

EFFECT OF EXOGENOUS PUTRESCINE ON WHEAT SEED GERMINATION AND PHYSIOLOGICAL CHARACTERISTICS UNDER SALT STRESS

YANG, X. F.¹ – YAO, C. J.¹ – LI, Y. J.¹ – ZHANG, S. J.^{1,2*}

¹*College of Environment and Life Sciences, Weinan Normal University, Weinan 714000, China*

²*Huayin Seed Management Station, Huayin 714200, China*

**Corresponding author
email: shujun-zhang@outlook.com*

(Received 9th Jun 2025; accepted 30th Jul 2025)

Abstract. In this experiment, the seeds of wheat cultivar “Fengdecunmai 1” were utilized as experimental materials. Under a saline stress condition of 50 mmol·L⁻¹, the impacts of five distinct concentrations of exogenous putrescine (0.1, 0.2, 0.5, 1.0 and 2.0 mmol·L⁻¹) on germination indices (including germination percentage, root length, and bud length) and physiological indices (such as root activity, chlorophyll content, malondialdehyde content, and proline content) were investigated at the seedling stage. The results are as follows: (1) Seed germination rate, root length and bud length were inhibited, root activity and chlorophyll content decreased, malondialdehyde and proline contents increased significantly under a salt stress condition of 50 mmol·L⁻¹. (2) 0.2-0.5 mmol·L⁻¹ putrescine could remarkably alleviate the suppressive impact of saline stress on the germination of wheat seeds and root length; 0.5 mmol·L⁻¹ putrescine significantly mitigated the inhibitory impact of salt stress on wheat bud length; putrescine at different concentrations of 0.1-2.0 mmol·L⁻¹ alleviated suppressive influence of saline stress on root functionality, promoted the synthesis of chlorophyll in wheat leaves and reduced the accumulation of malondialdehyde (MDA) content, 0.5 mmol·L⁻¹ putrescine had the most significant mitigation effect; 0.5 mmol·L⁻¹ exogenous putrescine could significantly increase the proline content under salt stress, so as to enhance the adaptability to salt stress. In conclusion, 0.5 mmol·L⁻¹ exogenous putrescine was the most effective in sodium chloride stress mitigation at 50 mmol·L⁻¹ on wheat seed germination and seedling growth.

Keywords: *Triticum aestivum*, sodium-chloride stress, exogenous putrescine, physiological characteristics, alleviation

Introduction

Global soil salinization is intensifying due to the impact of human activities and extreme climate. (Mushtaq et al., 2022). The build-up of excessive sodium ions (Na⁺) and chloride ions (Cl⁻) in the soil can reduce the soil water potential, cause osmotic stress, inhibit water absorption by plant roots, and lead to slow growth, abnormal physiological metabolism, even plant death (Łukasz et al., 2024; Kotakis et al., 2014). According to the statistics, currently there are over 380 million hectares of saline-alkali land and over 7 million hectares of salinized soil in China, accounting for about 20% of the arable land area, and increasing year by year. Common wheat is the second largest grain crop in China, its growth and development are often affected by various factors, while salinity impairs the yield and degrades the quality of wheat as a main stress factor. Therefore, how to mitigate the reduction in grain yield and quality caused by salt stress has become an urgent problem in production.

In recent years, substances to alleviate salt stress have been continuously explored, among them, polyamines (PAs) have been applied to effectively increase the salt-alkali

resistance of plants as a type of stress-resistant substance. PAs represent a category of low molecular-weight, aliphatic nitrogen-containing compounds with strong physiological activity synthesized during the metabolic pathways of organisms, which affects the development, growth and morphogenesis of plant, and is closely related to crop drought resistance, salt resistance, acid resistance and other stress resistances (Parrotta et al., 2023; Liu et al., 2024; Najafi et al., 2025). Putrescine (Put), Spermidine (Spd), along with Spermine (Spm), are among the common polyamines, and Put functions as the hub of the PAs biosynthesis pathway and acts as a precursor for the formation of Spm and Spd (Han et al., 2025; Khan et al., 2025). Relevant research results showed that exogenous Spm and Spd have certain alleviating effects on wheat (Lou, 2017; Li et al., 2023; Fatemeh et al., 2022), barley (Kiirat et al., 2007), rice (Mao et al., 2010; Najafi et al., 2025), beans (Hu et al., 2023), cucumber (Duan et al., 2009) and potato (Liu et al., 2023b) under stress. Nevertheless, investigations into the impact of Put on wheat's tolerance to salt stress remain scarce.

To investigate the impacts on the developmental and physiological parameters of wheat seeds during germination under 50 mmol·L⁻¹ NaCl stress, Fengdecunmai 1 (Zheng et al., 2017) seeds were selected as the materials in this experiment, and five separate concentrations of Put are applied for seed soaking. It can clarify the mitigating impact of exogenous Put on the suppressive impact of salt stress on wheat growth and germination, offer a foundation for the high-productivity cultivation of wheat in saline-affected soil, and establish a robust cornerstone for subsequent utilization of saline soil and safeguarding food security.

