https://doi.org/10.17221/312/2023-PSE

Putrescine improves salt tolerance of wheat seedlings by regulating ascorbate and glutathione metabolism, photosynthetic performance, and ion homeostasis

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Citation: Zhao X.L., Zhang Y.B., Zhang X.Q., Shan C.J. (2023): Putrescine improves salt tolerance of wheat seedlings by enhancing ascorbate and glutathione metabolism, photosynthetic performance, and ion homeostasis. Plant Soil Environ., 69: 512–521.

Abstract: To supply more insights into the roles of putrescine (Put) in alleviating salt stress in wheat crops, we explored the effects of Put on ascorbate (ASC) and glutathione metabolism, photosynthetic performance, and ion homeostasis in leaves of salt-stressed wheat seedlings. Our results displayed that salt stress increased the activities of enzymes in ASC and glutathione metabolism, including ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase, gamma-glutamylcysteine synthetase, and L-galactono-1,4--lactone dehydrogenase, which increased reduced ascorbate (AsA), dehydroascorbate (DHA), reduced glutathione (GSH), oxidised glutathione (GSSG), total ASC and total glutathione contents. Whereas, salt stress induced higher increases in the contents of DHA and GSSG than those of AsA and GSH, which led to reduced AsA/DHA and GSH/GSSG. Meanwhile, salt stress reduced photosynthetic rate (P_n), maximum photochemical efficiency of PSII (F_v/F_m), and the contents of chlorophyll and carotenoids, and destroyed Na^+/K^+ homeostasis, which further inhibited plant growth. In comparison with salt stress alone, Put strengthened the activities of the above enzymes, which further increased the above metabolites contents, as well as AsA/DHA and GSH/GSSG in leaves of salt-treated seedlings. In this way, Put reduced malondialdehyde content and electrolyte leakage. Besides, Put also increased P_n, F_v/F_m, and above pigments contents, and maintained Na^+/K^+ homeostasis. Meanwhile, Put increased plant height and biomass of salt-treated seedlings. The present findings clearly implied that Put enhanced salt tolerance of wheat crops by strengthening ASC and glutathione metabolism, photosynthetic performance, and maintaining ion homeostasis in leaves. Therefore, Put can be applied to strengthen the salt tolerance of wheat crops in production and cultivation.

Keywords: salinity; polyamine; resistance; antioxidant; *Triticum aestivum* L.

In the world, salt stress limits wheat growth and production. Salt stress often induces oxidative damage by leading to the accumulation of excess reactive oxygen species (Kapoor and Hasanuzzaman 2020). Fortunately, plants can counteract oxidative damage through the antioxidant system (Soliman et al. 2020). In the antioxidant system, reduced ascorbate (AsA) and reduced glutathione (GSH) are major antioxidants (Mohsin et al. 2020). Their contents can be regulated through their regeneration and biosynthesis (Zhu et al. 2021). L-galactono-1,4-lactone dehydrogenase (GalLDH) and gamma-glutamylcysteine synthetase (γ-ECS) are the key enzymes for the biosynthesis of AsA and GSH, respectively. Ascorbate-glutathione (AsA-GSH) cycle is in charge of their regeneration. In this cycle, ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) are in charge of their operation (Maslennikova et al. 2022). Through this cycle, plants can not only realise AsA and GSH regeneration but also scavenge hydrogen peroxide (H_2O_2). Therefore, the metabolism of ASC and glutathione in plants played vital roles in counteracting salt stress.

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Exogenous substances could regulate AsA and GSH contents in plants by regulating their metabolism in fighting against abiotic stresses, including rare earth elements, trace element selenium, signal molecules, and plant growth regulators (Wang et al. 2011, Shan and Yang 2017, Zhu et al. 2021, Alharbi and Alaklabi 2022). For salt stress, Alharbi and Alaklabi (2022) reported that plant growth regulator jasmonates strengthened the ascorbate and glutathione metabolism of wheat crops. Polyamines (PAs) are also important plant growth regulators in regulating plant growth, the absorption of inorganic ions, and plant resistance (Kaur and Das 2022, Li et al. 2022). Increasing evidence showed that PAs enhanced plant tolerance to salt, drought, and chilling stresses (Buffagni et al. 2022, Dong et al. 2022, Ozmen et al. 2022). As one of the PAs, putrescine (Put) is a ubiquitous low-molecular-weight polyamine and contains two amino groups. It is a central product of the polyamine biosynthetic pathway and acts as a precursor of spermidine and spermine. More and more studies also showed that Put played a vital role in protecting plants against salt stress and drought stress (Ghalati et al. 2020, Zhao et al. 2022). For wheat crops, it has been documented that Put could alleviate adversity injuries caused by heat and drought stresses (Doneva et al. 2021, Kolupaev et al. 2021). Nevertheless, it is still unclear whether Put can regulate salt tolerance of wheat crops through ASC and glutathione metabolism. Hence, it is of significance to explore the influences of Put on ASC and glutathione metabolism in salt-stressed wheat crops.

