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Thiourea and arginine synergistically preserve redox homeostasis and ionic balance for alleviating salinity stress in wheat

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Plant growth regulators are cost-effective and efficient methods for enhancing plant defenses under stress conditions. This study investigates the ability of two plant growth-regulating substances, thiourea (TU) and arginine (Arg), to mitigate salinity stress in wheat. The results show that both TU and Arg, particularly when used together, modify plant growth under salinity stress. Their application significantly increases the activities of antioxidant enzymes while decreasing the levels of reactive oxygen species (ROS), malondialdehyde (MDA), and relative electrolyte leakage (REL) in wheat seedlings. Additionally, these treatments significantly reduce the concentrations of Na^+ and Ca^{2+} and the Na^+/K^+ ratio, while significantly increasing K^+ levels, thereby preserving ionic osmotic balance. Importantly, TU and Arg markedly enhance the chlorophyll content, net photosynthetic rate, and gas exchange rate in wheat seedlings under salinity stress. The use of TU and Arg, either individually or in combination, results in a 9.03–47.45% increase in dry matter accumulation, with the maximum increase observed when both are used together. Overall, this study highlights that maintaining redox homeostasis and ionic balance are crucial for enhancing plant tolerance to salinity stress. Furthermore, TU and Arg are recommended as potential plant growth regulators to boost wheat productivity under such conditions, especially when applied together.

Keywords Salinity stress, Reactive oxide species, Antioxidant, Ionic balance

Dramatic climate change and agricultural practices have led to the increasing degradation of agro-ecosystems[1]. One of the most severe consequences is land salinization, which threatens global food security[2]. Salinity stress currently impacts approximately 20% of global cropland, a figure that could rise to 50% by 2050[3]. It induces osmotic stress in crop roots and results in ion imbalance within plants[4]. This unfavorable condition also leads to increased decomposition of plant chlorophyll, reduced photosynthetic rates, and metabolic disorders, ultimately decreasing plant yield[5,6]. Additionally, a common severe effect is the increased production of ROS, which can cause oxidative damage to a wide range of biomolecules, including DNA, proteins, and lipids[7].

Wheat (*Triticum aestivum*) is one of the most important cereal crops globally, not only as the most widely cultivated grain but also as a key economic commodity[8]. However, wheat is sensitive to salinity, which can inhibit its growth, disrupt physiological and biochemical processes, and significantly reduce yield. The primary strategies to mitigate the effects of salinity stress include transgenic technology and the use of plant growth regulators. The former method involves developing salinity-tolerant wheat varieties using techniques such as gene editing[9,10]. On the other hand, plant growth regulators enhance wheat's salinity tolerance by regulating physiological activities and related substance levels to alleviate stress damage[11]. These regulators are generally more accepted and widely used than transgenic approaches. They improve plant tolerance to various abiotic stresses like salinity, drought, and heavy metals, and enhance seed germination, nutrient uptake, and reproductive growth, which can lead to better crop yield and quality[12]. Because they are environmentally friendly, easy to use, cost-effective,

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and practical, plant growth regulators are crucial for ensuring crop growth and maintaining yield and quality[13]. However, due to the similar action modes of these regulators, using just one may not be effective. Identifying combinations of growth regulators that enhance salinity tolerance in wheat is essential for cultivating wheat in stressful environments, improving yields, and securing food security.

Thiourea (TU), a plant growth regulator consisting of 36% nitrogen and 42% sulfur, has gained attention for its ability to enhance plant growth under adverse conditions[14]. Its high water solubility allows easy penetration into plant tissues. Known for its strong bioactivity, TU helps maintain water homeostasis and redox balance through hormonal regulation[15–17]. Research has shown that TU supports plant growth under drought[18], heavy metals[19], and salinity stresses[20]. For example, Pandey, et al. found that TU altered the redox state in rice seedlings, enhancing their growth under salinity stress[20]. Additionally, Hussein reported that TU could mitigate the impact of salinity on the growth and yield traits of flax plants by enhancing antioxidant defense mechanisms. Due to these benefits, the use of TU in agriculture is increasing[21].

Arginine, a significant amino acid in organisms, exhibits the highest nitrogen-to-carbon ratio among amino acids[22]. Its metabolism is integral to numerous plant physiological processes, including the synthesis of proline[23], nitric oxide (NO)[24,25], and polyamines[26]. These substances play critical roles in improving plant performance under abiotic stress conditions by preventing the excessive accumulation of reactive oxygen species (ROS) and reducing oxidative damage. They primarily achieve this by maintaining osmotic potential and redox balance in plant cells[27–29]. For example, Silvera et al. reported that foliar applications of arginine enhanced leaf gas exchange during drought and bolstered antioxidant responses in roots during recovery[22]. Furthermore, Akladious and Hanafy observed that foliar sprays of arginine increased the tolerance of white lupin to salinity stress by activating antioxidant enzyme defenses[30]. However, since arginine's primary function is to promote the synthesis of proline and NO, its effectiveness may be less immediate compared to growth regulators like thiourea (TU). Thus, it is proposed that combining TU and arginine (Arg) could address each other's limitations and produce a synergistic effect.

