within germination boxes (12×12×5 cm), 10 mL of the corresponding treatment solution was added to the germination paper in each box, with six replicates per treatment. Germination boxes were placed in a light incubator under a cycle of 16±2°C darkness for 12 h and 25±2°C light for 12 h (light intensity: 60 μmol·m<sup>-2</sup>·s<sup>-1</sup>) for 14 days. Germination was counted every 24 h starting from the day of germination. An equal volume of sterile water was added at 24 h intervals.

## Measurement of indicators

**Seed germination stage indicators:** Germination potential was calculated on day 3. Germination rate and germination index were calculated on day 7. Radicle length, plumule length, single seedling fresh weight, and single seedling dry weight were also measured on day 7. After the germination test, a total of six uniform seedlings were randomly selected from each treatment group for the measurement of shoot / root length, and dry / fresh weight of seedlings.

**Malondialdehyde (MDA) and osmotic adjustment substance contents:** Fresh leaves from 14-day-old seedlings were used to determine MDA was measured as Heath & Packer's method (Heath & Packer, 1968). Proline content (Pro) was determined by sulfosalicylic acid method. Soluble sugar (SS) content was determined by anthrone method, soluble protein (SP) content was quantified using the Coomassie Brilliant Blue method (Sena *et al.*, 2024; Zhang *et al.*, 2024).

**Antioxidant enzyme activities:** Fresh leaves from 14-day-old seedlings were used to determine assessment of superoxide dismutase (SOD) activity utilizing nitroblue tetrazolium (Qu *et al.*, 2024); peroxidase (POD) activity assessment utilizing the guaiacol method (Tao *et al.*, 2018) and assessment of catalase (CAT) enzyme activity via the ammonium molybdate colorimetric assay (Liu *et al.*, 2025).

## Data analysis

Data were organized utilizing Excel 2019, and statistical analyses were conducted with SPSS 26. Draw with Origin 2019 ( $p<0.05$ ).

## Results

**Analysis of main components in PP-FB:** As shown in Table 1, PP-FB exhibited the highest polysaccharide content at  $54.89 \text{ mg}\cdot\text{g}^{-1}$ , followed by reducing sugar content at  $45.84 \text{ mg}\cdot\text{g}^{-1}$ . The soluble protein, total phenolic and flavonoid contents were  $2.21 \text{ mg}\cdot\text{g}^{-1}$ ,  $0.78 \text{ mg}\cdot\text{g}^{-1}$  and  $3.29 \text{ mg}\cdot\text{g}^{-1}$ , respectively.

**Effects of PP-FB on assessment of wheat seed germination rate, germination energy, and germination index under salt stress conditions:** As shown in Fig. 1, different concentrations of PP-FB significantly affected wheat seed germination under salt stress. The F2 treatment increased the above three indices under salt stress. Compared with CK0, CK1 showed obvious salt stress symptoms. In comparison to the salt stress control CK1, the PP-FB treatments significantly alleviated the negative effects of salt stress on the germination of wheat seeds. The three indices of the wheat seeds all increased to a peak and then declined. The most pronounced alleviation of salt stress was observed with 1.0% PP-FB, while both higher and lower concentrations resulted in diminished efficacy, indicating a concentration-dependent effect.

### Effects of PP-FB on wheat seedling growth

**Effects of PP-FB on root length and shoot length measurements of wheat seedlings:** As shown in Fig. 2, compared with CK0, root / shoot length in CK1 significantly decreased ( $p<0.05$ ), indicating salt stress severely inhibited root and shoot growth. Compared with CK1, the shoot / root length in the F2 treatment showed a remarkable increase ( $p<0.05$ ), by 106.35% and 93.98%, respectively. The F3 and F4 treatments were also significantly higher than CK1 but lower than F2. Therefore, the addition of PP-FB under salt stress conditions can substantially enhance the growth of

both shoots and roots.