Materials and methods

The material used in this experiment is wheat variety Fengdecunmai No.1, which is provided by the Laboratory of Molecular Chromosome Engineering, Northwest A&F University. China's Tianjin Kemiou Chemical Reagent Co., LTD. was the source from which NaCl was obtained, and Put was the product of Sigma Company, USA.

We selected Fengdecunmai No.1 seeds with full and intact grains, disinfected the seeds with 10% sodium hypochlorite for 15 min, washed them with distilled water for many times, soaked the seeds for 12 h and then used for reserve. The treated seeds were deposited in a clean petri dish (12 cm) containing 2 layers of moist filter paper. The treatment solution was added until the seeds were just submerged, and the solution was renewed each day to preserve the concentration. Thirty seeds were allocated to each petri dish, and the experiment consisted of seven treatment groups (*Table 1*), each with three replicates. The group under salt stress conditions employed a 50 mmol·L⁻¹ sodium chloride (NaCl) solution, based on preliminary tests using different concentrations (25, 50, 75 and 100 mmol·L⁻¹) of NaCl solution on Fengdecunmai 1 seed sprouting and sapling growth, which showed that low concentrations (25 mmol·L⁻¹) of NaCl exerted no notable suppressive impact on seed germination; high concentrations (75 and 100 mmol·L⁻¹) of sodium chloride (NaCl) markedly suppressed seed germination, even preventing germination and severely affecting subsequent growth and physiological indicator measurements; while medium concentrations (50 mmol·L⁻¹) of NaCl could both exert inhibitory stress and not affect subsequent seedling growth and physiological indicator measurements.

If there is no seed germination for three consecutive days, it will be considered as the end of germination, then germination rate was assessed. After germination, 10 seedlings

were randomly selected from each treatment, root length (RL) and bud length (BL) were measured using a ruler. Physiological indicators such as root activity, chlorophyll content, malondialdehyde (MDA) content and proline (Pro) content were measured using the TTC method, colorimetric method, thio barbituric acid method and sulfosalicylic acid method, respectively. The above methods were determined by referring to Zhang et al. (2016).

The experimental data were sorted by Microsoft Excel 2010, and SPSS statistics 26.0 was used for significant difference analysis ($P < 0.05$). Principal component analysis was used to evaluate the salt tolerance of wheat materials under different treatments, and Origin Pro 22 was used for graphing.

Table 1. Test treatment

Treatment	Contents
CK	Control group: Cultured in pure water
NaCl	Salt stress group: cultured with 50 mmol·L ⁻¹ NaCl solution
0.1 Put	Soaked with 0.1 mmol·L ⁻¹ Put, cultured in 50 mmol·L ⁻¹ NaCl solution
0.2 Put	Soaked with 0.2 mmol·L ⁻¹ Put, cultured in 50 mmol·L ⁻¹ NaCl solution
0.5 Put	Soaked with 0.5 mmol·L ⁻¹ Put, cultured in 50 mmol·L ⁻¹ NaCl solution
1.0 Put	Soaked with 1.0 mmol·L ⁻¹ Put, cultured in 50 mmol·L ⁻¹ NaCl solution
2.0 Put	Soaked with 2.0 mmol·L ⁻¹ Put, cultured in 50 mmol·L ⁻¹ NaCl solution

Results

Impact of diverse concentrations of Put on the germination percentage of seeds under saline stress

As presented in Table 2, when contrasted with the blank control, the germination ratio of wheat seeds subjected to 50 mmol·L⁻¹ saline stress drastically decreased, indicating that 50 mmol·L⁻¹ saline stress can inhibit seed germination. After soaking the seeds with different concentrations of exogenous Put solution, the germination rate of seeds was increased to different degrees compared to salt stress, indicating that different exogenous Put at different concentrations could mitigate the suppressive impact of saline stress on seed germination to a certain extent. When the exogenous Put concentration was 0.2 and 0.5 mmol·L⁻¹, the seed germination rate reached the highest value of 100% with a significant difference compared to saline stress, which suggested that the exogenous Put within this concentration range exerted the most optimal mitigating effect on Seed sprouting ratio in saline stress conditions. As the exogenous Put concentration continued to increase, the seed germination rate remained higher than that in the presence of saline stress, but the difference was not notable.