To date, no studies have explored the combined application of TU and Arg. It is uncertain whether this innovative combination can synergistically promote wheat growth under salinity stress. Therefore, this study aimed to determine whether these two growth regulators could collaboratively alleviate the detrimental effects of salinity stress on wheat. For this purpose, we conducted a short-term hydroponic experiment with wheat seedlings to investigate the benefits of combining TU and Arg on wheat subjected to salinity stress, with a focus on the plants' redox and ionic balance. We hypothesized that the TU and Arg combination could work synergistically to reduce oxidative damage caused by salinity stress and manage ionic imbalance, thereby enhancing the salinity tolerance of wheat.

Materials and methods

Plant materials and experimental design

The wheat cultivar Bainong 207, provided by the Wheat Genetic Improvement Research Center of the Henan Institute of Science and Technology, was used in this study. Healthy wheat seeds were soaked in 15% sodium hypochlorite for ten minutes and then rinsed five times with distilled water to eliminate any residues. Subsequently, thirty seeds were arranged on saturated $CaSO_4$-soaked blotting paper (45 cm long and 30 cm wide) and covered with another sheet of blotting paper. The paper was rolled up and incubated in the dark at 28 °C for seven days to facilitate germination. Uniform seedlings were then transferred to a hydroponic system and incubated with Hoagland's nutrient solution for seven days before treatment.

Wheat seedlings at the triticale stage were transferred into a nutrient solution containing TU, Arg, and NaCl for treatment. Five treatments were established in the experiment, each with five replications. The treatments included: control (without NaCl, TU, and Arg), NaCl (100 mM NaCl), TU (100 mM NaCl + 7.5 μM TU), Arg (100 mM NaCl + 10 μM Arg), and TU + Arg (100 mM NaCl + 7.5 μM TU + 10 μM Arg). The concentration of NaCl was chosen based on a previous study[10], and the beneficial effects of 7.5 μM TU were demonstrated by Pandey et al.[20]. The optimal concentration of Arg was determined through preliminary experimentation (data shown in Fig. S1). Throughout the experimental period, all seedlings were housed in a phytotron with day/night temperatures of 30 °C/28 °C and relative humidity of 60%. Samples were collected on days 3 and 6 after treatment initiation. For biomass determination, five samples were taken from the replicates of each treatment. The samples for determining physiological parameters and ion content included five biological replicates, each comprising leaves from three different seedlings. Biomass, relative electrolyte leakage, and ion content were measured immediately after sampling, while other samples were frozen in liquid nitrogen and stored at − 80 °C.

Determination of biomass

The roots and above-ground parts of the wheat seedlings were separated and blotted to remove surface water before being weighed to obtain fresh weight. The plant tissues were then individually placed into kraft paper bags and dried in an electric blast dryer (DHG-2200B-118, Zhengzhou Shengyuan Instrument Co., Ltd., Zhengzhou, China) at 80 °C until a constant weight was achieved to determine the dry weight.

Determination of photosynthetic pigment content and photosynthetic parameters

Photosynthetic pigments were extracted using 95% ethanol. Specifically, 0.1 g of fresh leaves was extracted in darkness using 3 mL of 95% ethanol for 48 h. The optical densities (OD) of the extracts at 470 nm, 644 nm, and 662 nm were then measured using a spectrophotometer (U-5100, Hitachi, Japan). The photosynthetic pigment content was calculated according to the method reported by Pongprayoon et al[31].

A Li-6400 portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA) was used to determine photosynthetic parameters. Measurements were taken just before each sampling, selecting the penultimate leaf from the bottom. Three readings were recorded for each leaf, and five different leaves were measured for each treatment.

Determination of ROS content

The contents of H_2O_2 and $O_2^{\cdot-}$ were measured separately to assess changes in ROS content under the various treatments.

The H_2O_2 content was determined using the dimethylphenol orange method[32]. Briefly, 0.1 g of fresh plant sample powder (prepared under liquid nitrogen) was accurately weighed and extracted in 1 mL of pre-cooled acetone for 10 min, then centrifuged at 10,000 g for 15 min at 4 °C to obtain the supernatant. Subsequently, 150 µL of the extract was mixed with 100 µL of distilled water, 250 µL of 400 mM sorbitol, 250 µL of 0.8 mM ammonium ferrous sulfate, and 150 µL of dimethylphenol orange. This mixture was incubated at 30 °C for 30 min. The OD of the solution at 560 nm was then measured. A standard curve was prepared using a 100 µM H_2O_2 solution to calculate the H_2O_2 concentration in the samples.

The $O_2^{\cdot-}$ levels were determined using the hydroxylamine hydrochloride method[33]. Specifically, 0.1 g of fresh sample powder was accurately weighed, suspended in 1 mL of 50 mM phosphate buffer (pH 7.8), and extracted for 10 min before being centrifuged at 10,000 g for 15 min to collect the supernatant. Then, 100 µL of the extract was thoroughly mixed with 200 µL of 0.25 mM hydroxylamine hydrochloride and 100 µL of 6.25 mM phosphate buffer (pH 7.8), and incubated in the dark at 25 °C for 1 h. Afterward, 200 µL of 4.25 mM sodium *p*-aminobenzenesulfonate and 200 µL of 1.75 mM α-naphthylamine solution were added to the mixture, which was further incubated in the dark for another 20 min. The OD of the solution at 530 nm was measured. A standard curve was prepared using a sodium nitrite solution.