Increasing literature showed that salt stress also inhibited plant growth by inducing a drop in the photosynthetic performance and destroying ion homeostasis (Ghalati et al. 2020, Mohsin et al. 2020). Meanwhile, increasing research also showed that exogenous substances could mitigate salt stress by enhancing the photosynthetic performance and maintaining ion homeostasis, which further enhanced plant growth. For Put, many reports showed that it also mitigated salt stress by enhancing the photosynthetic performance and ion homeostasis of plants (Yuan et al. 2019, Ghalati et al. 2020, Liu et al. 2020). While it is also still unclear whether Put affects the photosynthetic performance and ion homeostasis of wheat crops. Thus, it will also be of significance to explore the influences of Put on the photosynthetic performance and ion homeostasis of wheat crops.

In this study, we explored the roles of Put in affecting plant growth indicators, chlorophyll (*Chl*) and carotenoids (Car) contents, photosynthetic rate (P_n) and maximum photochemical efficiency of PSII (F_v/F_m), Na^+ and K^+ contents, Na^+/K^+ ratio, malondialdehyde (MDA) content, electrolyte leakage (EL), the activities of enzymes in ASC and glutathione metabolism, AsA, GSH, total ASC and total glutathione contents, as well as AsA/DHA and GSH/GSSG in leaves of salt-stressed wheat seedlings. The purpose of this research was to elucidate whether and how Put enhanced the salt tolerance of wheat crops, which will provide new insights into its application in wheat production and cultivation.

MATERIAL AND METHODS

Plant material and treatments. The seeds of wheat (*Triticum aestivum* L., cv. Bainong207) were germinated and cultured in an artificial climate chamber. The day/night temperature, photosynthetic active radiation, photoperiod, and relative humidity were set as 28–30/18–20 °C, 500 μmol/m^2/s, 12 h and 60%, respectively. When the second leaves were fully expanded, seedlings were watered by the half-strength Hoagland's solution which was exchanged every other day. When the third leaves were fully expanded, seedlings with similar height and growth status were used to explore the effects of Put alone or plus salt stress on ASC and glutathione metabolism, photosynthetic performance, and ion homeostasis in leaves of seedlings.

The treatment concentration of NaCl used in this study was selected from 30, 60, 90, and 120 mmol/L NaCl. Five groups of wheat seedlings were respectively treated with 0, 30, 60, 90, and 120 mmol/L NaCl. After 7 days of treatment, the seedlings treated with 120 mmol/L NaCl showed obvious wilting phenomenon, the seedlings treated with 30 and 60 mmol/L NaCl all showed no obvious wilting phenomenon, and the seedlings treated with 90 mmol/L NaCl showed slight wilting phenomenon. Therefore, we selected 90 mmol/L NaCl as the treatment concentration for the current study. The roots of wheat seedlings were placed in beakers containing 500 mL 90 mmol/L NaCl and then treated for 7 days. To ensure a dark environment for the root system, the beakers were all wrapped with aluminum foil. To investigate the influences of Put, three groups of plants were respectively treated with 0.5, 1.0, and 3.0 mmol/L Put for 6 h and then exposed to 90 mmol/L NaCl for 7 days. Control seedlings were treated with the half-strength Hoagland's solution alone. To investigate the effect

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of the most beneficial 1 mmol/L Put concentration alone on the activities of enzymes in ASC and glutathione metabolism, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 7 days. Each treatment was repeated with four replications, with 6 seedlings per replication. After 3 days of treatment, the third leaves of wheat seedlings were sampled and frozen with liquid nitrogen. All frozen leaf samples were then rapidly kept at –80 °C until the analyses of physiological and biochemical indicators. After 7 days of treatment, plant growth indicators of wheat seedlings were measured.

Measurement of APX, GR, DHAR, and MDHAR. The extraction and the activity of APX (EC 1.11.1.11) were measured according to Nakano and Asada (1981). The extraction and the activity of GR (EC 1.6.4.2) were analysed according to Grace and Logan (1996). The extraction and the activity of MDHAR (EC 1.6.5.4) were analysed according to Miyake and Asada (1992). The extraction and the activity of DHAR (EC 1.8.5.1) were analysed according to Dalton et al. (1986). The activities of the above enzymes were expressed as U/g fresh weight (FW).

Measurement of GalLDH and γ-ECS. GalLDH (EC 1.3.2.3) was extracted and analysed according to Tabata et al. (2001). γ-ECS (EC 6.3.2.2) was extracted and analysed according to Rüegsegger and Brunold (1992).

Measurement of AsA, GSH, total ASC, total glutathione, AsA/DHA, and GSH/GSSG. AsA and total ASC contents were analysed according to Hodges et al. (1996). DHA content was calculated as the difference between total ASC and AsA. AsA/DHA was expressed as the ratio of AsA content to DHA content. Total glutathione and GSSG contents were analysed according to Griffith (1980). GSH content was calculated as the difference between total glutathione and GSSG. GSH/GSSG was expressed as the ratio of GSH content to GSSG content.

Measurement of MDA content and EL. MDA content was measured according to Hodges et al. (1999). EL was measured according to Zhao et al. (2004).