Impact of diverse concentrations of Put on root and bus length under salt stress

As shown in Table 3, significant difference analysis indicated that 50 mmol·L⁻¹ saline stress significantly inhibited the root elongation and bud length of wheat seedlings in contrast to the blank control. Under salt-induced stress, compared to the blank control, the root length and bud length of wheat decreased by 59.35% and 41.12%, respectively, and the root length was more sensitive to salt concentration. Upon the addition of the exogenous Put solution, the root length and bud length of wheat

exhibited an initial increase followed by a subsequent decrease. When the level of externally supplied Putrescine reached $0.5 \text{ mmol}\cdot\text{L}^{-1}$, the root length of wheat seedlings was significantly promoted, which compared with the salt treatment control group. When the exogenous Put concentration reached $1.0 \text{ mmol}\cdot\text{L}^{-1}$, the bud elongation reached the maximum value, which was a significantly different from that salt stress, but had no significant difference from that under $0.5 \text{ mmol}\cdot\text{L}^{-1}$ exogenous Put concentration. The results indicated that exogenous Put of $0.5\text{-}1.0 \text{ mmol}\cdot\text{L}^{-1}$ had the optimal mitigation for root elongation and bud length under a salt stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$.

Table 2. *Impact of diverse concentrations of putrescine on the sprouting of wheat seeds under salt-induced stress*

Treatment	Seed germination percentage / %
CK	100.00 a
NaCl	94.43 ± 3.3 b
0.1 Put	97.80 ± 3.3 ab
0.2 Put	100.00 a
0.5 Put	100.00 a
1.0 Put	97.80 ± 3.3 ab
2.0 Put	97.80 ± 3.3 ab

Treatments with values trailed by distinct letters in the table exhibit significant disparities ($P < 0.05$)

Table 3. *Impact of diverse concentrations of exogenous putrescine on wheat root and bud length under salt stress*

Treatment	Root length / cm	Bud length / cm
CK	6.42 ± 0.05 a	3.38 ± 0.10 a
NaCl	2.61 ± 0.17 d	1.99 ± 0.05 d
0.1 Put	2.77 ± 0.06 d	2.22 ± 0.07 cd
0.2 Put	3.93 ± 0.09 c	2.17 ± 0.04 cd
0.5 Put	4.96 ± 0.15 b	2.37 ± 0.11 bc
1.0 Put	3.10 ± 0.12 d	2.67 ± 0.14 b
2.0 Put	2.67 ± 0.07 d	1.96 ± 0.09 d

Impact of diverse concentrations of Put on root activity of wheat under salt-induced stress

The growth status and vitality level of plant roots are directly related to plant growth, and root vitality is a direct indicator of root growth. As shown in *Table 4*, the analysis of significant differences in root activity demonstrated that the root activity under a salt stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$ significantly declined in contrast to that of the blank control, and the root activity of wheat after soaking in Put exhibited a pattern of initially ascending and subsequently descending as the putrescine concentration increased. Compared with $50 \text{ mmol}\cdot\text{L}^{-1}$ salt-induced stress treatment, the impact of exogenous Put treatment on the increase of root vitality reached a statistically significant level ($P < 0.05$), indicating that exogenous Put treatment might reduce the suppressive impact

of saline stress on wheat root activity to different degrees. Upon the exogenous Put concentration attaining $0.5 \text{ mmol}\cdot\text{L}^{-1}$, the root activity reached the maximum, and the root activity was significantly different from that under salt-induced stress, and it showed no substantial divergence from that under the blank control. The results indicated that $0.5 \text{ mmol}\cdot\text{L}^{-1}$ exogenous Put produced the strongest effect on root activity inhibited by $50 \text{ mmol}\cdot\text{L}^{-1}$ salt-induced stress. These findings aligned with the impact of Put on the root length of wheat seedlings under saline- induced stress, indicating that exogenous Put in the appropriate concentration range could effectively improve the root length and activity of wheat root under saline- induced stress.

Table 4. *Impact of diverse concentrations of putrescine on wheat root activity under saline-induced stress*

Treatment	Root activity / $\mu\text{gTTF}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$
CK	$135.95 \pm 5.02 \text{ a}$
NaCl	$65.42 \pm 1.57 \text{ e}$
0.1 Put	$81.51 \pm 2.76 \text{ d}$
0.2 Put	$99.48 \pm 3.89 \text{ c}$
0.5 Put	$125.88 \pm 4.74 \text{ a}$
1.0 Put	$111.56 \pm 4.03 \text{ b}$
2.0 Put	$90.06 \pm 3.11 \text{ cd}$

Impact of diverse concentrations of Put on chlorophyll content under saline stress

As presented in Table 5, in comparison with the blank control, the amount of overall chlorophyll (Chl), Chla and Chlb of wheat seedlings underwent a substantial reduction under a salt stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$. They were reduced by 34.21%, 46.90% and 40.43%, respectively. The chlorophyll content of seed soaked in Put initially increased and subsequently decreased as the concentration of exogenous Put varied. Upon the concentration attaining $0.5 \text{ mmol}\cdot\text{L}^{-1}$, the Chl content reached its peak, which was 1.59 times of that under salt stress, showing a significant difference from that under salt-induced stress. The results revealed that exogenous Put of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Achieved the greatest efficacy on chlorophyll's content inhibition under $50 \text{ mmol}\cdot\text{L}^{-1}$ salt-induced stress.

