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Poly-glutamic acid mitigates the negative effects of salt stress on wheat seedlings by regulating the photosynthetic performance, water physiology, antioxidant metabolism and ion homeostasis

Qidi Zhu, Yanyan Li, Niuniu Zhang, Yilin Wu, Xingqi Ou*

School of Agriculture, Henan Institute of Science and Technology, Xinxiang, Henan Province, P.R. China
**Corresponding author: zhuqidi@hist.edu.cn*

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Abstract: To uncover the regulatory metabolism of poly-glutamic acid (PGA) in protecting wheat crops against salt stress (SS) at the physiological level, we utilised hydroponic experiments to explore the roles of PGA in regulating the photosynthetic performance, water physiology, antioxidant metabolism and ion homeostasis of wheat seedlings exposed to SS for 10 days. The findings demonstrated that SS inhibited the photosynthetic performance of wheat seedlings. In contrast, different doses of PGA all improved the photosynthetic performance, especially for 0.3% PGA. Compared with SS, 0.3% PGA plus SS decreased nonphotochemical quenching (q_N) by 26.3% and respectively increased photosynthetic rate (P_n), soil and plant analyser development (SPAD) value, maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m), photochemical quenching (q_p) and actual photochemical efficiency of PSII (Y(II)) by 54.0, 27.8, 34.6, 42.4 and 25.8%. For water metabolism, SS destroyed the water balance of wheat seedlings. In contrast, different doses of PGA enhanced water balance, especially for 0.3% PGA. Compared with SS, 0.3% PGA plus SS decreased leaf water saturation deficit (LWSD) by 35.5% and respectively increased leaf relative water content (LRWC), transpiration rate (T_r), stomatal conductance (g_s) and the contents of soluble sugars (SSS) and proline (Pro) by 15.9, 94.7, 37.5, 44.6 and 62.3%. For antioxidant metabolism, SS induced the peroxide damage to wheat seedlings. In contrast, different doses of PGA all mitigated the SS-induced peroxide damage, especially for 0.3% PGA. Compared with SS, 0.3% PGA plus SS respectively decreased superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents and electrolyte leakage (EL) by 39.1, 29.6, 46.2 and 36.3%, and respectively increased superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductases (DHAR) and monodehydroascorbate reductase (MDHAR) activities, and antioxidants ascorbic acid (AsA) and glutathione (GSH) contents by 69.2, 49.2, 77.8, 80.6, 109.5, 121.7, 104.5, 63.8 and 39.6%. Besides, SS destroyed the ion homeostasis of wheat seedlings. In contrast, different doses of PGA all maintained ion homeostasis, especially for 0.3% PGA. Compared with SS, 0.3% PGA plus SS reduced Na^+ content by 40.7% and respectively increased K^+, Ca^{2+} and Mg^{2+} contents by 64.4, 82.6 and 105.6%, thereby respectively increasing K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios by 177.6, 209.4 and 244.8%. In the above ways, SS inhibited wheat height and biomass. In contrast, different doses of PGA all improved wheat height and biomass under SS, especially for 0.3% PGA. Compared with SS, 0.3% PGA plus SS, respectively, increased wheat height and biomass by 27.4% and 41.7%. In the above ways, PGA mitigated salt toxicity in wheat seedlings. The current findings implied that there was a potential for the use of PGA in real situations to improve wheat salt tolerance, especially for the 0.3% dose.

Keywords: *Triticum aestivum* L.; salinity; gas exchange parameters; antioxidant defence system; ionic imbalance

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Wheat (*Triticum aestivum* L.) is an annual or perennial herbaceous plant in the Poaceae and wheat genera. In the world, wheat is the second most cultivated cereal crop and is also a staple food for around 4.5 billion people worldwide (Nafees et al. 2023). However, it usually suffers from different abiotic and biotic stresses during growth. Among various abiotic stresses, salt stress (SS) is a common adversity wheat crops suffer from during growth and development. Meanwhile, the soil used to cultivate crops worldwide has become saltier, and more than 900 Mha of soil is affected by SS worldwide (Hopmans et al. 2021). By the year 2050, more than 50% of the arable agricultural land might be affected by salinity (Sadak et al. 2023). Thus, salinised soil will seriously affect the growth and productivity of crops, especially for wheat. As reported, SS usually disturbs plants' water balance, ion homeostasis, and antioxidant metabolism (Fairoj et al. 2023, Maqsood et al. 2023). In this way, SS induced damage to the photosynthetic performance, thereby inhibiting plant growth. To prevent wheat plants against SS, researchers found that many exogenous substances could be utilised to mitigate above negative effects of SS, such as melatonin (MT) (Yan et al. 2023), calcium (Ca) (Sadak et al. 2023), salicylic acid (SA) (Fairoj et al. 2023), spermine (Spm) (Talaat and Hanafy 2023) and hydrogen sulfide (H_2S) (Kumari et al. 2023). Thus, it will be very important to apply exogenous substances, especially for those which are friendly to plants and/or the environment, to improve wheat salt tolerance.

Poly-glutamic acid (PGA) is a high molecular weight polymer of glutamate. It has good water solubility and strong absorption, and its degradation product is non-toxic and pollution-free glutamate (Balogun-Agbaje et al. 2021). Previous research also showed that PGA promoted the growth of many crops, including cotton (*Gossypium herbaceum* L.), Chinese cabbage (*Brassica rapa* subsp. pekinensis), maize (*Zea mays* L.), Danshen (*Salvia miltiorrhiza*), and turnip (*Brassica rapa* L.) (Liang et al. 2019, Bai et al. 2020, 2022, Cao et al. 2022, Shan et al. 2024). PGA could also enhance the photosynthetic performance of crops, including Chinese cabbage, maize and *Salvia miltiorrhiza* (Ma et al. 2022, Quan et al. 2022, Shan et al. 2024). Lei et al. (2015) and Xu et al. (2020) displayed that PGA reinforced the antioxidant metabolism of rape seedlings. Shan et al. (2024) demonstrated that PGA also reinforced the antioxidant metabolism of *Salvia miltiorrhiza*. Besides, increasing research showed that PGA enhanced crop tolerance to stresses, including heat, cold, drought, and salt (Lei et al. 2015, Guo et al. 2017, Ma et al. 2022, Quan et al. 2022). As far as we have reviewed the literature, few papers have evaluated the effects of PGA on the salt tolerance of crops at the physiological level. For wheat crops, the work was only done on the effects of PGA on the K^+-Na^+ balance and the activities of antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) under SS at the physiological level (Guo et al. 2017). However, no work was done on the effects of PGA on the balance between Na^+ and other ions, the activities of antioxidant enzymes in the ascorbate-glutathione (AsA-GSH) cycle, the contents of non-enzymatic antioxidants ascorbic acid (AsA) and glutathione (GSH), photosynthetic performance and water physiology under SS. Therefore, more details are still needed to uncover and clarify the regulatory mechanism of PGA in enhancing wheat salt tolerance at the physiological level.

In this study, we postulated that PGA mitigated salt toxicity in wheat seedlings by modulating photosynthetic performance, water physiology, antioxidant metabolism, and ion homeostasis. To verify this hypothesis, we investigated the effects of different PGA concentrations on indicators related to photosynthetic performance, water physiology, antioxidant metabolism, ion homeostasis and plant growth. Through this investigation, we can uncover more details about the regulatory mechanism of PGA in alleviating salt toxicity in wheat seedlings, which will show more insights into the theoretical basis for the potential use of PGA in real situations to improve wheat salt tolerance.