Determination of membrane lipid peroxidation and REL

The thiobarbituric acid method was used to determine the MDA content in the samples. 0.1 g of fresh sample powder was accurately weighed, extracted with 1 mL of 10% trichloroacetic acid for 10 min, and then centrifuged at 10,000 g for 20 min to obtain the supernatant. The extract was then mixed in equal volumes with 0.75% thiobarbituric acid and incubated at 100 °C for 15 min. After incubation, the supernatant was collected by centrifugation, and its OD at 450 nm, 532 nm, and 600 nm was measured. The MDA concentration was calculated using the following equation:

$$\text{MDA concentration } (\mu M) = 6.45 \times (\text{OD532} - \text{OD600}) - 0.56 \times \text{OD450} \quad (1)$$

REL was measured according to a previous report[34]. Specifically, 0.2 g of fresh leaves were accurately weighed, fragmented, and transferred to a centrifuge tube containing 10 mL of deionized water. The samples were incubated on a shaker at 50 rpm for 2 h. The conductivity of the solution, L_0, was measured after incubation. The sample tubes were then boiled for 20 min. After cooling to room temperature, the conductivity, L_1, was measured. REL is expressed as $(L_0/L_1) \times 100\%$.

Determination of antioxidant enzyme activity

Antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), were determined using Solarbio micro kits with the following codes: APX (BC0225), POD (BC0095), CAT (BC0205), and SOD (BC0175). Measurements were conducted strictly in accordance with the instructions provided with the kits.

Measurement of ion content

The contents of Na^+, K^+, and Ca^{2+} in the samples were quantified using atomic absorption spectroscopy. Specifically, 0.2 g of dried sample powder was accurately weighed and placed in a decoction tube containing a 4:1 (V/V) mixture of 68% HNO_3 and 30% H_2O_2. The mixture was left at room temperature for 12 h and then boiled at high temperature until the solution became clear. The decocted solution was diluted to 50 mL with 0.1% HNO_3, and the absorbance at 589 nm, 766.5 nm, and 422.7 nm was measured using an atomic absorption spectrophotometer (ZA3000, Hitachi, Japan) to quantify the Na^+, K^+, and Ca^{2+} contents, respectively.

Statistical analysis

All collected data were initially organized and analyzed using Excel 2019. The data were then statistically processed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Differences between treatments were evaluated using Duncan's multiple range test at the $p < 0.05$ significance level. All data were checked for normal distribution prior to statistical analysis. Graphs were created using Origin 2022 software (OriginLab, MA, USA).

Results

Effects of TU and Arg on biomass accumulation in wheat seedlings under salinity stress

The results of the study showed that salinity stress significantly inhibited the growth of wheat seedlings, both root and shoot (Fig. 1a). Specifically, under salinity stress, the shoot and root dry weights of wheat seedlings decreased by 32.24% and 19.05% at 3 days after treatment (DAT), and by 38.75% and 38.21% at 6 DAT, respectively, compared to the control (Fig. 1d,e). The inhibition of wheat seedlings' growth became more severe over time. However, the addition of TU and Arg significantly enhanced the growth of wheat seedlings under salinity stress. For instance, at 3 DAT, the shoot dry weight of the TU and Arg treatment groups increased by 21.36% and 30.10%, respectively, relative to the NaCl group. A similar enhancement was observed in root growth, and