Table 5. *Impact of diverse concentrations of exogenous Put on synthesis of chlorophyll under salt stress*

Treatment	Chl a / $\text{mg}\cdot\text{L}^{-1}$	Chl b / $\text{mg}\cdot\text{L}^{-1}$	Chl content / $\text{mg}\cdot\text{g}^{-1} \text{ FW}$
CK	$22.89 \pm 0.32 \text{ a}$	$23.71 \pm 0.27 \text{ a}$	$0.94 \pm 0.25 \text{ a}$
NaCl	$15.06 \pm 0.15 \text{ e}$	$12.59 \pm 0.08 \text{ g}$	$0.56 \pm 0.04 \text{ e}$
0.1 Put	$17.83 \pm 0.14 \text{ d}$	$16.46 \pm 0.07 \text{ e}$	$0.64 \pm 0.09 \text{ d}$
0.2 Put	$20.42 \pm 0.11 \text{ b}$	$18.20 \pm 0.05 \text{ d}$	$0.74 \pm 0.03 \text{ c}$
0.5 Put	$22.69 \pm 0.06 \text{ a}$	$21.05 \pm 0.15 \text{ b}$	$0.89 \pm 0.04 \text{ b}$
1.0 Put	$18.78 \pm 0.12 \text{ c}$	$19.09 \pm 0.09 \text{ c}$	$0.74 \pm 0.03 \text{ c}$
2.0 Put	$17.69 \pm 0.08 \text{ d}$	$13.34 \pm 0.11 \text{ f}$	$0.62 \pm 0.06 \text{ d}$

Impact of diverse concentrations of Put on MDA content in wheat under salt stress

As shown in *Table 6*, the MDA content in wheat leaves was $0.83 \mu\text{g}\cdot\text{L}^{-1}$ under the blank control, and increased to $1.06 \mu\text{g}\cdot\text{L}^{-1}$ under a salt stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$, indicating that plant metabolism decelerated, ROS (reactive oxygen species) accumulated, and the harm of membrane lipid peroxidation was aggravated, thereby causing an elevation in MDA content under salt stress. The application of exogenous Put could reduce the accumulation of MDA in wheat leaves, indicating that exogenous Put exerted a notable suppressive influence on the accumulation of MDA. Under a saline stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$ treatment, the MDA amount of was notably greater than that of blank control, and along with the elevation of exogenous Put concentration in the presence of salt stress, MDA content of leaves decreased as a whole compared with that under salt stress. When the exogenous Put concentration reached $0.5 \text{ mmol}\cdot\text{L}^{-1}$, the content of MDA was the lowest, only $0.84 \mu\text{g}\cdot\text{L}^{-1}$, which showed a significant divergence from that under $50 \text{ mmol}\cdot\text{L}^{-1}$ salt stress ($P < 0.05$), however, it did not show any significant divergence from the blank control. The outcomes indicated that exogenous Put at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ could effectively reduce membrane lipid peroxidation and suppress the buildup of MDA in seedlings treated with salt damage.

Table 6. Impact of diverse concentrations of exogenous putrescine on malondialdehyde content

Treatment	MDA content / $\mu\text{g}\cdot\text{L}^{-1}$
CK	0.83 ± 0.03 d
NaCl	1.06 ± 0.05 a
0.1 Put	0.98 ± 0.02 b
0.2 Put	0.91 ± 0.03 c
0.5 Put	0.84 ± 0.04 d
1.0 Put	0.89 ± 0.04 c
2.0 Put	0.93 ± 0.03 bc

Impact of diverse concentrations of Put on proline content under saline- induced stress

As illustrated in *Table 7*, the proline content under $50 \text{ mmol}\cdot\text{L}^{-1}$ salt stress exhibited a notably higher level. Under salt stress, the level of free proline in leaves went up at first and later went down with the elevation of exogenous Put concentration. In Comparison with the saline stress treatment, the proline content significantly increased ($P < 0.05$) within $0.2 - 1 \text{ mmol}\cdot\text{L}^{-1}$ Put concentration range, reaching the maximum value at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Put concentration, with little to no significant difference at 0.1 and $2.0 \text{ mmol}\cdot\text{L}^{-1}$. In conclusion, the application of Put can further increase the content of proline in leaves, and it has the optimal effect when the Put concentration reaches.

Comparison of salt tolerance of wheat under different treatments

Using principal component analysis to comprehensively analyze seven indicators, the overall effect of different treatments on wheat salt tolerance capacity was appraised. The outcomes (*Table 8*) showed that the contribution rate of the first two components exceeds 85%, so the first two components can be extracted as indicators to evaluate wheat salt tolerance during the germination period.