MATERIAL AND METHODS

Plant material and treatments. Wheat seeds of cultivar Bainong207 were used in this study. Seeds with plump grains and consistent sizes were chosen. Then, the seeds were surface-sterilised in 96% ethanol for 10 min. After that, the seeds were washed thoroughly with distilled water. Then the seeds were germinated in Petri dishes containing distilled-water-moistened filter paper and cultured in an artificial climate chamber at day/night temperature of 28–30/18–20 °C, an irradiance of 500 μmol/m^2/s, a 12 h photoperiod and relative humidity of 60%. After the seedlings fully unfolded the first leaves, they were still grown in Petri dishes containing distilled water. After the seedlings fully unfolded the second leaves,

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they were all treated with half-strength Hoagland's solution by submersing their roots in the solution to provide the nutrients for growth. After the third leaves were fully unfolded, seedlings with similar height and growth status were chosen for the next experiments.

The treatment concentration of NaCl (90 mmol/L NaCl) was chosen according to Zhao et al. (2023). The solution of NaCl was prepared by dissolving 90 mmol NaCl in 1 L half-strength Hoagland's solution. The seedlings were treated by SS by submersing their roots in 500 mL 90 mmol/L NaCl contained in beakers for 10 days. To keep the roots dark, beakers were wrapped in aluminium foil. To explore the effects of PGA, the seedlings were treated by 0.1, 0.3 and 0.6% PGA for 12 h and then treated by SS for 10 days and expressed as SS + 0.1% PGA, SS + 0.3% PGA and SS + 0.6% PGA. Control seedlings were only treated with half-strength Hoagland's solution and expressed as control. Thus, there were 5 treatments: control, SS, SS + 0.1% PGA, SS + 0.3% PGA and SS + 0.6% PGA. The experiment was performed in a randomised block design with three replications for each treatment. After 5 and 10 days of treatment, all the third leaves were sampled and frozen with liquid N_2. The above-frozen samples were stored in an ultra-low temperature freezer at –80 °C until the analyses were completed. After 10 days of treatment, wheat growth indicators were recorded. Due to the need for many measurements of physio-biochemical indicators on the 5th day, the third leaves of 6 seedlings were sampled. Meanwhile, more measurements of physio-biochemical indicators were taken on the 10th day than on the 5th day, and the third leaves of 9 seedlings were sampled. Therefore, for each replication per treatment, there were a total of 16 seedlings. On the 5th day, 6 leaves sampled from 6 seedlings with one leaf per seedling were used to measure physio-biochemical indicators per replicate. On the 10th day, 9 leaves sampled from 9 seedlings with one leaf per seedling were used to measure physio-biochemical indicators, and 1 seedling was used to measure the growth indicators.

Determination of soil and plant analyser development (SPAD) value, photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s) and chlorophyll fluorescence parameters. The SPAD value was measured using SPAD-502 Plus Chlorophyll Meter (Tokyo, Japan). Licor-6400 photosynthetic instrument (Lincoln, USA) was used to determine the values of P_n, T_r and g_s. The above indicators were determined from 9:30 a.m. to 11:00 a.m. Parameters maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (q_p), nonphotochemical quenching (q_N) and actual photochemical efficiency of PSII (Y(II)) were measured by a PAM-2500 portable modulated chlorophyll fluorometer (Effeltrich, Germany). For dark adaptation, the leaves were covered for 30 min. Then, the above parameters were measured. The above parameters were also determined from 9:30 a.m. to 11:00 a.m.

Measure leaf relative water content (LRWC) and leaf water saturation deficit (LWSD). Fresh leaves were first weighed and recorded as fresh weight (FW). Then, the fresh leaves were immersed in water for 12 h and recorded as the saturated weight (SW). Then, the leaves were dried in an oven at 105 °C for 5 min, followed by 65 °C until constant weight and recorded as the dry weight (DW). The values of LRWC and LWSD were respectively calculated according to the following Eqs. (1) and (2).

$$LRWC = [(FW - DW)/(SW - DW)] \times 100\% \quad (1)$$

$$LWSD = [(SW - FW)/(SW - DW)] \times 100\% \quad (2)$$

Determination of proline and soluble sugars. Soluble sugars were measured according to the anthrone-sulfuric acid method. Proline was measured according to the acidic-ninhydrin method.

Measurement of SOD, POD and CAT. Fresh samples (0.5 g) were homogenised and centrifugated, according to Lu et al. (2020). Then the supernatant was used to measure the activities of SOD, POD and CAT, according to Lu et al. (2020). POD activity was measured by spectrophotometrically monitoring the changes in the absorbance at 470 nm in 3 min. CAT activity was analysed by monitoring the changes in the absorbance at 240 nm in 3 min. SOD activity was analysed by monitoring the absorbance at 560 nm. The above enzymes' activities were expressed as U/g FW.

APX, GR, DHAR and MDHAR analysis. ascorbate peroxidase (APX) activity was analysed according to Nakano and Asada (1981). Glutathione reductase (GR) activity was analysed according to Grace and Logan (1996). Monodehydroascorbate reductase (MDHAR) activity was analysed according to Miyake and Asada (1992). Dehydroascorbate reductases (DHAR) activity was analysed according to Dalton et al. (1986). The above four enzymes' activities were also expressed as U/g FW.

AsA and GSH analysis. The contents of antioxidants ascorbic acid (AsA) and glutathione (GSH) were analysed according to Hodges et al. (1996) and Griffith (1980).

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Measurement of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents and electrolyte leakage (EL). H_2O_2 and O_2^- contents were analysed according to the methods of Zheng et al. (2009) and Ke et al. (2007). According to Hodges et al. (1999) and Zhao et al. (2004), MDA content and EL were measured, respectively.

Analysis of Na^+, K^+, Ca^{2+} and Mg^{2+} contents and the ratios of K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+. Na^+, K^+, Ca^{2+} and Mg^{2+} contents were analysed according to Afrangan et al. (2023). Dry samples (0.5 g) were milled and ashed in the electric oven. The ash was digested by 1 mol/L HCl. The contents of Na^+, K^+, Ca^{2+} and Mg^{2+} were determined using the flame photometry. Then the ratios of K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ were calculated.

Plant height and dry biomass analysis. Plant height was measured using the meter ruler. Whole seedlings were oven-dried for 72 h at 80 °C, and the dry weight was recorded as dry biomass.

Statistical analysis. Data in this article was the mean of three replications. Data was examined by the Kolmogorov-Smirnov test before the analysis of variance (ANOVA). Means were compared by one-way ANOVA and Duncan's multiple range test at the 5% significance level.

Comprehensive evaluation for the effects of PGA on wheat salt tolerance. The membership function method and the inverse membership function method were used to comprehensively evaluate the effects of PGA on wheat salt tolerance, according to Zhang et al. (2024). The membership function was used for the positive correlation index, including P_n, SPAD, F_v/F_m, q_p, Y(II), LRWC, T_r, g_s, the contents of soluble sugars, proline, AsA, GSH, K^+, Ca^{2+} and Mg^{2+}, the activities of SOD, POD, CAT, APX, GR, DHAR and MDHAR, the ratios of K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+, plant height and dry biomass. The evaluation value for the positive correlation index was calculated by using Eq. (3). The inverse membership function method was used for the negative correlation index, including q_N, LWSD, the contents of $O_2^{\cdot-}$, H_2O_2, MDA and Na^+, EL. The evaluation value for the negative correlation index was calculated by using Eq. (4).

$$R(X_i) = (X_i - X_{min})/(X_{max} - X_{min}) \quad (3)$$

$$R(X_i) = 1 - (X_i - X_{min})/(X_{max} - X_{min}) \quad (4)$$

Where: X_i – measured value of a specific indicator; X_{max} and X_{min} – maximum and minimum values of that same indicator, respectively. The higher the average value of the membership function, the better salt tolerance for wheat seedlings.