Measurement of *Chl*, Car, P_n, and F_v/F_m. *Chl* and Car contents were analysed according to Song et al. (2016). P_n was measured by using the photosynthesis system (Licor-6400, Lincoln, USA). Top fully expanded leaves were equilibrated and then P_n was recorded. *Chl* fluorescence parameter F_v/F_m was measured by using a Yaxin-1161G fluorometer (Yaxin, China). After leaf dark adaptation for 30 min, F_v/F_m was measured.

Measurement of Na^+ and K^+ contents and Na^+/K^+ ratio in leaves. Na^+ and K^+ contents were determined according to Afrangan et al. (2023). Oven-dried leaf samples (0.5 g) were milled and ashed in the electric oven. Then the ash was digested with 1 mol/L HCl. K^+ and Na^+ contents were measured by using flame photometry. Na^+/K^+ was expressed as the ratio of Na^+ content to K^+ content.

Measurement of plant height and biomass. Plant height was measured by using a meter ruler. The fresh biomass of seedlings was first weighed and recorded. Then seedlings were oven dried for 72 h at 80 °C. Finally, dry biomass was weighed and recorded.

Statistical analysis. Data shown in tables and figures was the mean of four replications. Means were compared by one-way analysis of variance and Duncan's multiple range test at the 5% level of significance.

RESULTS

Effects of Put on the activities of enzymes responsible for ASC and glutathione metabolism. In comparison with control, salt stress markedly increased APX, GR, DHAR, MDHAR, γ-ECS, and GalLDH activities (Table 1). Compared to salt stress alone, all treatments with Put plus salt stress markedly further increased the activities of the above enzymes. Among different concentrations, 1.0 mmol/L Put showed better effects on the above enzymes than other concentrations. Compared to salt stress alone, 1.0 mmol/L Put increased APX, GR, DHAR, MDHAR, γ-ECS, and GalLDH activities by 89.5, 60.7, 85.7, 85.7, 90.9 and 92.9%, respectively. Compared with control, 1.0 mmol/L Put alone also increased APX, GR, DHAR, MDHAR, γ-ECS, and GalLDH activities. The above findings suggested that Put could enhance salt tolerance by enhancing ASC and glutathione metabolism.

Effects of Put on the contents of AsA, total ASC, GSH, and total glutathione and the ratios of AsA/DHA and GSH/GSSG. In comparison with control, salt stress markedly increased AsA, total ASC, GSH, and total glutathione contents (Table 2). However, salt stress markedly reduced AsA/DHA and GSH/GSSG. In comparison with salt stress alone, pretreatments with different concentrations of Put significantly increased the contents of the above metabolites, as well as AsA/DHA and GSH/GSSG. Among different concentrations, 1.0 mmol/L Put plus salt stress showed better effects on the above indicators than

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Table 1. Effects of putrescine (Put) on the activities of enzymes in ascorbate (ASC) and glutathione metabolism in salt-stressed wheat leaves

Treatment	APX	DHAR	MDHAR	GR	γ-ECS	GalLDH
	(U/g FW)					
Control	2.5 ± 0.29^{d}	1.5 ± 0.11^{d}	1.0 ± 0.12^{d}	2.0 ± 0.22^{d}	1.5 ± 0.20^{d}	1.0 ± 0.09^{d}
SS	3.8 ± 0.33^{c}	2.1 ± 0.23^{c}	1.4 ± 0.15^{c}	2.8 ± 0.31^{c}	2.2 ± 0.27^{c}	1.4 ± 0.15^{c}
0.5 Put + SS	5.1 ± 0.58^{b}	2.8 ± 0.22^{b}	1.8 ± 0.21^{b}	3.6 ± 0.30^{b}	3.1 ± 0.40^{b}	2.0 ± 0.24^{b}
1.0 Put + SS	7.2 ± 0.61^{a}	3.9 ± 0.43^{a}	2.6 ± 0.32^{a}	4.5 ± 0.51^{a}	4.2 ± 0.37^{a}	2.7 ± 0.31^{a}
3.0 Put + SS	6.0 ± 0.53^{b}	3.1 ± 0.28^{b}	2.0 ± 0.19^{b}	3.4 ± 0.39^{b}	3.0 ± 0.26^{b}	2.1 ± 0.21^{b}
1.0 Put	4.7 ± 0.41^{b}	2.9 ± 0.34^{b}	1.9 ± 0.25^{b}	3.2 ± 0.36^{b}	2.8 ± 0.33^{b}	1.8 ± 0.22^{b}