Table 7. Impact of diverse concentrations of exogenous putrescine on the proline content

Treatment	Proline content / $\mu\text{g}\cdot\text{g}^{-1}$ FW
CK	2.17 ± 0.01 e
NaCl	2.69 ± 0.02 cd
0.1 Put	2.73 ± 0.04 c
0.2 Put	2.96 ± 0.05 b
0.5 Put	3.27 ± 0.03 a
1.0 Put	2.91 ± 0.02 b
2.0 Put	2.69 ± 0.02 d

Table 8. Total variance explained of salt tolerance capacity in wheat seeds at germination stage

Composition	Initial eigenvalue			Extract sum of squares and load it		
	Total	Percentage of the variance (%)	Accumulation (%)	Total	Percentage of the variance (%)	Accumulation (%)
1 (Germination percentage)	5.152	79.598	79.598	4.944	70.632	70.632
2 (Root length)	1.342	19.172	92.770	1.550	22.137	92.770
3 (Bud length)	0.261	3.735	96.504			
4 (Root activity)	0.183	2.620	99.124			
5 (Chlorophyll content)	0.060	0.861	99.985			
6 (MDA content)	0.001	0.014	99.999			
7 (Proline content)	0.000	0.001	100.00			

According to the principal component score coefficient matrix (Table 9), the expression of the principal component function for wheat salt tolerance during the germination period was obtained: $Y_1 = 0.221X_1 + 0.150X_2 + 0.079X_3 + 0.202X_4 + 0.199X_5 - 0.219X_6 + 0.135X_7$, $Y_2 = -0.214X_1 + 0.153X_2 + 0.372X_3 - 0.026X_4 - 0.012X_5 + 0.127X_6 - 0.700X_7$ (where X_1 - X_7 stand for separately the seven indicator variables with the same dimension after standardized processing, and Y represents the salt tolerance score of the extracted principal component).

Table 9. Score coefficient matrix of principal components related to salt tolerance of wheat seeds in the germination period

Factor	Principal component 1	Principal component 2
Germination percentage	0.221	-0.214
Root length	0.150	0.153
Bud length	0.079	0.372
Root activity	0.202	-0.026
Chlorophyll content	0.199	-0.012
MDA content	-0.219	0.127
Proline content	0.135	-0.700

A single principal component (Y_1 Y_2) could not comprehensively appraise the salt-enduring capacity of wheat seeds during the germination phase. Thus, by using the ratio of the eigenvalues of each principal component to the cumulative sum of the

eigenvalues of all extracted principal components as the weighting factor, the equation for computing the salt tolerance of wheat during the germination period is $Y = 0.793Y_1 + 0.207Y_2$. Thus, the comprehensive score (Y value) and the order of salt tolerance for wheat in the germination phase with different treatments was derived (Table 10). In Table 10, The salt tolerance of wheat under $50 \text{ mmol}\cdot\text{L}^{-1}$ NaCl salt stress at germination stage is $\text{CK} > 0.5 \text{ Put} > 1.0 \text{ Put} > 0.2 \text{ Put} > 2.0 \text{ Put} > 0.1 \text{ Put}$. That is, $0.5 \text{ mmol}\cdot\text{L}^{-1}$ concentration of Put can effectively reduce the toxicity of seedlings after salt damage treatment.

Table 10. Comprehensive factor scores and rankings of salt tolerance of wheat under different treatments

Treatment	Synthesis score	Ranking of salt tolerance
CK	1.28	1
0 Put	-1.06	7
0.1 Put	-0.48	6
0.2 Put	0.04	4
0.5 Put	0.58	2
1.0 Put	0.08	3
2.0 Put	-0.44	5

Discussion

Impact of diverse concentrations of Put on growth indicators under salt stress

The germination period and seedling stage are the phases of wheat that are most vulnerable to salt stress, and the key to wheat germination and emergence depends on wheat's ability to tolerate salt stress (Wang et al., 2021). The germination percentage serves as a crucial metric for evaluating seed quality and an important basis for the actual seed usage in production (Yu et al., 2019; Siddique et al., 2018). Under saline stress conditions, excessive buildup of Sodium ions (Na^+) and chloride ions (Cl^-) in the soil can reduce the soil water potential, thereby causing osmotic stress. On the one hand, seeds face challenges in water absorption and may even undergo exosmosis, which severely inhibits the normal germination of seed. On the other hand, this impedes the water absorption of wheat roots and restricts the growth of both roots and shoots. This study shows that the germination percentage, root elongation and bud length of wheat seeds under a saline-induced stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$ were discovered to be remarkably less than those of the CK group, suggesting that salt-induced stress inhibits seed germination and growth. Exogenous Put can mitigate the suppressive impact of saline on seed germination, root and bud growth, with the best alleviating effect at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Put concentration. This corresponds to the outcomes of Liu et al. (2022) using melatonin, Sun et al. (2024) using fulvic acid, melatonin and salicylic acid to enhance the germination index of wheat seeds subjected to saline stress, and Hayati et al. (2022) spraying exogenous polyamines to alleviate the progress of soybean seedlings under salt-induced stress. These outcomes demonstrated that appropriate exogenous Put solution can reduce cell membrane permeability, resist germination barriers caused by salt stress, improve the metabolic capacity, and enhance the stretching and expansion of roots and buds when exposed to salt-induced stress.