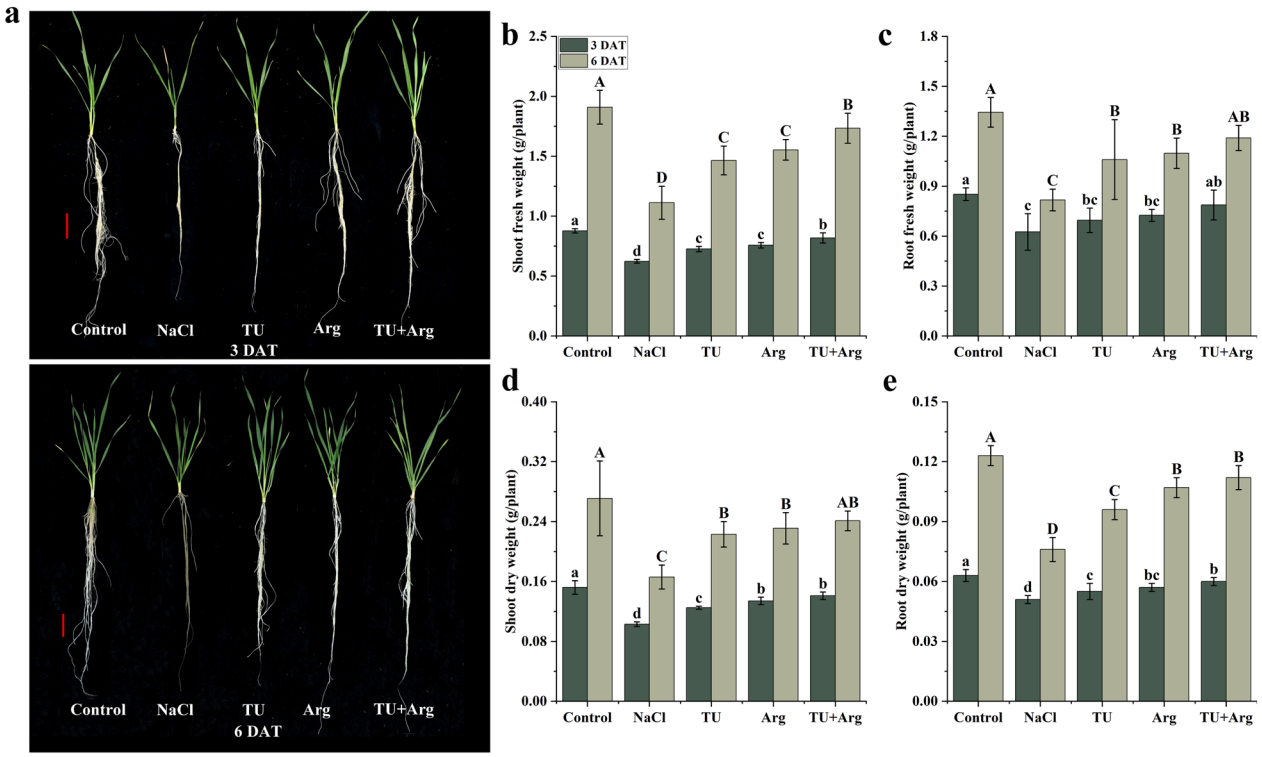

Fig. 1. Phenotype and biomass of wheat seedlings under different treatments. Phenotypes (**a**), stem fresh weight (**b**), root fresh weight (**c**), stem dry weight (**d**) and root dry weight (**e**) of wheat seedlings after 3 and 6 days of treatment. The length of the red bar in Fig. a is 5 cm. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. *DAT* days after treatment.

this promotion was more pronounced with extended treatment duration. At 6 DAT, the shoot dry weight in the TU and Arg groups had increased by 34.34% and 39.16%, respectively, compared to the NaCl group. Remarkably, the combination of TU and Arg exhibited a significant synergistic effect. At 6 DAT, the shoot dry weight of the combined TU + Arg group increased by 8.07% and 4.33%, and the root dry weight by 16.67% and 4.67% compared to the TU and Arg groups, respectively (Fig. 1d,e).

Effects of TU and Arg on photosynthetic pigment content in wheat seedlings under salinity stress

The contents of chlorophyll a, chlorophyll b, and carotenoids in wheat plants were significantly diminished under salinity stress. However, the application of TU and Arg markedly mitigated the decline in photosynthetic pigment content due to salinity stress. In comparison with the NaCl group, the chlorophyll content increased by 11.07–15.24% and 7.46–10.78%, chlorophyll b content by 12.71–15.31% and 7.59–9.34%, and the carotenoid content by 10.39–40.32% and 5.19–35.48% in the TU and Arg groups, respectively (Fig. 2). Additionally, the combination of TU and Arg demonstrated synergistic effects, but these were only observed at 6 DAT, except in the case of carotenoids, where the synergistic effects were noted at both 3 DAT and 6 DAT (Fig. 2).

Effects of TU and Arg on photosynthetic parameters in wheat seedlings under salinity stress

Unexpectedly, all photosynthetic parameters were significantly reduced under salinity stress, including the net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration (Fig. 3). Conversely, the application of TU and Arg significantly alleviated the adverse effects of salinity stress on these parameters. The net photosynthetic rate for the TU and Arg groups increased by 15.17% and 11.75% at 3 DAT, and by 14.13% and 11.71% at 6 DAT, respectively, compared to the NaCl group (Fig. 3a). The other three parameters also increased correspondingly (Fig. 3b–d). Moreover, the combined use of TU and Arg outperformed their individual applications, although this beneficial effect was not observed in the net photosynthetic rate at 3 DAT (Fig. 3).

Effects of TU and Arg on ROS in wheat seedlings under salinity stress

The accumulation of $O_2^{\cdot-}$ in the leaves increased significantly under salinity stress (Fig. 4a). However, the use of TU and Arg notably decreased the $O_2^{\cdot-}$ content in leaves under such stress. At 3 DAT, the $O_2^{\cdot-}$ content in the leaves of the TU, Arg, and combined TU + Arg treated groups was reduced by 3.06%, 3.94%, and 4.96%, respectively, in