The seedlings were treated as follows: control – half-strength Hoagland's solution; SS – 90 mmol/L NaCl; 0.5 Put + SS – 0.5 mmol/L Put + 90 mmol/L NaCl; 1.0 Put + SS – 1.0 mmol/L Put + 90 mmol/L NaCl; 3.0 Put + SS – 3.0 mmol/L Put + 90 mmol/L NaCl, 1.0 Put – 1.0 mmol/L Put. The plants were pretreated with different concentrations of Put for 6 h, and then exposed to 90 mmol/L NaCl for 3 days. In order to investigate the effect of the most beneficial 1 mmol/L Put concentration alone on the activities of enzymes in ASC and glutathione metabolism, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 3 days. Values represent mean ± standard deviation (n = 4), small letters indicate statistical difference at $P < 0.05$; APX – ascorbate peroxidase; DHAR – dehydroascorbate reductase; MDHAR – monodehydroascorbate reductase; GR – glutathione reductase; γ-ECS – gamma-glutamylcysteine synthetase; GalLDH – L-galactono-1,4-lactone dehydrogenase; FW – fresh weight

other concentrations. Compared to salt stress alone, 1.0 mmol/L Put plus salt stress increased AsA/DHA, GSH/GSSG, AsA content, total ASC content, GSH content, and total glutathione content by 98.7, 109.5, 57.1, 47.0, 63.0 and 50.8%, respectively. Compared with the control, 1.0 mmol/L Put alone also increased AsA/DHA, GSH/GSSG, AsA content, total ASC content, GSH content, and total glutathione content. These findings suggested that Put could increase AsA and GSH contents and maintain their redox state, which further enhanced salt tolerance. The above results once again suggested that Put could enhance ASC and glutathione metabolism in salt-stressed wheat crops.

Effects of Put on MDA content and EL. In comparison with control, salt stress markedly made a rise

Table 2. Effects of putrescine (Put) on the contents of ascorbate (AsA), total ascorbate (ASC), reduced glutathione (GSH) and total glutathione and the ratios of AsA/dehydroascorbate (DHA) and glutathione (GSH)/oxidised glutathione (GSSG) in leaves of salt-stressed wheat seedlings

Treatment	AsA	Total ascorbate	AsA/DHA	GSH	Total glutathione	GSH/GSSG
	(μmol/g FW)			(μmol/g FW)		
Control	2.70 ± 0.29^{d}	2.84 ± 0.23^{d}	19.00 ± 1.75^{b}	0.43 ± 0.04^{d}	0.45 ± 0.05^{d}	21.50 ± 2.32^{b}
SS	3.50 ± 0.28^{c}	4.02 ± 0.31^{c}	6.69 ± 0.59^{f}	0.54 ± 0.06^{c}	0.63 ± 0.06^{c}	6.00 ± 0.50^{f}
0.5 Put + SS	4.30 ± 0.37^{b}	4.83 ± 0.45^{b}	8.09 ± 0.77^{e}	0.69 ± 0.07^{b}	0.78 ± 0.07^{b}	7.67 ± 0.71^{e}
1.0 Put + SS	5.50 ± 0.57^{a}	5.91 ± 0.53^{a}	13.29 ± 1.52^{c}	0.88 ± 0.08^{a}	0.95 ± 0.08^{a}	12.57 ± 1.38^{c}
3.0 Put + SS	4.40 ± 0.40^{b}	4.84 ± 0.40^{b}	10.11 ± 1.10^{d}	0.72 ± 0.07^{b}	0.80 ± 0.07^{b}	9.00 ± 0.97^{d}
1.0 Put	3.82 ± 0.34^{bc}	3.98 ± 0.36^{c}	23.88 ± 2.67^{a}	0.60 ± 0.06^{b}	0.62 ± 0.07^{c}	30.00 ± 3.75^{a}

The seedlings were treated as follows: control – half-strength Hoagland's solution; SS – 90 mmol/L NaCl; 0.5 Put + SS – 0.5 mmol/L Put + 90 mmol/L NaCl; 1.0 Put + SS – 1.0 mmol/L Put + 90 mmol/L NaCl; 3.0 Put + SS – 3.0 mmol/L Put + 90 mmol/L NaCl, 1.0 Put – 1.0 mmol/L Put. The plants were pretreated with different concentrations of Put for 6 h, and then exposed to 90 mmol/L NaCl for 3 days. In order to investigate the effect of the most beneficial 1 mmol/L Put concentration alone on the contents of AsA, total ASC, GSH and total glutathione and the ratios of AsA/DHA and GSH/GSSG, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 3 days. Values represent mean ± standard deviation (n = 4), small letters indicate statistical difference at $P < 0.05$; FW – fresh weight