RESULTS

Effect of PGA on the photosynthetic performance. Compared with control, SS significantly declined P_n, SPAD value, F_v/F_m, q_p and Y(II) but increased q_N after 5 days and 10 days of treatment (Figures 1 and 2). Compared with SS alone, different dosages of PGA all significantly decreased q_N and increased above other indicators of salt-stressed wheat seedlings. Among different dosages, 0.3% PGA showed better effects on the above indicators. After 10 days of treatment, 0.3% PGA significantly decreased q_N to 0.29 and respectively increased P_n, SPAD value, F_v/F_m, q_p and Y(II) to 8.5 μmol/m²/s, 26.0, 0.68, 0.44 and 0.37, compared with SS alone.

Effect of PGA on water physiological characteristics. Compared with the control, SS significantly

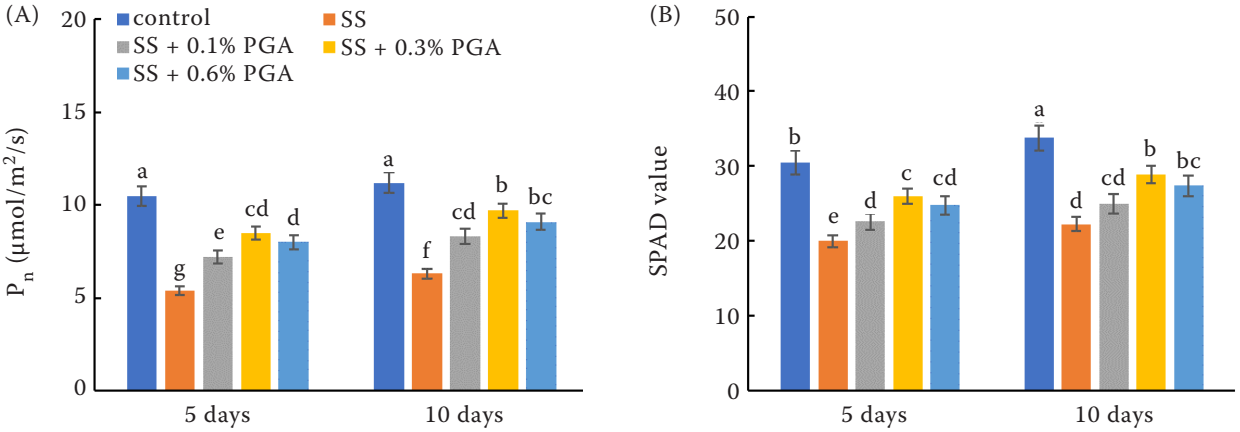

Figure 1. Effects of poly-glutamic acid (PGA) on (A) photosynthetic rate (P_n) and (B) soil and plant analyser development (SPAD) value of salt-stressed (SS) wheat seedlings. Different letters stand for the significant difference at $P < 0.05$

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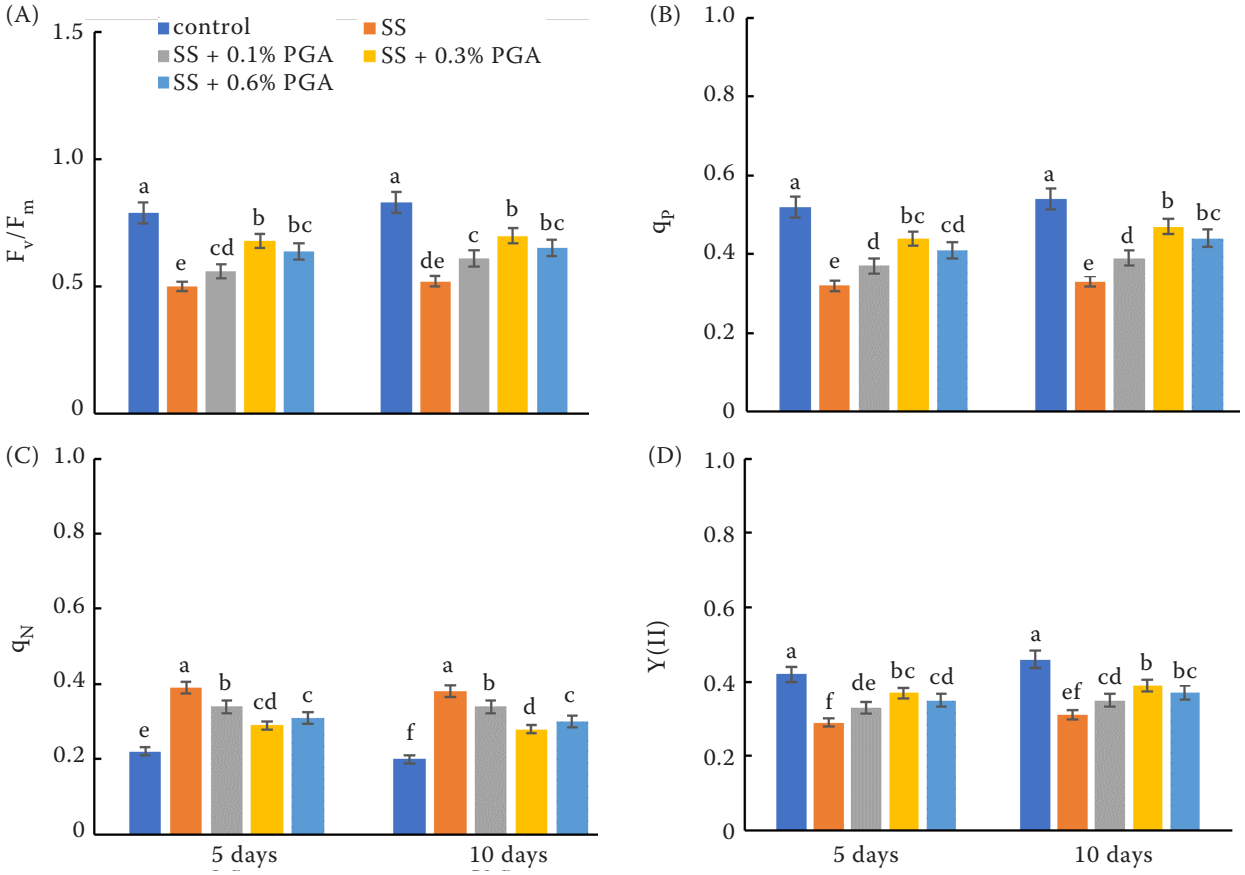

Figure 2. Effects of poly-glutamic acid (PGA) on (A) maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m); (B) photochemical quenching (q_p); (C) nonphotochemical quenching (q_N), and (D) actual photochemical efficiency of PSII Y(II) of salt-stressed (SS) wheat seedlings. Different letters stand for the significant difference at $P < 0.05$

increased LWSD and osmolytes soluble sugar and proline contents, and LRWC, T_r and g_s declined after 5 days and 10 days of treatment (Figures 3 and 4). Compared with SS alone, different dosages of PGA all significantly decreased LWSD and increased above other indicators of salt-stressed wheat seedlings. Among different dosages, 0.3% PGA showed better effects on the above indicators. After 10 days of treatment, 0.3% PGA significantly decreased LWSD to 20.0% and respectively increased LRWC, T_r, g_s, soluble sugar content and proline content to 80.0%, 4.0 mmol/m^2/s, 0.21 mol/m^2/s, 588.0 mg/g FW and 278.0 μg/g FW, compared with SS alone.