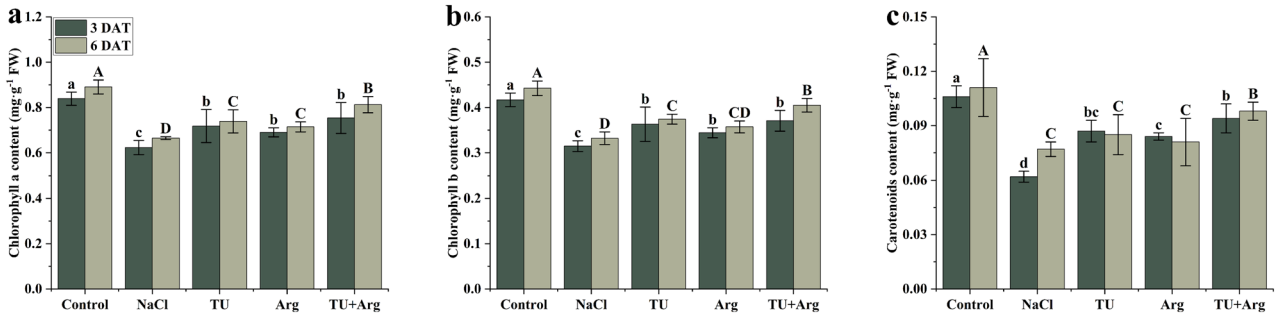

Fig. 2. Effects of TU and Arg on chlorophyll a (**a**), chlorophyll b (**b**), and carotenoid (**c**) contents of wheat seedlings under salinity stress. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. *DAT* days after treatment. FW: fresh weight.

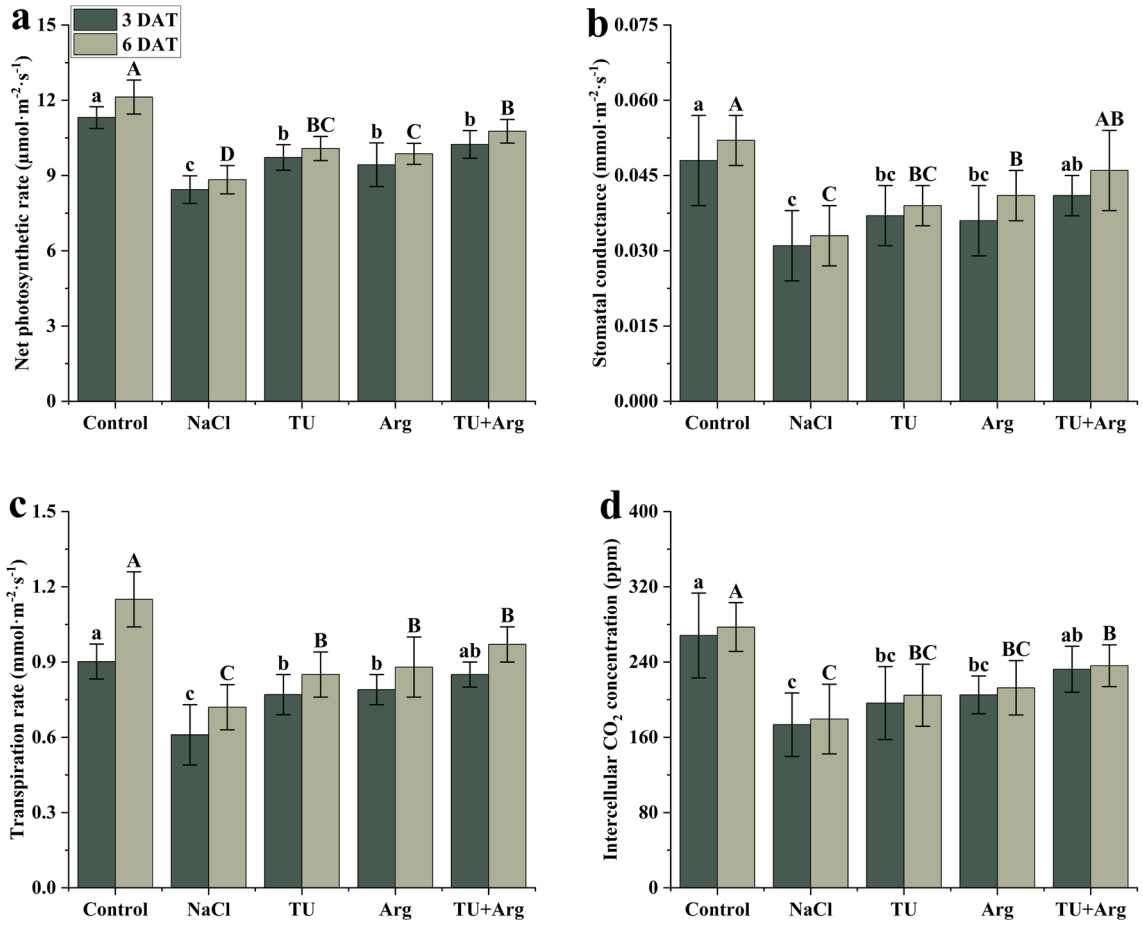

Fig. 3. Effects of TU and Arg on net photosynthetic rate (**a**), stomatal conductance (**b**), transpiration rate (**c**), and intercellular CO_2 concentration (**d**) in wheat seedlings under salinity stress. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. *DAT* days after treatment.

comparison to the NaCl group. At 6 DAT, the $O_2^{\cdot-}$ content in the treatment groups did not exhibit a significant change (Fig. 4a).

H_2O_2 content in the leaves also rose significantly under salinity stress (Fig. 4b). Similarly, the application of TU and Arg substantially reduced the H_2O_2 content in the leaves, with the combined treatment being the most effective. At 3 DAT and 6 DAT, the use of TU and Arg together resulted in significant decreases of 9.81% and 16.87% in H_2O_2 content in the leaves compared to the NaCl group (Fig. 4b).