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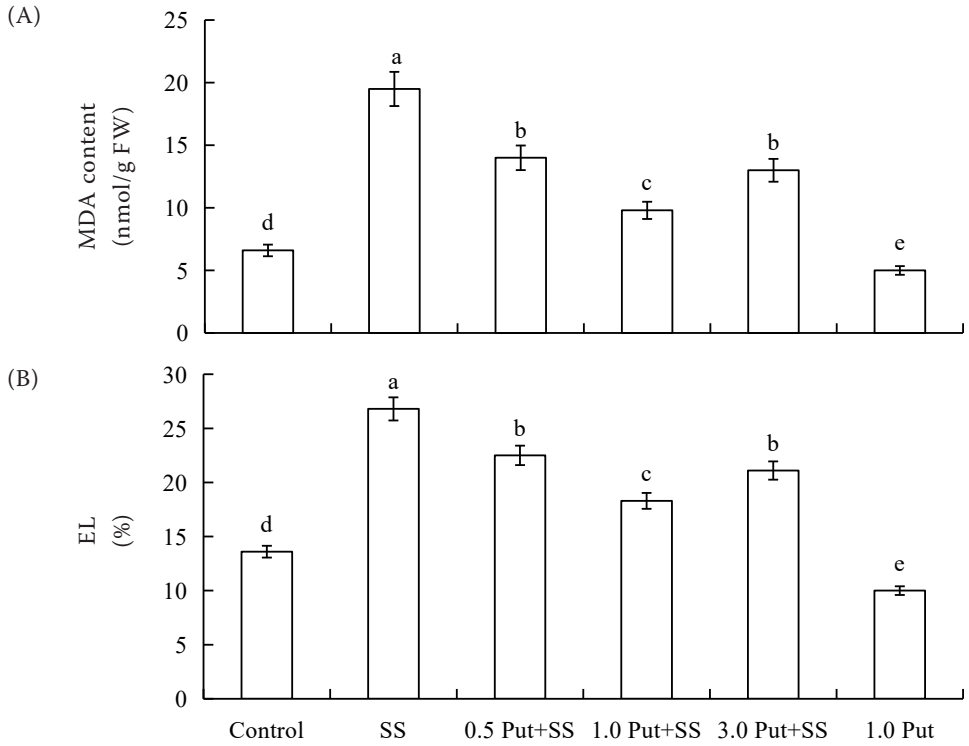

Figure 1. Effects of putrescine (Put) on malondialdehyde (MDA) content and electrolyte leakage (EL) of wheat seedlings leaves under salt stress. The seedlings were treated as follows: control – half-strength Hoagland's solution; SS – 90 mmol/L NaCl; 0.5 Put + SS – 0.5 mmol/L Put + 90 mmol/L NaCl; 1.0 Put + SS – 1.0 mmol/L Put + 90 mmol/L NaCl; 3.0 Put + SS – 3.0 mmol/L Put + 90 mmol/L NaCl, 1.0 Put – 1.0 mmol/L Put. The plants were pretreated with different concentrations of Put for 6 h and then exposed to 90 mmol/L NaCl for 3 days. To investigate the effect of the most beneficial 1 mmol/L Put concentration alone on malondialdehyde content and electrolyte leakage, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 3 days. Values represent mean ± standard deviation (n = 4), small letters indicate statistical difference at $P < 0.05$; FW – fresh weight

in MDA content and EL (Figure 1). In comparison with salt stress alone, all treatments with Put plus salt stress markedly decreased the above indicators under salt stress. Among different concentrations, the application of 1.0 mmol/L Put plus salt stress showed better effects on the above indicators than other concentrations. Compared to salt stress alone, 1.0 mmol/L Put plus salt stress reduced MDA content and EL by 49.7% and 31.7%, respectively. Compared with control, 1.0 mmol/L Put alone also reduced MDA content and EL. The above results suggested that Put could increase the antioxidant capacity of wheat seedlings, which further enhanced salt tolerance.

Effect of Put on the photosynthetic performance. In comparison with the control, salt stress markedly made a drop in P_n, F_v/F_m, *Chl*, and Car contents (Table 3). In comparison with salt stress alone, all treatments with Put plus salt stress markedly increased above indicators under salt stress. Among different concentrations, the application of 1.0 mmol/L Put plus salt stress showed better effects on the above indicators than other concentrations. Compared to salt stress alone, 1.0 mmol/L Put increased P_n, F_v/F_m, *Chl* content, and Car content by 55.4, 44.6, 41.6, and 72.0%, respectively. Compared with the control, 1.0 mmol/L Put alone also increased P_n, F_v/F_m, *Chl* content, and Car content. The above findings suggested that Put enhanced the photosynthetic performance of salt-stressed wheat seedlings.

Effects of Put on Na^+ and K^+ contents and Na^+/K^+ ratio in leaves. In comparison with control, salt stress markedly increased Na^+ content and reduced K^+ content in wheat leaves (Table 4). Thus, salt stress significantly increased the Na^+/K^+ ratio. Compared to salt stress alone, all treatments with Put plus salt stress markedly decreased Na^+ content and increased K^+ content, which further decreased the Na^+/K^+ ratio. Among different concentrations, the application of

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Table 3. Effects of putrescine (Put) on photosynthetic rate (P_n), maximum photochemical efficiency of PSII (F_v/F_m) and the contents of chlorophyll (*Chl*) and carotenoids (Car) in leaves under salt stress

Treatment	*Chl* (mg/g FW)	Car (mg/g FW)	P_n (μmol/m^2/s)	F_v/F_m
Control	1.90 ± 0.16^{b}	0.55 ± 0.05^{b}	20.3 ± 1.40^{b}	0.76 ± 0.03^{b}
SS	1.13 ± 0.10^{e}	0.25 ± 0.03^{e}	11.2 ± 1.22^{e}	0.55 ± 0.01^{e}
0.5 Put + SS	1.37 ± 0.10^{d}	0.33 ± 0.03^{d}	14.5 ± 1.13^{d}	0.61 ± 0.02^{d}
1.0 Put + SS	1.60 ± 0.12^{c}	0.43 ± 0.05^{c}	17.4 ± 1.25^{c}	0.70 ± 0.02^{c}
3.0 Put + SS	1.50 ± 0.11^{cd}	0.38 ± 0.04^{cd}	15.6 ± 1.40^{cd}	0.64 ± 0.02^{d}
1.0 Put	2.28 ± 0.18^{a}	0.68 ± 0.07^{a}	23.8 ± 1.80^{a}	0.83 ± 0.03^{a}