**Effects of PP-FB on dry / fresh weight of wheat seedlings:** As shown in Fig. 3, different concentrations of PP-FB significantly affected seedling dry and fresh weight. Compared with CK0, dry and fresh weight in CK1 significantly decreased, indicating salt stress inhibited biomass accumulation. Compared with CK1, dry and fresh weight under different PP-FB treatments all increased to a peak and then declined. All PP-FB treatments increased fresh and dry weight, with the F2 treatment increasing by 87.40% and 87.23%. Therefore, adding PP-FB under salt stress promotes biomass accumulation.

As shown in Fig. 4B~D, under salt stress, compared with CK0, SS, SP and Pro contents in CK1 increased by 17.27%, 3.88% and 51.70%, respectively. Compared with CK1, these contents in the F2 treatment significantly increased ( $p<0.05$ ) by 31.27%, 7.71% and 55.29%, respectively. Therefore, adding PP-FB under salt stress enhances the content of osmotic adjustment substances, mitigating salt stress damage.

**Effects of PP-FB on antioxidant enzyme activities in wheat plants at the seedlings stage:** As depicted in Fig. 5A, compared with CK0, SOD activity increased in CK1. Compared with CK0, SOD activity in all PP-FB treatments increased to varying degrees, with the F2 treatment showing a 9.65% increase. Compared with CK1, SOD activity in F2 increased by 6.33%. SOD activity in PP-FB treatments increased to a peak and then declined. Thus, PP-FB physiologically enhances SOD activity, protecting the plasma membrane from oxidative damage.

As presented in Fig. 5B and 5C, the treatment groups exhibited significant changes relative to the CK0 control, POD and CAT activities significantly increased in CK1. Compared with CK0, CAT and POD activities in PP-FB treatments notably increased ( $p<0.05$ ). The F2 treatment exhibited the highest POD and CAT activities,  $144.392 \text{ U}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  and  $47.572 \text{ U}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ , respectively. The CAT and POD contents in the PP-FB treatments displayed an inverted "V"-shaped trend. Therefore, when wheat seedlings are subjected to salt stress, PP-FB treatment enhances their antioxidative capacity.

**Table 1. Content of main active substances in fermentation broth of *P. pulmonarius*.**

Ingredients	Soluble protein	Polysaccharide	Reducing sugar	Total phenols	Flavone
Content ( $\text{mg}\cdot\text{g}^{-1}$ )	$2.21 \pm 0.03$	$54.89 \pm 1.26$	$45.84 \pm 1.95$	$0.78 \pm 0.04$	$3.29 \pm 0.69$

Note: The figures in the table represent the average  $\pm$  standard error

**Table 2. Impact of different PP-FB concentrations on wheat seedlings under salt stress photosynthetic pigments.**

Treatment	Chl a ( $\text{mg}\cdot\text{g}^{-1}$ )	Chl b ( $\text{mg}\cdot\text{g}^{-1}$ )	Chl (a+b) ( $\text{mg}\cdot\text{g}^{-1}$ )	Car ( $\text{mg}\cdot\text{g}^{-1}$ )
CK0	$3.17 \pm 0.38\text{a}$	$1.20 \pm 0.08\text{a}$	$4.37 \pm 0.46\text{a}$	$1.00 \pm 0.41\text{a}$
CK1	$3.09 \pm 0.29\text{a}$	$1.24 \pm 0.12\text{a}$	$4.33 \pm 0.40\text{a}$	$0.80 \pm 0.18\text{ab}$
F1	$2.97 \pm 0.72\text{a}$	$1.03 \pm 0.23\text{a}$	$4.00 \pm 0.96\text{a}$	$0.49 \pm 0.09\text{b}$
F2	$3.32 \pm 0.12\text{a}$	$1.34 \pm 0.17\text{a}$	$4.66 \pm 0.28\text{a}$	$1.14 \pm 0.07\text{a}$
F3	$3.20 \pm 0.16\text{a}$	$1.24 \pm 0.15\text{a}$	$4.44 \pm 0.32\text{a}$	$1.08 \pm 0.13\text{a}$
F4	$2.96 \pm 0.46\text{a}$	$1.18 \pm 0.36\text{a}$	$4.15 \pm 0.82\text{a}$	$1.01 \pm 0.53\text{a}$