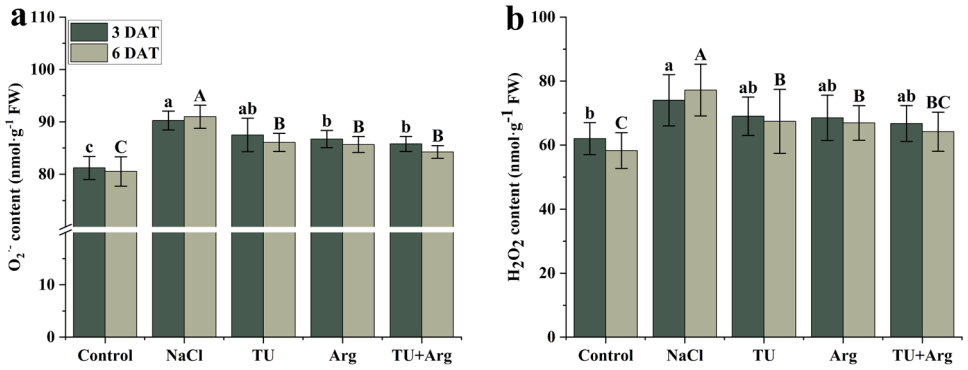

Fig. 4. Effects of TU and Arg on $O_2^{\cdot-}$ (**a**) and H_2O_2 (**b**) in wheat seedlings under salinity stress. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. DAT: days after treatment. *FW* fresh weight.

Effects of TU and Arg on MDA content and REL in wheat seedlings under salinity stress

The MDA content in the leaves increased significantly under salinity stress and continued to rise with the duration of the stress (Fig. 5a). The application of growth regulators TU and Arg significantly lowered the MDA levels in the leaves. Among these treatments, a combination of TU and Arg was the most effective, particularly at 6 DAT. At 6 DAT, the MDA content in the TU + Arg-treated group was further reduced by 12.82% and 5.56% compared to the TU and Arg groups, respectively (Fig. 5a).

REL exhibited a similar pattern to MDA, showing a significant increase under salinity stress that escalated with the stress duration (Fig. 5b). Likewise, both TU and Arg significantly decreased REL when applied individually; however, there was no significant difference in efficacy between the two. The combination of TU and Arg, however, displayed a significant synergistic effect. Relative to the TU and Arg groups, the REL in the TU + Arg group was significantly lowered by 6.78% and 7.96% at 3 DAT, and by 11.83% and 11.78% at 6 DAT, respectively (Fig. 5b).

Effects of TU and Arg on antioxidant enzyme activity in wheat seedlings under salinity stress

A significant increase in the activities of SOD, POD, CAT, and APX was detected in the leaves under salinity stress, with increases ranging from 14.54% (APX) to 30.08% (SOD) at 3 DAT (Fig. 6). The application of TU and Arg led to further enhancements in the activities of these four antioxidant enzymes. At 3 DAT, TU improved the activities of these enzymes by more than 28% relative to the control, with the most substantial increase observed in CAT activity at 55.85%. Arg's effects were comparable to those of TU, showing an overall increase in antioxidant enzyme activities of more than 27% and a peak increase in SOD activity of 59.24% over the control (Fig. 6). As expected, the combination of TU and Arg exhibited the best performance, with a 39.03% increase in APX activity over the control and impressive increases of 76.91%, 86.94%, and 79.55% for SOD, CAT, and POD, respectively (Fig. 6).

Similar to 3 DAT, at 6 DAT, the application of Arg and Tu also significantly increased the antioxidant enzyme activities of the wheat seedlings. The combination of TU and Arg remained the most effective. However, on day 6

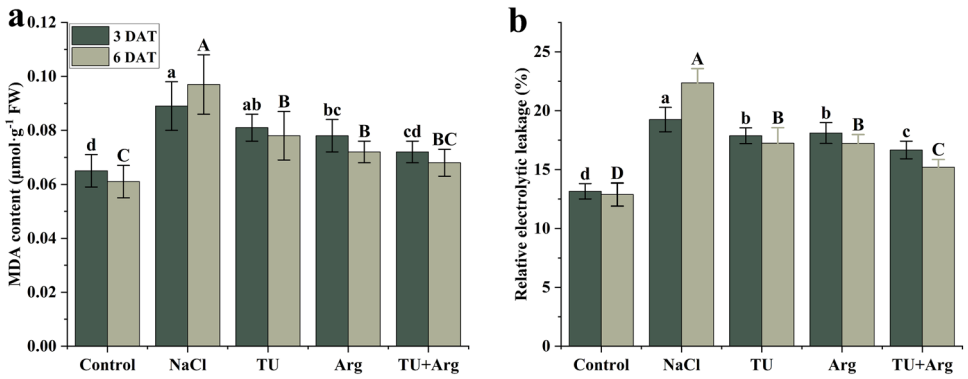

Fig. 5. Effects of TU and Arg on MDA (**a**) and REL (**b**) in wheat seedlings under salinity stress. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. DAT: days after treatment. *FW* fresh weight.