The seedlings were treated as follows: control – half-strength Hoagland's solution; SS – 90 mmol/L NaCl; 0.5 Put + SS – 0.5 mmol/L Put + 90 mmol/L NaCl; 1.0 Put + SS – 1.0 mmol/L Put + 90 mmol/L NaCl; 3.0 Put + SS – 3.0 mmol/L Put + 90 mmol/L NaCl, 1.0 Put – 1.0 mmol/L Put. The plants were pretreated with different concentrations of Put for 6 h, and then exposed to 90 mmol/L NaCl for 3 days. In order to investigate the effect of the most beneficial 1 mmol/L Put concentration alone on P_n, F_v/F_m and the contents of *Chl* and Car, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 3 days. Values represent mean ± standard deviation (n = 4), small letters indicate statistical difference at $P < 0.05$; FW – fresh weight

1.0 mmol/L Put plus salt stress showed better effects on the above indicators than other concentrations. Compared to salt stress alone, 1.0 mmol/L Put plus salt stress increased K^+ content by 55.6% and decreased Na^+ content by 46.1%, which further decreased the Na^+/K^+ ratio by 65.5%. Compared with control, 1.0 mmol/L Put alone also increased K^+ content and decreased Na^+ content. These results indicated that Put maintained Na^+/K^+ homeostasis in leaves, which further enhanced the salt tolerance of wheat crops.

Effects of Put on plant height and biomass. In comparison with the control, salt stress markedly led to decreases in plant height and biomass (Figure 2). In comparison with salt stress alone, all treatments with Put plus salt stress markedly increased plant height and biomass. Among different concentrations, 1.0 mmol/L Put plus salt stress showed better effects on the above indicators than other concentrations. Compared to salt stress alone, 1.0 mmol/L Put plus salt stress increased plant height and biomass by 33.5% and 69.3%, respectively. Compared with control, 1.0 mmol/L Put alone also increased plant height and biomass. The above findings directly suggested that Put enhanced the salt tolerance of wheat seedlings.

DISCUSSION

Increasing research demonstrated that salt stress leads to oxidative damage in plants. The findings of this study also suggested that salt stress-induced oxidative damage in wheat seedlings is indicated by MDA and EL. Increasing studies showed that Put could alleviate oxidative damage in stressed plants by strengthening the antioxidant capacity, including heat, drought and salt stresses (Hassan et al. 2020, Jahan et al. 2022). It has been reported that Put could be used

Table 4. Effects of putrescine (Put) on Na^+ and K^+ contents and Na^+/K^+ ratio in leaves under salt stress

Treatment	Na^+ content	K^+ content	Na^+/K^+ ratio
	(mg/g DW)		
Control	0.8 ± 0.09^{d}	16.8 ± 1.50^{a}	0.05 ± 0.01^{e}
SS	2.6 ± 0.23^{a}	9.0 ± 0.80^{d}	0.29 ± 0.03^{a}
0.5 Put + SS	2.1 ± 0.18^{b}	11.5 ± 1.00^{c}	0.18 ± 0.02^{b}
1.0 Put + SS	1.4 ± 0.10^{c}	14.0 ± 1.10^{b}	0.10 ± 0.01^{d}
3.0 Put + SS	1.8 ± 0.16^{b}	13.0 ± 1.00^{bc}	0.14 ± 0.02^{c}
1.0 Put	0.6 ± 0.07^{e}	18.8 ± 2.20^{a}	0.03 ± 0.00^{f}

The seedlings were treated as follows: control – half-strength Hoagland's solution; SS – 90 mmol/L NaCl; 0.5 Put + SS – 0.5 mmol/L Put + 90 mmol/L NaCl; 1.0 Put + SS – 1.0 mmol/L Put + 90 mmol/L NaCl; 3.0 Put + SS – 3.0 mmol/L Put + 90 mmol/L NaCl, 1.0 Put – 1.0 mmol/L Put. The plants were pretreated with different concentrations of Put for 6 h, and then exposed to 90 mmol/L NaCl for 3 days. In order to investigate the effect of the most beneficial 1 mmol/L Put concentration alone on Na^+ and K^+ contents and Na^+/K^+ ratio, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 3 days. Values represent mean ± standard deviation (n = 4), small letters indicate statistical difference at $P < 0.05$; DW – dry weight