Note: In the same column, different lowercase letters indicate statistically significant differences ( $p<0.05$ )

### Effects of PP-FB on photosynthesis in wheat seedlings:

As shown in Table 2, while the contents of Chl a, Chl (a+b) and Car showed slight decreases in comparison to CK0, the content of Chl b in CK1 slightly increased. Compared with CK1, the addition of PP-FB resulted in increases of 7.56%, 7.69% and 7.62% in Chl a, Chl b and Chl (a+b), respectively, and a 42.47% increase in Car content within the F2 treatment. Therefore, the addition of PP-FB under salt stress conditions can enhance the content of photosynthetic pigments.

**Effects of PP-FB on MDA and osmotic adjustment substance contents in wheat seedlings:** As shown in Fig. 4A, MDA content was lowest in CK0 ( $5.583 \mu\text{mol}\cdot\text{g}^{-1}$ ). CK1 showed a sharp increase ( $p<0.05$ ) to  $22.868 \mu\text{mol}\cdot\text{g}^{-1}$ , a 309.62% increase over CK0, indicating severe membrane damage due to salt stress. Compared to CK1, the MDA content in the PP-FB treatment groups showed an initial decrease followed by

an increase. MDA content was lowest in F2 ( $12.512 \mu\text{mol}\cdot\text{g}^{-1}$ ), a significant 45.3% decrease compared to CK1. MDA contents in F1, F3 and F4 were  $18.430 \mu\text{mol}\cdot\text{g}^{-1}$ ,  $13.163 \mu\text{mol}\cdot\text{g}^{-1}$ , and  $14.365 \mu\text{mol}\cdot\text{g}^{-1}$ , respectively, all significantly lower than CK1 but higher than F2 ( $p<0.05$ ). Therefore, PP-FB can alleviate salt stress-induced membrane damage by inhibiting lipid peroxidation.

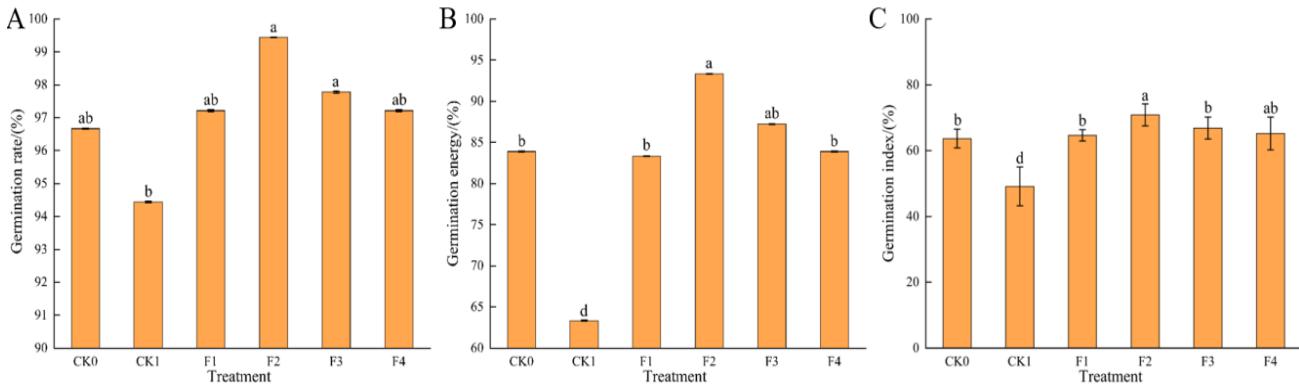


Fig. 1. The impact of varying concentrations of PP-FB on three germination indices of wheat seed under salt stress conditions.  
Note: Diverse lowercase letters represent statistically meaningful variations at  $p<0.05$  among different treatments.