Effect of PGA on the antioxidant metabolism. In contrast with control, SS significantly enhanced wheat antioxidant metabolism by reinforcing SOD, POD, CAT, APX, GR, DHAR and MDHAR activities, and AsA and GSH contents. However, SS also enhanced the overproduction of O_2^- and H_2O_2, thereby increased MDA content and EL (Figures 5–8). Compared with SS alone, different dosages of PGA all significantly increased the activities of the above enzymes and AsA and GSH contents in leaves of salt-stressed wheat seedlings. This way, PGA reduced the overproduction of O_2^- and H_2O_2, thereby alleviating the SS-induced peroxide damage. Among different dosages, 0.3% PGA showed better positive effects on wheat antioxidant capacity under SS. After 10 days of treatment, 0.3% PGA respectively significantly increased the activities of SOD, POD, CAT, APX, GR, DHAR and MDHAR, and the contents of AsA and GSH to 46.9 U/g FW, 17.1 U/g FW, 5.7 U/g FW, 4.8 U/g FW, 3.9 U/g FW, 4.4 U/g FW, 4.7 U/g FW, 4.89 μmol/g FW and 0.66 μmol/g FW, compared with SS alone. Meanwhile, 0.3% PGA respectively significantly reduced EL and the contents of O_2^-, H_2O_2 and MDA to 15.6%, 9.1 μmol/g FW, 13.2 μmol/g FW and 9.6 nmol/g FW.

Effects of PGA on Na^+, K^+, Ca^{2+} and Mg^{2+} contents and the ratios of K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+. Compared with the control, SS significantly increased Na^+, Ca^{2+}, and Mg^{2+} content and decreased K^+ content after 10 days of treatment (Table 1). In this

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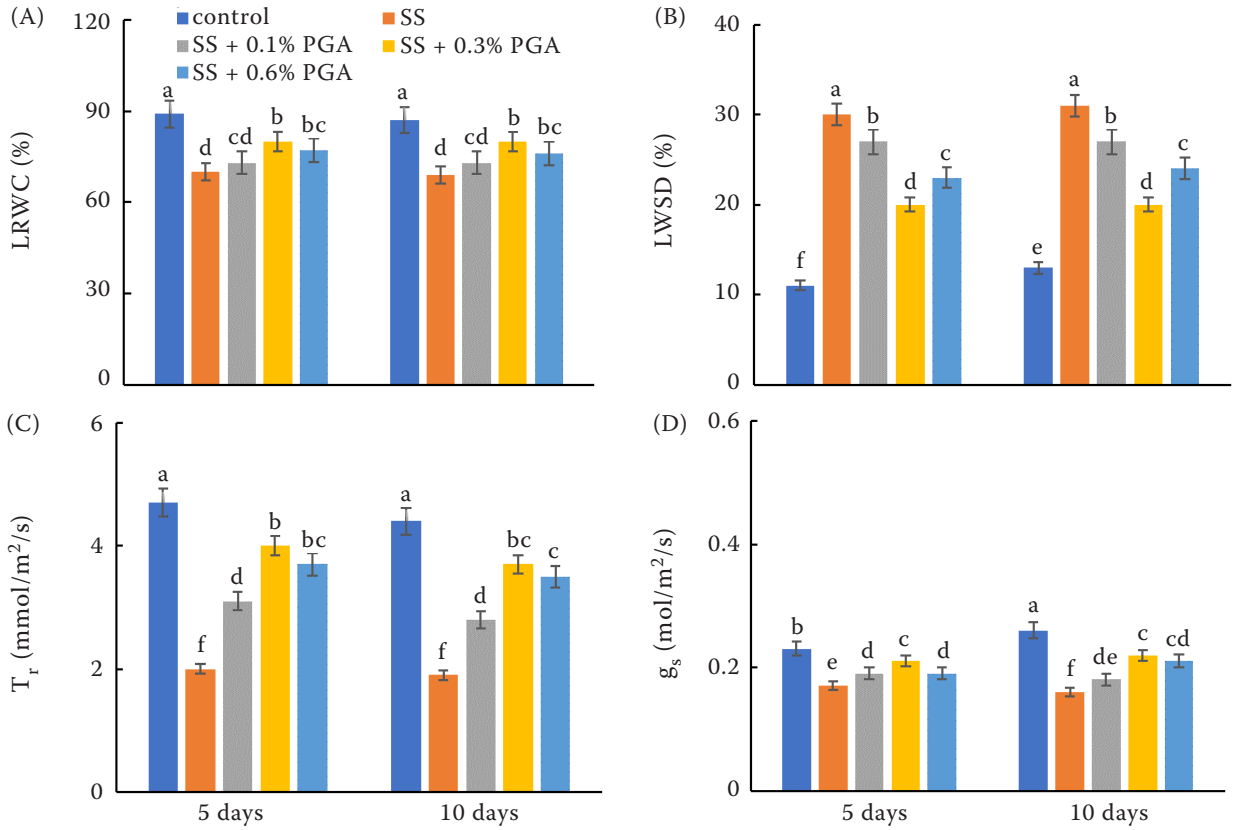

Figure 3. Effects of poly-glutamic acid (PGA) on water physiological parameters (A) leaf relative water content (LRWC); (B) leaf water saturation deficit (LWSD); (C) transpiration rate (T_r) and (D) stomatal conductance (g_s) of salt-stressed (SS) wheat seedlings. Different letters stand for the significant difference at $P < 0.05$

way, SS destroyed the ion homeostasis indicated by lower K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios. Compared with SS alone, different dosages of PGA all significantly reduced Na^+ content and improved K^+, Ca^{2+} and Mg^{2+} contents in leaves of salt-stressed wheat seedlings, thereby increased K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios. Among different dosages, 0.3% PGA showed better positive effects on ion homeostasis. In contrast with SS alone, 0.3% PGA significantly decreased Na^+ content to 1.6 mg/g DW and respectively increased K^+, Ca^{2+} and Mg^{2+} contents to 14.3, 4.2 and 3.7 mg/g DW, thereby respectively

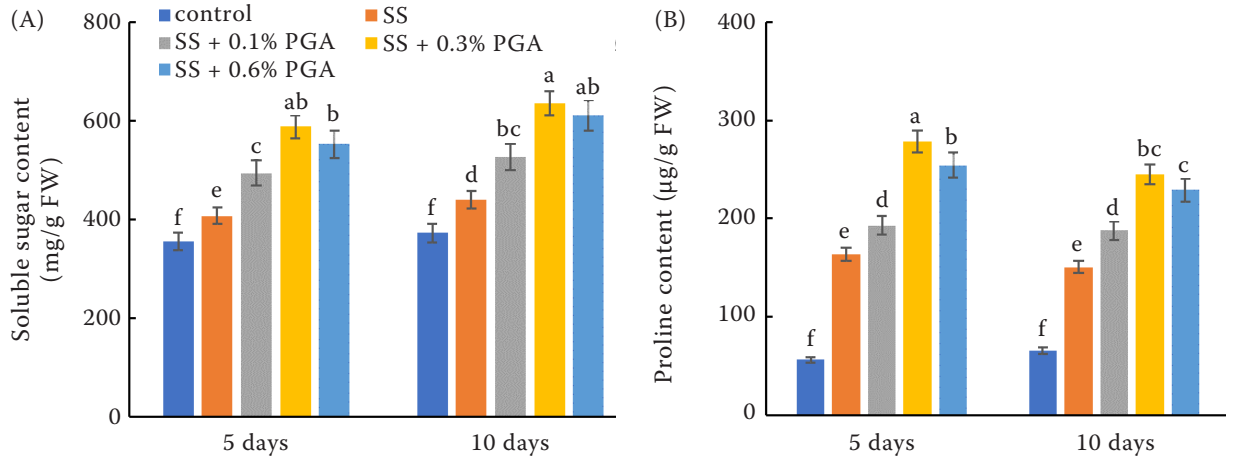

Figure 4. Effects of poly-glutamic acid (PGA) on (A) the contents of osmolytes soluble sugar and (B) proline in salt-stressed (SS) wheat seedlings leaves. Different letters stand for the significant difference at $P < 0.05$. FW – fresh weight