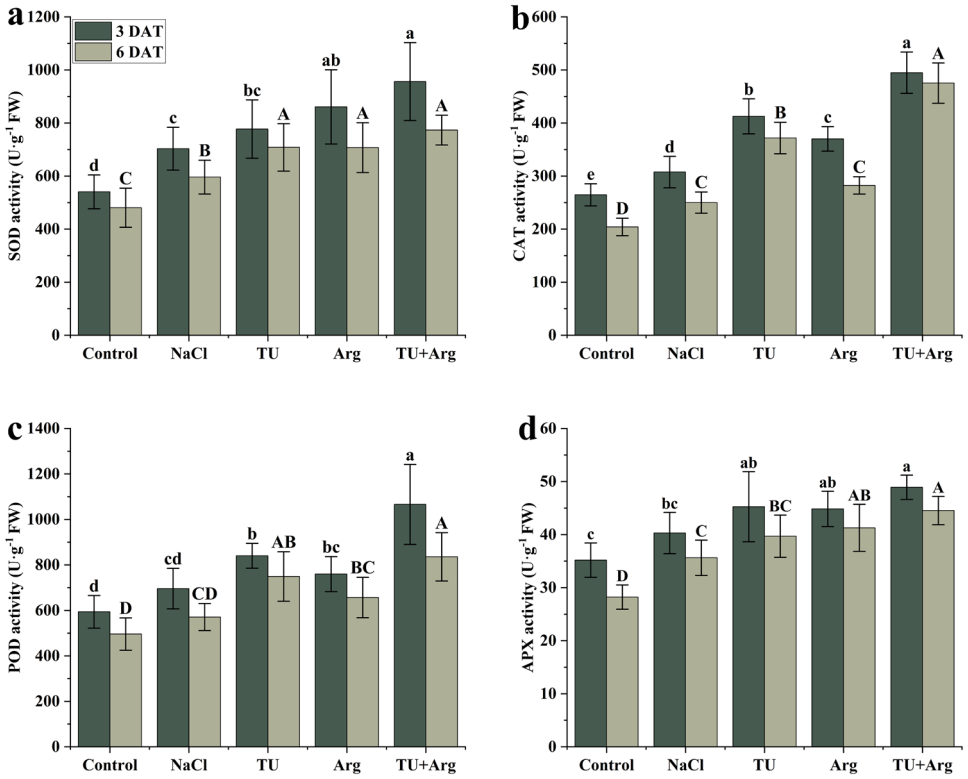

Fig. 6. Effects of TU and Arg on SOD (**a**), CAT (**b**), POD (**c**), and APX (**d**) activities in wheat seedlings under salinity stress. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. *DAT* days after treatment. *FW* fresh weight.

after treatment, a decreasing trend in the activities of all four antioxidant enzymes was observed under different treatment conditions compared to day 3 (Fig. 6).

Effects of TU and Arg on ion content in wheat seedlings under salinity stress

The contents of Na^+ and K^+ displayed distinct patterns under salinity stress, with a significant rise in Na^+ and a marked reduction in K^+ (Fig. 7a,b). At 3 DAT, Na^+ content increased by 236.51%, while K^+ content decreased by 59.06% compared to the control. However, the application of TU and Arg significantly curtailed the rise in Na^+ content and the decline in K^+ content. With TU and Arg, the increase in Na^+ was only 149.92% and 146.65%, respectively, while the reduction in K^+ was limited to 33.03% and 36.67% compared to the control. Not unexpectedly, the combined application of TU and Arg minimized the adverse effects of salinity stress. Under the combined influence of TU and Arg, Na^+ content increased by only 100.20%, while K^+ content decreased by just 22.83% (Fig. 7a,b). In addition, the Na^+/K^+ ratio generally followed the trend of Na^+ under different treatments, which increased significantly under salinity stress (Fig. 7c). Conversely, the application of TU and Arg significantly restrained this increase. The trends between treatment groups did not change markedly with extended treatment duration, with the effects of TU and Arg becoming more noticeable (Fig. 7c).

Ca^{2+} also rose significantly under salinity stress, but the increase was not as pronounced as that of Na^+ (Fig. 7d). There was an increase of 73.43% and 42.73% in Ca^{2+} content at 3 and 6 days after treatment, respectively. In the presence of TU and Arg, this increase was much less. With TU, the increments at the two-time points were only 42.57% and 15.24%, respectively, and with Arg, they were 44.91% and 16.36%. The increase in Ca^{2+} was even more drastically curbed by the combination of TU and Arg, with increases of just 31.67% and 4.55% at 3 DAT and 6 DAT, respectively, when compared with the control (Fig. 7d).