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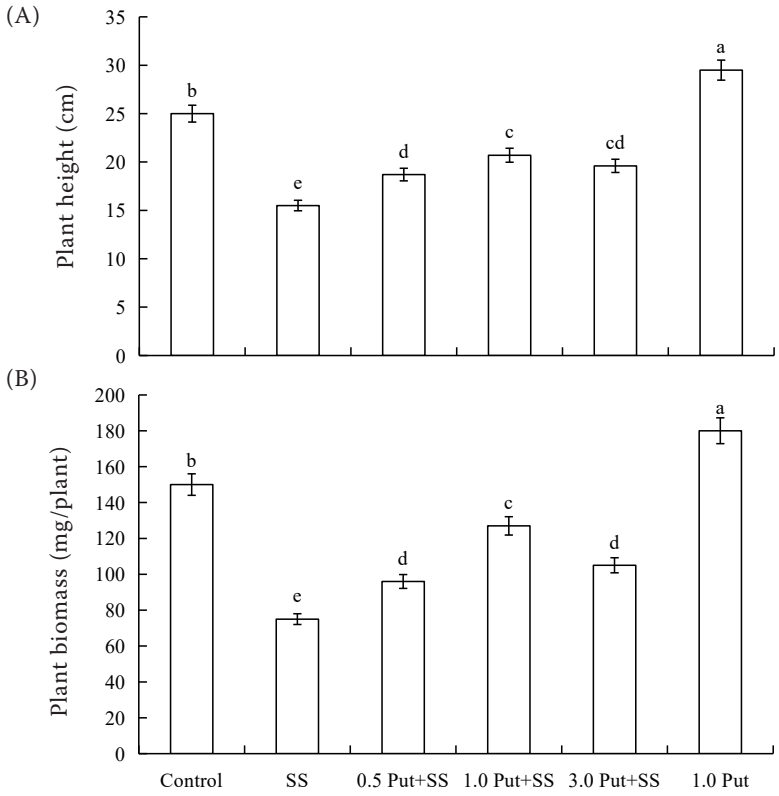

Figure 2. Effects of putrescine (Put) on plant height and biomass of salt-stressed wheat seedlings. The seedlings were treated as follows: control – half-strength Hoagland's solution; SS – 90 mmol/L NaCl; 0.5 Put + SS – 0.5 mmol/L Put + 90 mmol/L NaCl; 1.0 Put + SS – 1.0 mmol/L Put + 90 mmol/L NaCl; 3.0 Put + SS – 3.0 mmol/L Put + 90 mmol/L NaCl, 1.0 Put – 1.0 mmol/L Put. The plants were pretreated with different concentrations of Put for 6 h and then exposed to 90 mmol/L NaCl for 7 days. To investigate the effect of the most beneficial 1 mmol/L Put concentration alone on plant height and biomass, the plants were pretreated with 1 mmol/L Put for 6 h, and then exposed to half-strength Hoagland's solution for 3 days. Values represent mean ± standard deviation (n = 4), small letters indicate statistical difference at $P < 0.05$

to fight against salt stress in guava, cucumber, and tea (Xiong et al. 2018, Wu et al. 2019, Ghalati et al. 2020). Kapoor and Hasanuzzaman (2020) found that Put could alleviate salt stress in *Luffa acutangula* by regulating ASC and glutathione metabolism *via* APX and GR. While, there is still no knowledge about the effects of Put on ASC and glutathione metabolism in salt-stressed wheat plants. For this research, the results demonstrated that Put also increased APX and GR activities in leaves of salt-stressed wheat, which agreed with the results of Kapoor and Hasanuzzaman (2020) on *Luffa acutangula*. In addition, the present study also demonstrated that Put increased MDHAR, DHAR, γ-ECS, and GalLDH activities in leaves of salt-stressed seedlings. Meanwhile, current findings also displayed that Put increased the contents of metabolites in ASC and glutathione metabolism, as well as AsA/DHA and GSH/GSSG. Our current results indicated that Put enhanced salt tolerance of wheat seedlings through ASC and glutathione metabolism, which induced the increases in AsA and GSH contents, as well as AsA/DHA and GSH/GSSG. In this way, Put strengthened the antioxidant capacity and maintained the redox state of salt-stressed wheat seedlings. Thus, our findings implied that Put could be applied to enhance wheat salt tolerance in production.

The influences of salt stress on plants can also be reflected in plant photosynthetic performance and growth. Salt stress reduced photosynthetic pigments *Chl* and Car contents in plants, as well as P_n and F_v/F_m, which further inhibited plant growth (Shu et al. 2019, Ghalati et al. 2020, da Silva et al. 2023). For current research, our results also displayed that salt stress reduced P_n, F_v/F_m, *Chl*, and Car contents, as well as plant height and biomass of wheat seedlings, which