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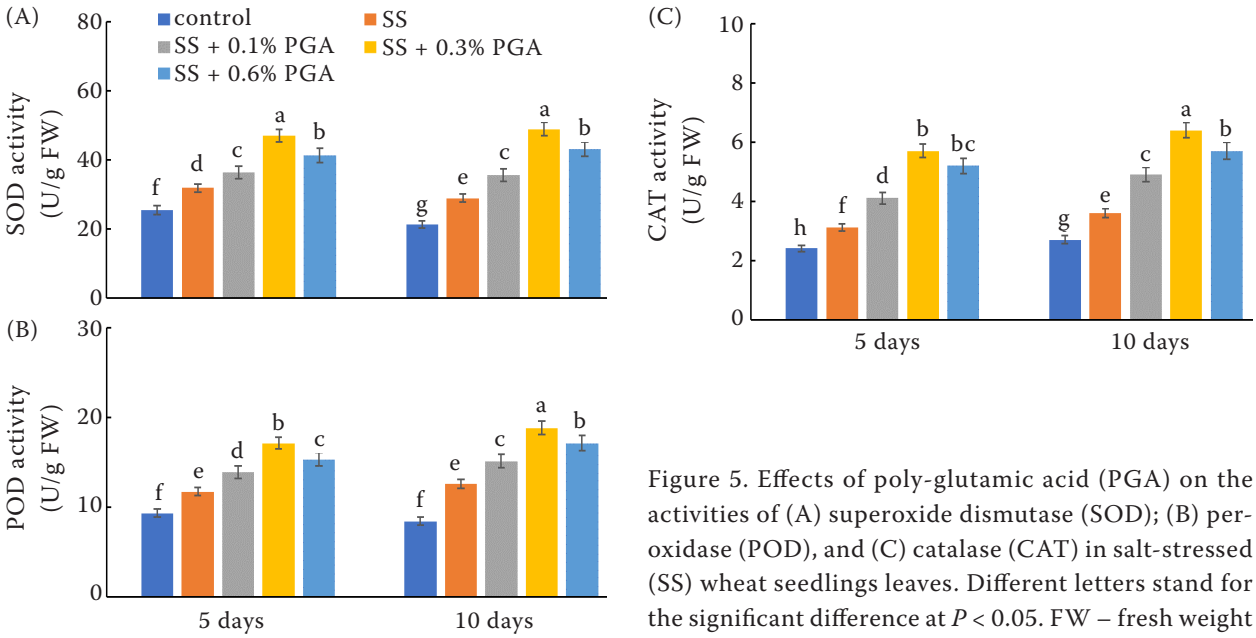

Figure 5. Effects of poly-glutamic acid (PGA) on the activities of (A) superoxide dismutase (SOD); (B) peroxidase (POD), and (C) catalase (CAT) in salt-stressed (SS) wheat seedlings leaves. Different letters stand for the significant difference at $P < 0.05$. FW – fresh weight

increased K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios to 8.94, 2.63 and 2.31. The above findings suggested that PGA application could maintain wheat ion homeostasis under SS.

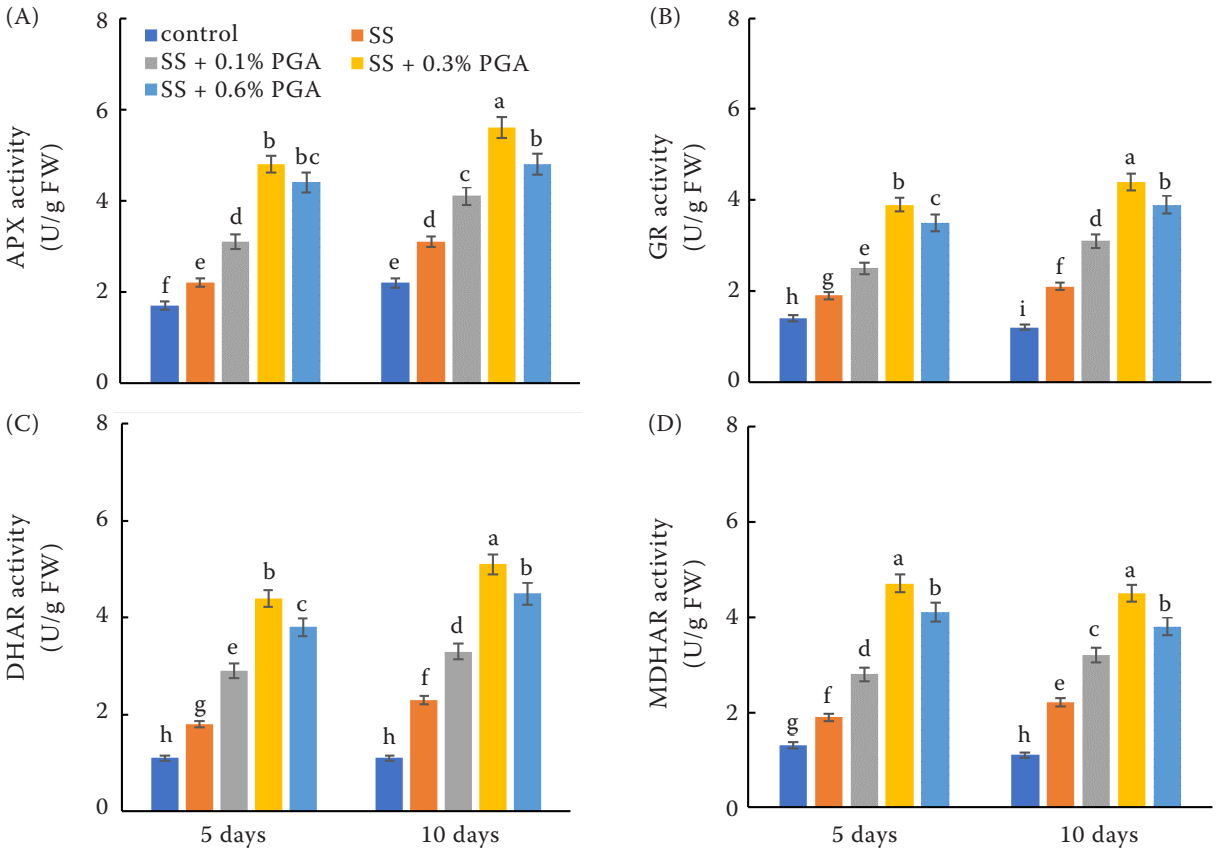

Figure 6. Effects of poly-glutamic acid (PGA) on (A) ascorbate peroxidase (APX); (B) glutathione reductase (GR); (C) dehydroascorbate reductases (DHAR), and (D) monodehydroascorbate reductase (MDHAR) activities in salt-stressed (SS) wheat seedlings leaves. Different letters stand for the significant difference at $P < 0.05$. FW – fresh weight

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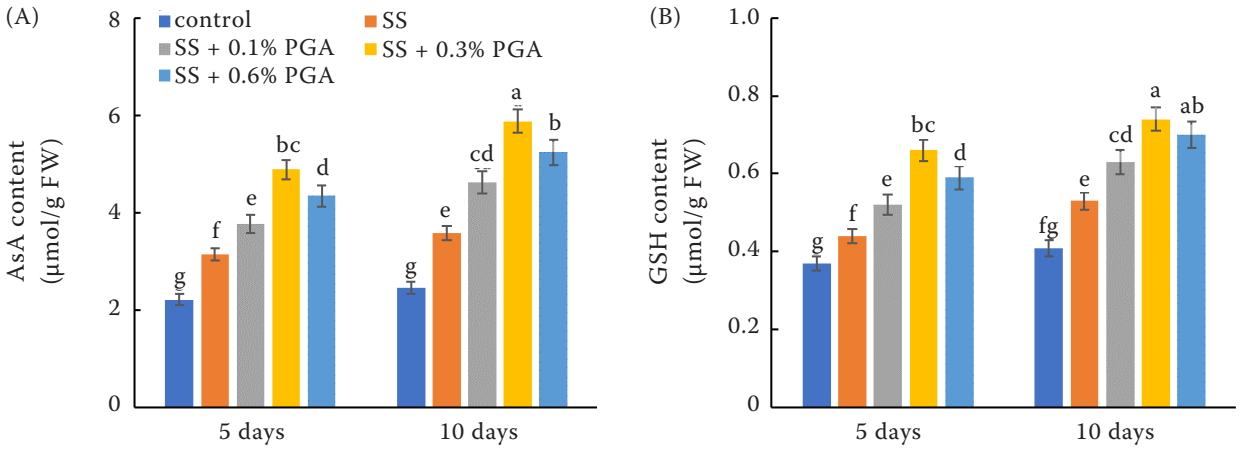