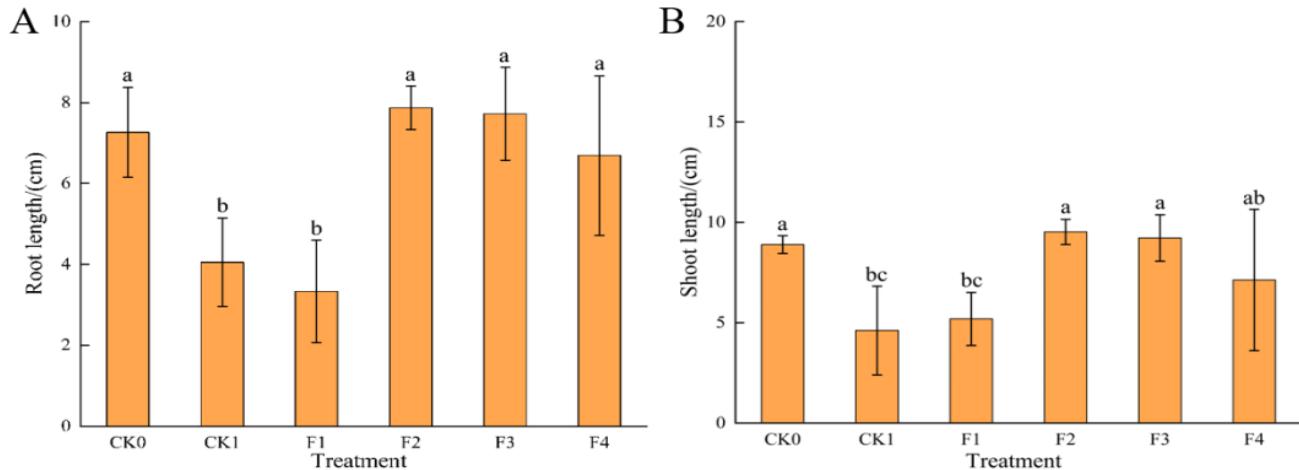


Fig. 2. Effects of PP-FB on the growth of wheat seedlings roots and shoots under salt stress.  
Note: Diverse lowercase letters represent statistically meaningful variations at  $p<0.05$  among different treatments.

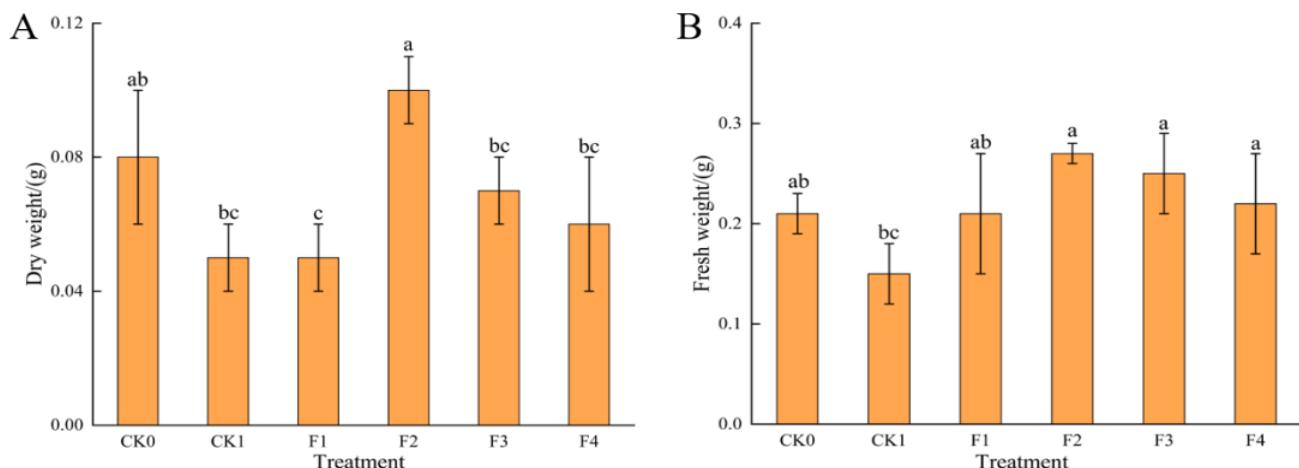


Fig. 3. Effects of PP-FB on dry / fresh weight of wheat seedlings under salt stress.  
Note: Diverse lowercase letters represent statistically meaningful variations at  $p<0.05$  among different treatments.