Impact of diverse concentrations of Put on root activity under saline stress

The growth status and roots activity level are directly related to plant growth. Root activity is a direct indicator of root growth, and stress can lead to the decline of plant root activity (Khan et al., 2025; Grygoruk et al., 2016). In this study, it was discovered that the root activity under a saline stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$ declined notably, when contrasted with the control group, signifying that saline-induced stress impeded plant growth by affecting root activity. Exogenous Put was capable of mitigating the suppressive influence of saline stress on the root functionality. Notably, at a Put concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Put having the strongest alleviating effect on the inhibited root activity under a saline-induced stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$. This corresponds to the outcomes of alleviating cucumber seedlings under salt-induced stress with Put by Yuan et al. (2017), indicating that Put can improve root vitality and maintain vigorous root metabolism by reducing root cell death caused by salt stress. Consequently, investigating the impact of exogenous putrescine on plant root vitality and growth under saline stress holds substantial significance.

Impact of diverse concentrations of Put on chlorophyll content under salt stress

Chlorophyll functions as a vital pigment in plants, facilitating the absorption, transfer, and transduction of luminous energy in photosynthesis, thereby playing a pivotal function in plant development. In face of stress, partial decomposition and synthesis of chlorophyll are hindered and photosynthetic rate decreases, and the change of chlorophyll value can be an important index of plant stress resistance (Ghassemi et al., 2024; Liu et al., 2023a). In the present investigation, the levels of Chl a, Chl b, and total Chl in the leaves under a saline-induced stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$ were found to decline significantly when juxtaposed with those of the control group, which may be due to the direct damage of Na^+ and Cl^- to chloroplasts or the enhancement of related Chl enzyme activity under certain salt-induced stress promoted the further degradation of chlorophyll (Si et al., 2020). Exogenous Put can reduce the inhibitory impact of saline-induced stress on Chl amount to varying degrees, with $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Put having the best alleviating effect on the inhibitory effect of chlorophyll content under a saline-induced stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$. The studies of Hua et al. (2017), Kotakis et al. (2014) and Shah et al. (2022) also demonstrated that Put could effectively reduce damage of photosynthetic cells, thereby delaying damage in *Scutellaria baicalensis*, cucumber and apple young leaves, and enhancing the salt tolerance.

Impact of diverse concentrations of Put on MDA content under saline stress

MDA is the final decomposition product of membrane lipid peroxidation in plants under stress conditions. It can cross-link lipids and proteins, causing varying degrees of damage to the structure and function of the plasma membrane. The level of MDA content can demonstrate the intensity of stress imposed on the plant (Hu et al., 2023; Wang et al., 2021). The findings of this investigation revealed that MDA accumulated significantly in plants under a saline-induced stress condition of $50 \text{ mmol}\cdot\text{L}^{-1}$, meaning that salt-induced stress led to metabolic dysfunction, aggregation of oxygen-reactive species, heightened damage degree of membrane lipid peroxidation, and elevated MDA content. Appropriate concentration of exogenous Put can reduce MDA content in vivo, and with the elevation of Put concentration, the level of MDA dropped at first and later increased steadily, that is, the MDA content decreased the most at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Put

concentration. This corresponds to the discoveries of Hu et al. (2023) and Ma (2012), who reported that Put could reduce MDA accumulation in beans and wheat under salt stress. This suggests that Put may alleviate membrane lipid peroxidation damage inflicted by saline-induced stress by eliminating ROS or enhancing the activity of related enzymes, thereby reducing MDA production.

Impacts of diverse concentrations of Put on proline content under salt stress

Proline is an adaptive solute produced by plants under stress conditions. Its primary function is to regulate the osmotic equilibrium between the vacuole and cytoplasm, so as to resist osmotic stress caused by stress, which is conducive to maintaining a high salt stress resistance (Hu et al., 2023; Wang et al., 2021). In this research, the proline content under a saline-induced stress condition of 50 mmol·L⁻¹ was remarkably accumulated comparison with the blank control group, and the proline content of wheat leaves soaked with different concentrations of Put was further increased, and the most substantial increment was attained at a Put concentration of 0.5 mmol·L⁻¹. This conclusion aligns with the findings reported by Hu et al. (2023), who studied the effects of exogenous Put on proline accumulation in kidney beans under salt stress, and Najafi et al. (2025), who found that low concentrations of exogenous Put stimulated proline accumulation in tomato leaves under salt stress. These outcomes indicate that exogenous Put can alleviate membrane damage or toxic effects caused by salt stress by maintaining or increasing proline content in plants.