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agreed with previous studies on other plants (Ghalati et al. 2020, Kapoor and Hasanuzzaman 2020). It has been reported that Put could enhance the photosynthetic performance by increasing P_n, F_v/F_m, *Chl,* and Car contents, which further promoted the growth of salt-stressed plants (Xiong et al. 2018, Wu et al. 2019, Ghalati et al. 2020, Kapoor and Hasanuzzaman 2020). However, it is still unclear whether Put affects the photosynthetic performance of salt-stressed wheat seedlings. For this study, the results also demonstrated that Put enhanced the photosynthetic performance of salt-stressed wheat seedlings by increasing P_n, F_v/F_m, *Chl,* and Car contents, which, in turn, enhanced the increases in plant height and biomass. As plant height and biomass are the most direct indicators to evaluate plant salt tolerance, the results of previous studies and current studies all suggested that Put enhanced salt tolerance of many plants, including wheat, guava, cucumber, tea, and *Luffa acutangula*. Previous research also displayed that Put could enhance the photosynthetic performance of salt-stressed cucumber seedlings by alleviating photoinhibition *via* the cooperation with cyclic electron flow, dissipating excess excitation energy and improving chlorophyll metabolism and xanthophyll cycle (Yuan et al. 2018, Shu et al. 2019, Wu et al. 2019). However, it is still unclear whether there is the same mechanism for the effect of Put on the photosynthetic performance of wheat seedlings as that of cucumber seedlings. Thus, it will also be interesting to further study the roles of Put in regulating cyclic electron flow, excess excitation energy, chlorophyll metabolism, and xanthophyll cycle of wheat seedlings, which will show more information for the roles of Put in strengthening salt tolerance of wheat crops.

For plants, salt stress often induces the discruption of ion homeostasis. Mohsin et al. (2020) found that salt stress destroyed K^+/Na^+ homeostasis by increasing the Na^+/K^+ ratio in wheat. Our research also displayed the same results as that of Mohsin et al. (2020). Maintaining ion homeostasis is an important mechanism to improve salt tolerance. It has been documented that Put could enhance the salt tolerance of cucumber seedlings by maintaining K^+/Na^+ homeostasis (Yuan et al. 2019). Liu et al. (2020) found that Put also played an important role in enhancing the salt tolerance of sugar beet by maintaining ion homeostasis (Liu et al. 2020). However, it is still unclear whether there is the same effect of Put on K^+/Na^+ homeostasis of salt-stressed wheat seedlings as that of other plants. In this study, our findings demonstrated that Put also maintained K^+/Na^+ homeostasis by reducing the Na^+/K^+ ratio, which was consistent with the results on other plants (Hassan et al. 2020). Previous studies also showed that Put maintained K^+/Na^+ homeostasis by interacting with ion channels and inducing the generation of H_2O_2 in cucumber seedlings exposed to salt stress (Yuan et al. 2019). However, it is still unclear whether there is the same mechanism for the role of Put in maintaining K^+/Na^+ homeostasis of wheat seedlings as that of cucumber seedlings. Thus, it will also be interesting to study the roles of Put in regulating K^+/Na^+ homeostasis of salt-stressed wheat seedlings, which can provide new information for the roles of Put in strengthening the salt tolerance of wheat crops.

In this study, we found that 0.5 and 1.0 mmol/L Put had positive effects on the antioxidant capacity, photosynthetic performance, growth, and K^+/Na^+ homeostasis of wheat seedlings under salt stress. While, 3 mmol/L Put rather decreased the parameters in combination with salt stress, indicating the negative effect. Thus, our results indicated that Put had dose effects on the salt tolerance of wheat crops. This phenomenon suggested that the selection of a proper Put concentration was important for its application in enhancing salt tolerance of crops in production and cultivation.

The exogenous application methods of Put include foliar application and root-zone application. For foliar application, many studies demonstrated that Put could enhance plant antioxidant capacity, photosynthetic performance, growth, and K^+/Na^+ homeostasis under stresses (Sharma et al. 2011, Xiong et al. 2018, Wu et al. 2019, Hassan et al. 2020, Jahan et al. 2022). For root-zone application, our current study and other studies also disclosed that Put enhanced plant antioxidant capacity, growth, and K^+/Na^+ homeostasis under stresses (Zhang et al. 2014, Yuan et al. 2019, Jahan et al. 2021). In addition, our current study also showed that root-zone application of Put enhanced the photosynthetic performance under salt stress. The above results of ours and other studies indicated that there was no difference in the effect of foliar application of Put and application to the roots. Thus, we can apply Put in the production and cultivation of crops through foliar or root-zone application. As we all know, a high concentration of Put will induce health and hygiene risks for humans or animals, a low concentration of Put will not induce these risks. When applied to plants, the suitable concentration of Put is usually low. Therefore, there are no health and

https://doi.org/10.17221/312/2023-PSE

hygiene risks for humans or animals when applied to plants in production and cultivation.

Our findings demonstrated that Put enhanced ASC and glutathione metabolism by increasing APX, GR, MDHAR, DHAR, GalLDH, and γ-ECS activities. Meanwhile, Put enhanced wheat salt tolerance by maintaining K^+/Na^+ homeostasis. Through the above mode of action, Put improved the photosynthetic performance and growth of wheat seedlings under salt stress. In the above Put-influenced process/pathway in wheat seedlings exposed to salt stress, ASC and glutathione metabolism were the most important, which was due to their importance in maintaining the redox state of salt-stressed wheat seedlings and further influenced other processes. The above results show new knowledge of the roles of Put in enhancing the salt tolerance of wheat crops. Therefore, Put can be applied to strengthen the salt tolerance of wheat crops in production and cultivation.

Acknowledgement. We thank Dr. Li for his valuable help in the English language. This research was supported by 2022 Henan Province Undergraduate University Research Teaching Demonstration Course "Biochemistry".

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Received: August 1, 2023
Accepted: October 23, 2023
Published online: November 1, 2023