Figure 7. Effects of poly-glutamic acid (PGA) on the contents of (A) antioxidants ascorbic acid (AsA) and (B) glutathione (GSH) in salt-stressed (SS) wheat seedlings leaves. Different letters stand for the significant difference at $P < 0.05$. FW – fresh weight

Effect of PGA on wheat growth parameters. Compared with the control, SS significantly decreased plant height and dry biomass after 10 days of treatment (Figure 9). Compared with SS alone, different dosages of PGA all significantly increased wheat height and dry biomass under SS. Among different dosages, 0.3% PGA showed better effects on wheat growth. Compared with SS alone, 0.3% PGA significantly increased plant height and dry biomass to 20.9 cm and 136.0 mg/plant, respectively.

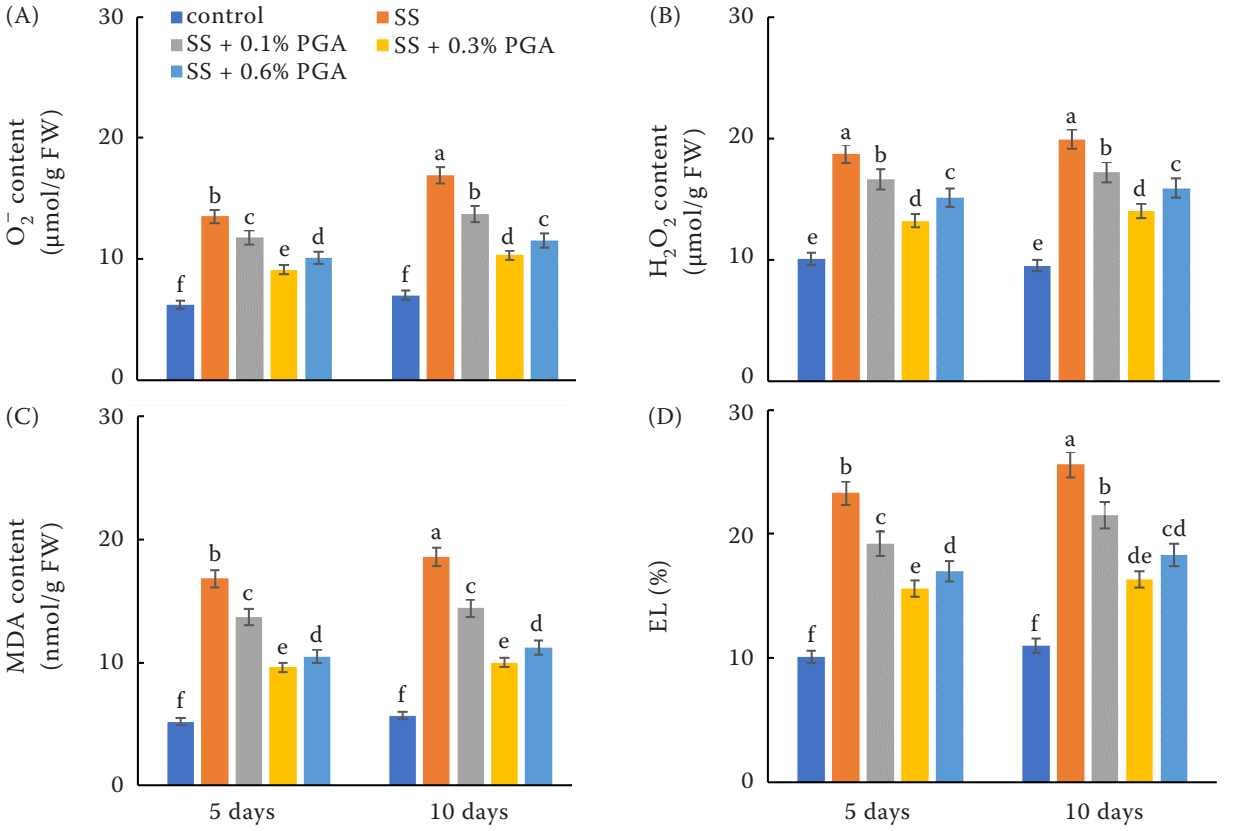

Figure 8. Effects of poly-glutamic acid (PGA) on the contents of (A) superoxide anion (O_2^-); (B) hydrogen peroxide (H_2O_2); (C) malondialdehyde (MDA) and (D) electrolyte leakage (EL) in salt-stressed (SS) wheat seedlings leaves. Different letters stand for the significant difference at $P < 0.05$. FW – fresh weight

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Table 1. Effects of poly-glutamic acid (PGA) on Na^+ and K^+ contents and Na^+/K^+ ratio in leaves of salt-stressed (SS) wheat seedlings

Treatment	Na^+ content	K^+ content	Ca^{2+} content	Mg^{2+} content	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	Mg^{2+}/Na^+ ratio
	(mg/g DW)						
Control	1.2 ± 0.04^e	17.4 ± 1.10^a	1.8 ± 0.12^e	1.4 ± 0.10^e	14.50 ± 0.91^a	1.50 ± 0.09^c	1.17 ± 0.07^c
SS	2.7 ± 0.15^a	8.7 ± 0.58^d	2.3 ± 0.18^d	1.8 ± 0.14^d	3.22 ± 0.20^e	0.85 ± 0.05^e	0.67 ± 0.03^d
SS + 0.1% PGA	2.3 ± 0.17^b	10.6 ± 0.70^c	3.0 ± 0.18^c	2.6 ± 0.19^c	4.61 ± 0.32^d	1.30 ± 0.07^d	1.13 ± 0.06^c
SS + 0.3% PGA	1.6 ± 0.11^d	14.3 ± 0.87^b	4.2 ± 0.26^a	3.7 ± 0.25^a	8.94 ± 0.61^b	2.63 ± 0.17^a	2.31 ± 0.15^a
SS + 0.6% PGA	1.9 ± 0.13^c	13.5 ± 0.95^b	3.6 ± 0.22^b	3.2 ± 0.22^b	7.11 ± 0.41^c	1.89 ± 0.13^b	1.68 ± 0.11^b

Different letters stand for the significant difference at $P < 0.05$. DW – dry weight

The above findings directly indicated that PGA application could reverse the inhibitory effect of SS on wheat growth.

The comprehensive evaluation of the effects of PGA on wheat salt tolerance. We comprehensively evaluated the effects of PGA dosages on wheat salt tolerance through the membership function method. The final evaluation order for wheat salt tolerance under different treatments from high to low as SS + 0.3% PGA > SS + 0.6% PGA > control > SS + 0.1%PGA > SS (Table 2). As the control seedlings did not suffer from SS, we only compared wheat salt tolerance among SS, SS + 0.1% PGA, SS + 0.3% PGA and SS + 0.6% PGA. The final evaluation order for wheat salt tolerance under different treatments except for control from high to low as SS + 0.3% PGA > SS + 0.6% PGA > SS + 0.1% PGA > SS. Above results implied that all concentrations of PGA enhanced wheat salt tolerance, compared with SS alone. Among different concentrations, 0.3% PGA showed a better effect on wheat salt tolerance than other PGA concentrations. These results suggested that PGA could be used as an effective agent to prevent wheat crops from being salinated in productive practice.