Conclusions

In summary, exogenous Put of 0.5 mmol·L⁻¹ maintained stress adaptability by increasing seed germination rate, bud length and root length, root activity and seedling leaf proline content under 50 mmol·L⁻¹ salt-induced stress, and reducing MDA content of membrane lipid peroxidation products, and had the most significant effect on alleviating adverse factors in wheat growth and development. However, the specific mechanisms of Put to elevate the salt tolerance adaptability of wheat is still further explored.

Funding. This study was supported by Key R&D Program Project of Shaanxi Provincial Department of Science and Technology (2025NC-YBXM-004); Provincial University Student Innovation and Entrepreneurship Training Program (S202310723043); Shaanxi Science and Technology Association Youth Talent Promotion Program Project (20200207).

REFERENCES

- [1] Duan, J. J., Guo, S. R., Kang, Y. Y., Zhou, G. X. (2009): Effects of exogenous spermidine on active oxygen scavenging system and bound polyamine contents in chloroplasts of cucumber under salt stress. – *Acta Ecologica Sinica* 29(2): 653-661.
- [2] Fatemeh, G., Gholizadeh, F., Janda, T., Gondor, O. K., Pál, M., Szalai, G., Sadeghi, A., Turkoglu, A. (2022): Improvement of drought tolerance by exogenous spermidine in germinating wheat (*Triticum aestivum* L.) plants is accompanied with changes in metabolite compositions. – *International Journal of Molecular Sciences* 23(16): 9047.

- [3] Ghassemi, S., Raei, Y. (2024): How can biochar and polyamine treatments mitigate salt toxicity by changing the physiological traits in garlic plants? – Environment, Development and Sustainability, (prepublish): 1-18.
- [4] Grygoruk, D. (2016): Root vitality of *Fagus sylvatica* L., *Quercus petraea* Liebl. and *Acer pseudoplatanus* L. in mature mixed forest stand. – Nephron Clinical Practice 58(2): 50-61.
- [5] Han, X. F., Wang, Z., Shi, L. Y., Wei, Z. Y., Shangguan, J. L., Zhao, M. W. (2025): Spermidine enhances the heat tolerance of *Ganoderma lucidum* by promoting mitochondrial respiration driven by fatty acid β -oxidation. – Applied and Environmental Microbiology e0097924.
- [6] Hayati, R., Rosa, E., Rahmiati, R., Savitri, S., Fitri, S., Khumaira, K. (2022): The polyamine compound to response for biotic and abiotic stresses in plant. – IOP Conference Series: Earth and Environmental Science 1116(1).
- [7] Hu, J. Y., Feng, G. J., Liu, D. J., Yang, X. X., Yan, Z. S., Liu, C. (2023): Effects of exogenous Put on seed germination and resistance of snap bean seeds under salt stress. – Chinese Agricultural Science 39(15): 52-58.
- [8] Hua, Z. R., Li, X. L. (2017): Effects of exogenous putrescine on photosynthetic characteristics of *Scutellaria baicalensis* under salt stress. – Acta Agriculture Jiangxi 29(12): 59-62.
- [9] Khan, H., Khan, Z., Eman, I., Eman, I., Ahmad, L., Shah, T., Wang, G. P., Feng, L., Alarfaj, A., Alharbi, S. A., Ansari, M. J. (2025): Synthetic bacterial communities regulate polyamine metabolism and genes encoding antioxidant defense system to enhance arsenic tolerance of rice. – South African Journal of Botany 178: 148-161.
- [10] Kiirat, A., Kilic, S., Kabar, K., Çavuşoğlu, K., Kılıç, S., Kabar, K. (2007): Some morphological and anatomical observations during alleviation of salinity (NaCl) stress on seed germination and seedling growth of barley by polyamines. – Acta Physiologiae Plantarum 29(6): 551-577.
- [11] Kotakis, C., Theodoropoulou, E., Tassis, K., Oustamanolakis, C. E., Ioannidis, N., Kotzabasis, K. (2014): Putrescine, a fast-acting switch for tolerance against osmotic stress. – Journal of Plant Physiology 171(2): 48-51.
- [12] Li, Y. B., Chen, B. X., Kurtenbach, R. (2023): Spermidine and spermine converted from putrescine improve the resistance of wheat seedlings to osmotic stress. – Russian Journal of Plant Physiology 3(70): 658.
- [13] Liu, J. Q., Li, L., Yang, H. H., Sun, M., Feng, H. Q. (2022): Effect of melatonin on seed germination and seedling physiological characteristics of wheat under salt stress. – Journal of Triticeae Crop 42(7): 857-863.
- [14] Liu, L. P., Wang, J. C., Si, E. J., Yao, L. R., Lu, Z. H., Qi, T. T., Ma, X. L., Wang, H. J., Li, B. C., Zhao, C. M., Shang, X. W., Meng, Y. X. (2023a): Effect of exogenous betaine and proline on seed germination and seedling growth of barley under salt stress. – Journal of Triticeae Crops 43(6): 766-774.
- [15] Liu, T. B., Qu, J., Fang, Y. Y., Yang, H., Lai, W. T., Pan, L. Y., Liu, J. H. (2024): Polyamines: the valuable bio-stimulants and endogenous signaling molecules for plant development and stress response. – Journal of Integrative Plant Biology 67(3): 582-595.
- [16] Liu, Y., Jiang, Y. H., Wang, Y. L., Yang, R. W., Wu, Y. (2023b): Effects of exogenous spermidine on growth, stress resistance physiological characteristics of potato seedlings under salt stress. – Acta Botanica Boreali-Occidentalia Sinica 43(12): 2079-2087.
- [17] Lou, X. Y. (2017): The effects of Polyamine soaking on seed germination of Zhoumai 18 wheat under drought stress. – Jiangsu Agricultural Sciences 45(9): 73-76.
- [18] Łukasz, W., Karolina, W., Sławomir, B., Małgorzata, G. (2024): Polyamine seed priming: a way to enhance stress tolerance in plants. – International Journal of Molecular Sciences 25(23): 12588-12588.