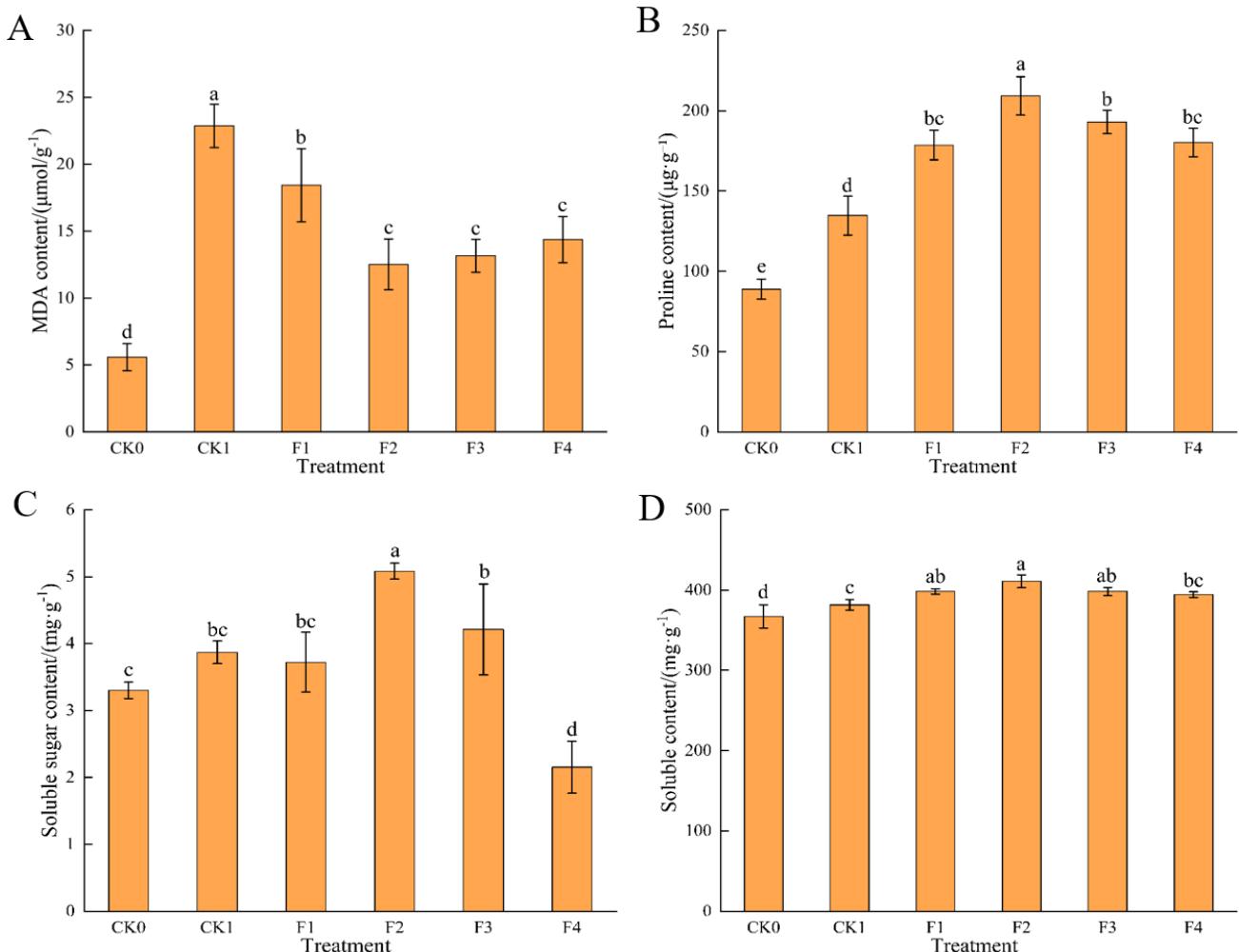


Fig. 4. Effects of PP-FB on MDA, soluble protein (SP), soluble sugar (SS) and proline (Pro) levels in salt-stressed wheat seedlings.  
Note: Diverse lowercase letters represent statistically meaningful variations at  $p<0.05$  among different treatments.