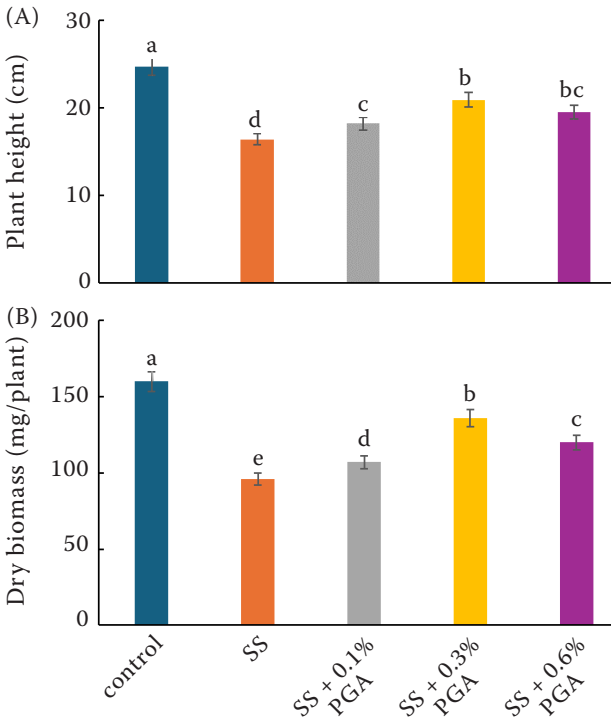

Figure 9. Effects of poly-glutamic acid (PGA) on plant height and dry biomass of salt-stressed (SS) wheat seedlings. Different letters stand for the significant difference at $P < 0.05$

DISCUSSION

P_n is the most reflective indicator of plant photosynthetic performance. Meanwhile, P_n has a close relationship with the content of chlorophylls (*Chl*). Under heat stress, Quan et al. (2022) found that PGA could improve Chinese cabbage's P_n and *Chl* content. Under drought stress, Ma et al. (2022) showed that PGA also improved maize's P_n and *Chl* content. In the current study, our findings demonstrated that PGA also improved P_n and *Chl* content, as indicated by the SPAD value under SS (Figure 1). As the main pigment for plants to absorb light energy, *Chl* content had an important influence on the ability of plants to absorb light energy. Thus, our results also indicated that PGA could enhance the ability of plants to absorb light energy by increasing *Chl* content under SS. Previous studies have shown that PGA could improve plant photosynthetic performance by increasing *Chl* content and P_n under various stresses. Besides, chlorophyll fluorescence parameters can effectively characterise the intrinsic relationship between SS and photosynthesis. q_p and NPQ represent the photochemical consumption and heat dissipation, respectively. F_v/F_m and Y(II)

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Table 2. The comprehensive evaluation of the effects of different dosages of poly-glutamic acid (PGA) on wheat salt tolerance

Indicator	Control	SS	SS + 0.1% PGA	SS + 0.3% PGA	SS + 0.6% PGA
P_n	0.902	0.052	0.399	0.642	0.532
SPAD	0.885	0.081	0.270	0.543	0.438
F_v/F_m	0.885	0.070	0.307	0.543	0.412
q_P	0.906	0.066	0.306	0.626	0.506
q_N	0.950	0.066	0.266	0.566	0.466
Y(II)	0.877	0.087	0.298	0.508	0.403
LRWC	0.832	0.134	0.289	0.562	0.406
LWSD	0.832	0.134	0.289	0.562	0.406
T_r	0.916	0.035	0.345	0.666	0.595
g_s	0.888	0.083	0.250	0.583	0.500
Soluble sugars content	0.059	0.271	0.550	0.899	0.822
Proline content	0.016	0.452	0.643	0.936	0.854
SOD activity	0.034	0.280	0.492	0.922	0.736
POD activity	0.039	0.395	0.607	0.920	0.776
CAT activity	0.039	0.253	0.563	0.928	0.753
APX activity	0.027	0.261	0.531	0.936	0.720
GR activity	0.017	0.276	0.556	0.932	0.797
DHAR activity	0.013	0.287	0.522	0.945	0.804
MDHAR activity	0.018	0.324	0.585	0.936	0.747
AsA content	0.033	0.325	0.597	0.923	0.756
GSH content	0.068	0.376	0.632	0.914	0.811
O_2^- content	0.968	0.073	0.362	0.670	0.564
H_2O_2 content	0.958	0.083	0.310	0.579	0.419
MDA content	0.979	0.065	0.363	0.674	0.591
EL	0.966	0.078	0.327	0.645	0.524
Na^+ content	0.964	0.079	0.317	0.727	0.552
K^+ content	0.913	0.043	0.233	0.602	0.523
Ca^{2+} content	0.033	0.217	0.477	0.922	0.700
Mg^{2+} content	0.027	0.184	0.498	0.928	0.733
K^+/Na^+ ratio	1.000	0.000	0.124	0.506	0.345
Ca^{2+}/Na^+ ratio	0.365	0.000	0.257	1.000	0.590
Mg^{2+}/Na^+ ratio	0.303	0.000	0.283	0.998	0.619
Plant height	0.881	0.083	0.256	0.516	0.381
Dry biomass	0.896	0.060	0.203	0.580	0.376
The average membership function value	0.543	0.155	0.391	0.745	0.592
Evaluation order	3	5	4	1	2

SS – salt stress; P_n – photosynthetic rate; SPAD – soil and plant analyser development; F_v/F_m – maximum photochemical efficiency of photosystem II (PSII); q_P – photochemical quenching; q_N – nonphotochemical quenching; Y(II) – actual photochemical efficiency of PSII; LRWC – leaf relative water content; LWSD – leaf water saturation deficit; T_r – transpiration rate; g_s – stomatal conductance; SOD – superoxide dismutase; POD – peroxidase; CAT – catalase; APX – ascorbate peroxidase; GR – glutathione reductase; DHAR – dehydroascorbate reductases; MDHAR – monodehydroascorbate reductase; AsA – antioxidants ascorbic acid; GSH – glutathione; MDA – malondialdehyde; EL – electrolyte leakage

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represent the maximum photochemical efficiency of PSII and the actual photochemical efficiency of PSII, respectively. Under heat stress, Quan et al. (2022) found that PGA could reduce NPQ and improve the q_P, F_v/F_m and Y(II) values of Chinese cabbage. This study also showed that PGA had the same effects on the above chlorophyll fluorescence parameters under SS (Figure 2). Our current results indicated that PGA could enhance photochemical consumption and reduce heat dissipation, improving wheat seedlings' light energy utilisation rate under SS. From the above results of previous and current studies, we can deduce that PGA had a universal role in enhancing plant photosynthetic performance under stresses. For this study, we clearly manifested that PGA could enhance photosynthetic performance by improving the light absorption capacity and light energy utilisation rate of wheat seedlings under SS. However, we also found no significant difference in the effects of PGA on the above indicators related to the photosynthetic performance between days 5 and 10 under SS. This phenomenon indicated that the pretreatment with PGA had a relatively constant promoting influence on wheat photosynthetic performance under SS. Previous studies showed that the growth of plants had a close relationship with their photosynthetic performance (Fairoj et al. 2023, Sadak et al. 2023). This study found that PGA also improved wheat height and biomass (Figure 9). Therefore, our current findings implied that PGA enhanced wheat salt tolerance by improving the photosynthetic performance, which further promoted wheat growth.