- [19] Ma, Y. (2012): Effects exogenous application of putrescine on active oxygen metabolism in wheat roots under salt stress. – Journal of Shaanxi University of Technology (Natural Science Edition) 28(4): 64-69.
- [20] Mao, S. G., Wang, R. L., Zhou, Q. C., Zhou, F., Hua, C., Liu, Q., Zhang, Q. (2010): Effects of spermidine on free polyamine content in rice leaves with different salt tolerance under salt stress. – Jiangsu Agricultural Science (6): 101-104.
- [21] Mushtaq, A., Sabir, N., Kousar, T., Rizwan, S., Rizwan, S., Jabeen, U., Bashir, F., Sabir, S., Shahwani, N. (2022): Effect of sodium silicate and salicylic acid on sodium and potassium ratio in wheat (*Triticum aestivum* L.) grown under salt stress. – Silicon 14(10): 5595-5600.
- [22] Najafi, R., Kappel, N., Mozafarian, M. (2025): The role of exogenously applied polyamines to improve heat tolerance in tomatoes: a review. – Agriculture 15(9): 988.
- [23] Parrotta, L., Sobieszczuk-Nowicka, E., Cai, G. (2023): Editorial: polyamines and longevity—role of polyamine in plant survival. – Frontiers in Plant Science 14: 3. DOI: <https://dx.doi.org/10.3389/fpls.2023.1232386>
- [24] Shah, M. J., Mahadi, M. H., Fahad, S. A., Nadiyah, M. A., Basmah, M. A., Khaled, M. R., Eslam, S. A. B., Dikhnah, A., Dilduza, J., Doha, A. A., Eldessoky, S. D., Mohamed, F. M. I., Shirong, G. (2022): Exogenous putrescine increases heat tolerance in tomato seedlings by regulating chlorophyll metabolism and enhancing antioxidant defense efficiency. – Plants 11(8): 1038-1038.
- [25] Si, L. B., Li, J. M., Li, G. Y., Jiang, X. Y., Lv, L. R., Yang, Y. L. (2020): Effects of tea polyphenols on physiological characteristics in leaves of wheat seedlings under salt stress. – Acta Ecologica Sinica 40(11): 3747-3755.
- [26] Siddique, A., Kumar, P. (2018): Physiological and biochemical basis of pre-sowing soaking seed treatments-an overview. – Plant Archives 18(2): 1931-1935.
- [27] Sun, Y., Liu, Y., Wang, Q. J., Di, Y. H., Wang, C. H., Wang, J. (2024): Effects of different soaking agents on salt tolerance of wheat seeds during germination under Na₂SO₄ stress. – Journal of Triticeae Crops 44(1): 101-109.
- [28] Wang, M. M., Zhao, G. Q., Liang, G. L., Chai, J. K., Li, N. J., Zhou, X. R. (2021): Physiological response of different salt-tolerant oats to salt stress. – Acta Pratacultural Science 38(11): 2200-2209.
- [29] Yu, Y. H., Wang, S. Y., Yan, X. N., Cong, R. Z., Wang, X. H., Huang, Y. (2019): Effects of different treatments on seed germination of *Pinus pumila*. – Journal of Temperate Forestry Research 2(3): 52-57.
- [30] Yuan, Y. Q. (2017): Physiological and Proteomic bases of Putrescine Alleviating Salt Stress Induced Injuries in Cucumber Plants. – Nanjing Agricultural University, Nanjing .
- [31] Zhang, Z. L., Li, X. F. (2016): Direction for Plant Physiological Experiments. 5rd Ed. – Higher Education Press, Beijing.
- [32] Zheng, J. Z., Zhang, T. F., Yang, W. T., Zheng, T. C. (2017): National authorized new wheat variety ‘Fengdecunmail’. – Journal of Triticeae Crops 37(06): 855.