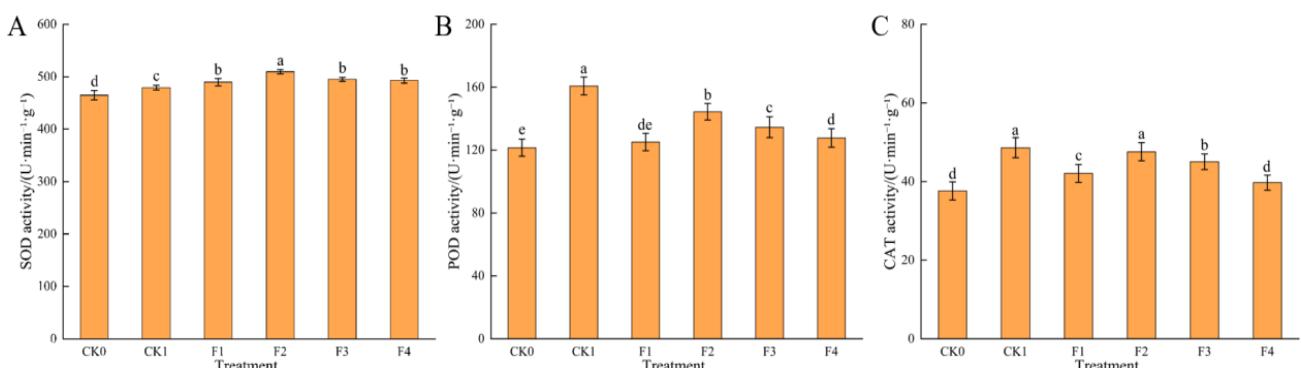


Fig. 5. The effects of PP-FB on SOD, POD and CAT of wheat seedlings under salt stress.  
Note: Diverse lowercase letters represent statistically meaningful variations at  $p<0.05$  among different treatments.

## Discussion

The alleviating effects of microbial fermentation broths are closely related to the varieties and contents of their bioactive components (Cilerdzic *et al.*, 2016). Polysaccharides, as major active components in fungal fermentation broths, possess strong antioxidant and immunomodulatory functions (Mu *et al.*, 2021). Proteins and reducing sugars can serve as nutrients or osmotic adjustment substances, providing energy and material basis for plant growth (Chen *et al.*, 2020). This study found that

polysaccharides were the most abundant component in PP-FB, followed by reducing sugars. In this study, the 1.0% PP-FB treatment showed the best effect, which may be related to the content and ratio of its active ingredients. As shown in Table 1, PP-FB is rich in polysaccharides (54.89 mg·g<sup>-1</sup>) and reducing sugars (45.84 mg·g<sup>-1</sup>). These substances may alleviate salt stress through the following mechanisms. Firstly, polysaccharides and reducing sugars can act as osmotic adjustment substances to maintain cellular osmotic balance, secondly, phenolic (0.78 mg·g<sup>-1</sup>) and flavonoid (3.29 mg·g<sup>-1</sup>) compounds, as natural

antioxidants, may directly scavenge ROS or activate the plant antioxidant system (Mehmood *et al.*, 2022). Thus, through their contributions to the overall efficacy, these bioactive components further enhance wheat's resistance to salt stress during germination and seedling growth.