In plants, osmolytes play important roles in fighting against SS. In this study, the findings showed that PGA enhanced the accumulation of osmolytes proline and soluble sugars in the leaves of wheat seedlings under SS (Figure 3). In this way, PGA reduced cellular water potential and enhanced water absorption capacity, further improving LRWC, T_r and g_s and decreasing LWSD of salt-stressed wheat seedlings (Figure 4). These results indicated that PGA showed an important influence on water physiology by maintaining the water balance of salt-stressed wheat crops through the above way. T_r and g_s had a close relationship with the stomatal aperture. The larger the stomatal aperture, the greater T_r and g_s are. Meanwhile, the stoma is the gateway for carbon dioxide to enter to provide raw materials for photosynthesis. Thus, our results also indicated that PGA could maintain wheat water balance and improve photosynthesis under SS by promoting the accumulation of osmolytes proline and soluble sugars. However, we also found no significant difference in the effects of PGA on the above indicators related to water physiology except proline content between days 5 and 10 under SS. This phenomenon indicated that the pretreatment with PGA also had a relatively constant promoting influence on wheat water balance under SS. It has been documented that there were many osmolytes in plant cells, such as proline, soluble sugars, betaine, soluble protein, free amino acids, etc. Our study only explored the effects of PGA on osmolytes proline and soluble sugars in wheat seedlings under SS. Thus, further investigation should be done on the effects of PGA on other osmolytes, which can provide more insights into the role of PGA in enhancing wheat water balance under SS. Moreover, Xu et al. (2017) showed that signal molecules Ca^{2+}, H_2O_2, brassinolide (BR), and jasmonic acid (JA) participated in PGA-promoted the accumulation of proline in canola. However, whether the above four signal molecules participated in PGA-improved proline accumulation and other osmolytes in salt-stressed wheat seedlings is still unclear. Therefore, it is also interesting to explore this part of the work, which can provide more insights into the role of PGA in enhancing wheat water balance under SS at the signal transduction level.

The activities of antioxidant enzymes SOD, POD and CAT play important roles in scavenging O_2^- and H_2O_2. In the current study, we showed that PGA enhanced the activities of SOD, POD and CAT in the leaves of wheat seedlings under SS to scavenge O_2^- and H_2O_2 (Figure 5), which was consistent with the results of Guo et al. (2017). Besides, the AsA-GSH cycle also plays a vital role in scavenging H_2O_2 and maintaining AsA and GSH contents in stressed plants (Punia et al. 2021, Maslennikova et al. 2022). For this study, we showed that PGA reinforced the activity of AsA-GSH cycle by improving APX, GR, DHAR and MDHAR activities in salt-stressed wheat seedlings (Figure 6), which indicated that PGA also enhanced the capacity of wheat seedlings to scavenge H_2O_2 through AsA-GSH cycle. Meanwhile, our results also displayed that PGA improved AsA and GSH contents in salt-stressed wheat seedlings (Figure 7), which indicated that PGA enhanced the accumulation of AsA and GSH by improving the activity of AsA-GSH cycle in salt-stressed wheat seedlings. Therefore, our results clearly implied that PGA improved the antioxidant capacity by enhancing the capacity of wheat seedlings to scavenge O_2^- and H_2O_2 under

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SS through the above antioxidant enzymes (SOD, POD, CAT, APX, GR, DHAR and MDHAR) and non-enzymatic antioxidants (AsA and GSH). In this way, PGA mitigated the peroxide damage of wheat seedlings induced by SS, indicated by O_2^-, H_2O_2, MDA and EL (Figure 8). Meanwhile, we also found that there was a significant difference in the effects of PGA on the activities of antioxidant enzymes and the contents of antioxidants except SOD activity between days 5 and 10 under SS. Except for SOD activity, the values of the activities of antioxidant enzymes and the contents of antioxidants on the 10th day were significantly higher than those on the 5th day after PGA treatment under SS. However, there was no significant difference in the effects of PGA on EL and the contents of O_2^-, H_2O_2 and MDA between days 5 and 10 under SS. This phenomenon indicated that the pretreatment with PGA had a longer time to enhance wheat antioxidant capacity under SS, thereby keeping the peroxide damage and the contents of O_2^-, H_2O_2 at low levels. Besides, it has been reported that the contents of AsA and GSH also had close relationships with the activities of their key biosynthetic enzymes L-galactono-1,4-lactone dehydrogenase (GalLDH) and gamma-glutamylcysteine synthetase (γ-ECS) in plants (Shan and Liang 2010). However, it is still unknown for the effects of PGA on GalLDH and γ-ECS activities in salt-stressed wheat seedlings. Moreover, signal molecules Ca^{2+}, H_2O_2, BR and JA participated in PGA-promoted canola antioxidant capacity (Xu et al. 2017). However, it is still unclear whether Ca^{2+}, H_2O_2, brassinolide, and jasmonic acid were involved in the PGA-improved activities of antioxidant enzymes and the contents of AsA and GSH in salt-stressed wheat seedlings. Therefore, it is also interesting to explore these parts of the work, which will also show more insights into the role of PGA in reinforcing wheat antioxidant capacity under SS at the signal transduction level.

Increasing research has demonstrated that SS disturbed plant ion homeostasis (Guo et al. 2021, 2023, Talaat and Hanafy 2023). For wheat crops, it has been reported that SS also disturbed the ion homeostasis by increasing Na^+ content and decreasing K^+ content, thereby lowering the ratio of K^+/Na^+ (Guo et al. 2017, Talaat and Hanafy 2023). Our current study demonstrated that SS had the same effects on ion homeostasis as the previous research (Table 1). Besides, Guo et al. (2017) reported that PGA could maintain the ion homeostasis of salt-stressed wheat crops by increasing K^+ content and decreasing Na^+ content, thereby increasing the ratio of K^+/Na^+. Our current findings also showed the same effects of PGA on the ion homeostasis of salt-stressed wheat crops as Guo et al. (2017). Meanwhile, we also found that PGA could maintain the ion homeostasis of salt-stressed wheat crops by decreasing Na^+ content and increasing Ca^{2+} and Mg^{2+} contents, which further increased Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios (Table 1). As plant salt tolerance has close relationships with the above ratios, our results clearly demonstrated that PGA improved wheat salt tolerance by maintaining ion homeostasis.

For this study, we found that different doses of PGA improved wheat seedlings' overall performance under SS. However, there were significant differences between the effects of three doses of PGA on overall performance. The application of 0.3% PGA showed a more positive influence on seedling performance under SS than 0.1% and 0.6% PGA. The application of 0.6% PGA showed a more positive influence on seedling performance under SS than 0.1% PGA. Therefore, this study showed an interesting phenomenon that the 0.3% PGA treatment was the most effective in improving seedling performance, with no further improvement found in the 0.6% PGA. Based on the results of our current study, the difference in the influence on seedling performance between 0.3% and 0.6% PGA was due to the more positive role of 0.3% PGA in enhancing the antioxidant capacity, improving the photosynthetic performance, and maintaining water balance and ion homeostasis of wheat seedlings under SS. Therefore, our results indicated that PGA had dose effects on wheat salt tolerance. This phenomenon demonstrated that screening the suitable PGA concentration was important for its use in enhancing wheat salt tolerance. However, we only investigated the effects of different doses of PGA on seedling performance at the physiological level, which can not fully explain why the 0.3% PGA treatment was the most effective in improving seedling performance, with no improvement found in the 0.6% PGA. As an important tool to elucidate the molecular mechanism of plants, the transcriptome can be further used to deeply explain this interesting phenomenon at the molecular level.

Our research clearly showed that different doses of PGA enhanced wheat salt tolerance by improving the photosynthetic performance, reinforcing the antioxidant capacity, and maintaining water balance and ion homeostasis, especially for 0.3% PGA. Hence, our results provided an important theoretical basis for the potential use of PGA in real situations to improve wheat salt tolerance.

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