Salt stress is one of the abiotic stresses that will have a major impact on wheat growth and yield (Khan *et al.*, 2019). The seed germination stage is the most critical phase in plant development, greatly impacting seedling emergence (Wang *et al.*, 2022). Various indicators related to seed germination have been used in germination studies (Rehmani *et al.*, 2022; Rehmani *et al.*, 2023). Among them, germination rate indicates final germination capacity, germination potential indicates germination speed and uniformity, germination index indicates overall germination vigor (Penfield, 2017). During germination, salt stress induces osmotic stress and oxidative stress, disrupting membrane integrity, interfering with metabolism, and affecting normal germination. Under salt stress, wheat seedling roots show increased sensitivity to  $\text{Na}^+$  and  $\text{Cl}^-$ , inhibiting root tip cell division and elongation (Yue *et al.*, 2021), significantly shortening root and shoot length. Impaired water and mineral absorption weakens photosynthetic capacity and assimilate accumulation, reducing plant fresh weight. Disrupted carbon and nitrogen metabolism and reduced dry matter translocation and allocation efficiency (Shao *et al.*, 2016) lead to significantly lower dry weight. This study found that different concentrations of PP-FB promoted wheat seed germination under salt stress.

Photosynthesis is a vital physiological process in plant growth (Hameed *et al.*, 2021). The contents of Chl a, Chl b and Car directly reflect the photosynthetic capacity of leaves. Salt stress can degrade photosynthetic pigments, reducing photosynthetic efficiency. Adding exogenous substances under salt stress can effectively protect chloroplasts and slow chlorophyll degradation (Jiang *et al.*, 2021). For example, adding the fungus *Aspergillus welwitschiae* BK considerably raised the amount of chlorophyll in salt-stressed maize (Gul *et al.*, 2023). This study found that chlorophyll content in PP-FB treatments was higher than in CK1. Adding PP-FB helps maintain chlorophyll content, sustaining photosynthesis in wheat seedlings under salt stress and providing necessary materials and energy for growth.

MDA content indicates the degree of cellular damage and is often used as an indicator of crop stress severity (Goncharuk *et al.*, 2022). Salt stress causes membrane lipid peroxidation, increasing MDA production and damaging crops. Maintaining cellular osmotic potential under salt stress is achieved by plants through the accumulation of compatible solutes, including soluble sugars, proline and soluble proteins (Bao *et al.*, 2020). Soluble sugars reflect both nutritional status and osmotic adjustment capacity, effectively mitigating salt stress damage (Ji *et al.*, 2021). Soluble protein content reflects the overall level of crop metabolism (Zhang *et al.*, 2023). This study found that the MDA content in PP-FB-treated groups was significantly lower than that in the salt stress control CK1, while the contents of SS, SP and Pro were higher. This indicates that PP-FB effectively alleviates salt stress-induced cellular damage and protects wheat seedlings from osmotic stress.

Antioxidant enzymes play crucial roles in scavenging ROS, SOD, POD and CAT are important enzymes that are essential to the enzymatic system (Sadak *et al.*, 2023). Their activities significantly increase when crops encounter salt stress (Ashraf, 2009). According to this study, wheat seedlings under salt stress had significantly higher SOD, POD and CAT activities than CK0. Consistent with its role as the primary defense in the plant antioxidant system, SOD initiates the enzymatic response against oxidative stress (Hasanuzzaman *et al.*, 2020). The negative effects of salt stress on growth are lessened when PP-FB increases the antioxidant capacity of wheat seedlings through increased SOD activity.

## Conclusion

The effects of PP-FB on wheat seed germination and seedling development under salt stress were investigated in this study. Salt stress induced a series of physiological responses: a marked decline in seedling root vigor, an increase in MDA content, activation of the antioxidant enzyme system, and enhanced accumulation of osmotic adjustment substances. Wheat exposed to salt stress showed improved germination and seedling development when various PP-FB concentrations were added. The highest germination potential, germination rate, and germination index were obtained with the 1.0% PP-FB treatment. It significantly promoted the accumulation of SS, SP and Pro, while reducing MDA content, increasing photosynthetic pigment content and antioxidant enzyme activities, which suggests that proper PP-FB application markedly alleviates salt-induced injury, stimulate healthy seedling growth and boost salt stress resistance in wheat.

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