Discussion

Salinity stress is one of the most detrimental abiotic stresses affecting plant growth, negatively impacting not only the aboveground growth but also causing severe damage to the roots. The use of plant growth regulators or exogenous chemical substances has been shown to effectively mitigate the damage caused by salinity stress and enhance the salinity resistance of plants. Previous studies have demonstrated that thiourea (TU) can enhance the tolerance of rice seedlings to salinity stress by maintaining their redox homeostasis[20], while arginine (Arg) can improve plant salinity tolerance by activating their antioxidant enzyme defense mechanisms[30,35]. Our study found that salinity stress significantly reduced the dry matter mass, photosynthetic pigment content, and photosynthetic rate of wheat seedlings. However, these effects were alleviated by the application of TU and Arg,

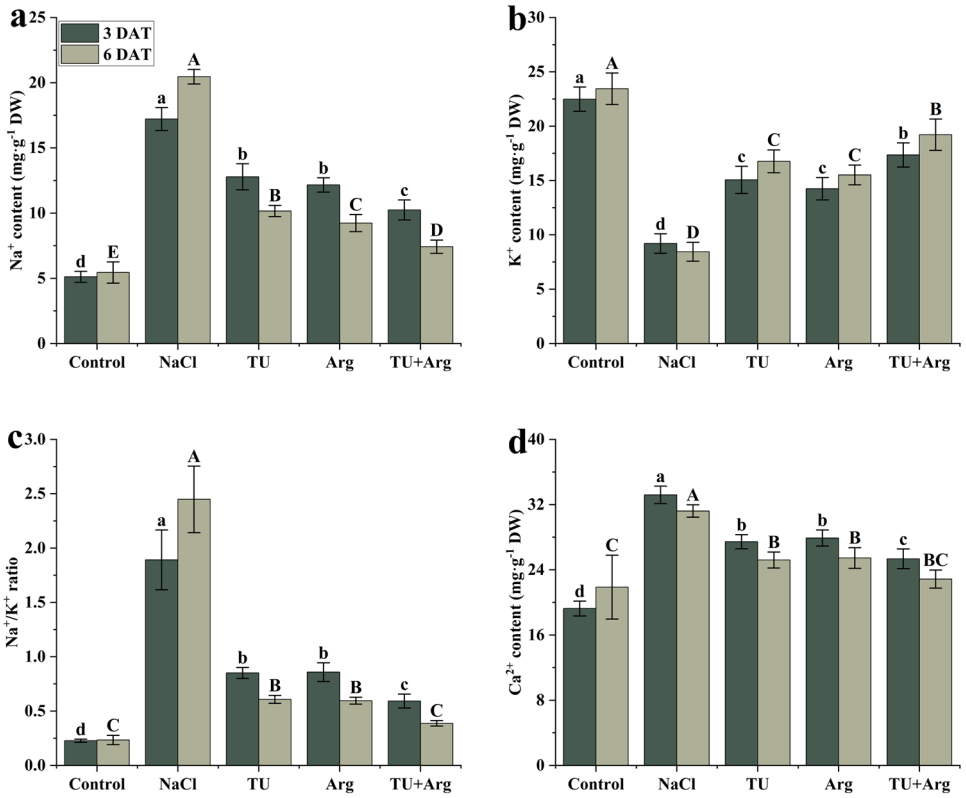

Fig. 7. Effects of TU and Arg on Na^+ (**a**), K^+ (**b**), Na^+/k^+ (**c**), and Ca^{2+} (**d**) in wheat seedlings under salinity stress. Different lowercase letters indicate significant differences between groups at the $p < 0.05$ level after 3 days of treatment; different uppercase letters indicate significant differences between groups at the $p < 0.05$ level after 6 days of treatment. Data are shown as mean ± SD, n = 5. *DAT* days after treatment. *DW* dry weight.

especially when used in combination. These results suggest that TU and Arg can improve the tolerance of wheat seedlings to salinity stress and exhibit a synergistic effect when applied together.

Photosynthesis, fundamental to dry matter accumulation in plants, occurs in chloroplasts, which are extremely sensitive to salinity. Salinity stress leads to oxidation of the plasma membrane, disruption of cellular osmotic balance, and damage to the ultrastructure of chloroplasts[36], induces chlorophyll degradation, reduces the activities of Calvin cycle enzymes including Rubisco, and decreases electron transfer from PS II to PS I[37]. Furthermore, salinity stress can cause stomatal closure, which reduces the concentration of CO_2 in the leaves and subsequently diminishes photosynthesis[38]. Our results corroborate previous findings that salinity stress reduced wheat stomatal conductance, leading to decreased leaf transpiration rates and intercellular CO_2 concentration, ultimately resulting in a decrease in photosynthetic capacity and a reduction in wheat biomass (Figs. 1 and 3). Interestingly, applying TU and Arg improved the photosynthetic efficiency of wheat plants under salinity stress. This enhancement in photosynthetic efficiency was particularly notable when TU and Arg were applied together (Fig. 3). This may be attributed to the fact that TU and Arg adjusted stomatal opening and closing, thus improving photosynthetic efficiency, a finding supported by previous studies. For example, Benzarti, et al. found that TU significantly increased stomatal conductance, CO_2 assimilation rate, and the maximum quantum efficiency of PS II photochemistry in *Atriplex portulacoides* L. under salinity stress[39]. Although there are no direct reports demonstrating that Arg can regulate stomatal opening and closing in plants stressed by salinity, Silveira, et al. indicated that Arg can promote gas exchange in leaves under drought stress[22].

Additionally, we found that TU and Arg increased the content of photosynthetic pigments in wheat plants under salinity stress (Fig. 2). This may be another significant reason for the enhanced photosynthesis in plants under salinity stress. The role of TU and Arg in increasing the photosynthetic pigment content of plants under salinity stress has already been demonstrated[40–42]. This may be attributed to the fact that the introduction of TU and Arg prevents the accumulation of ROS and reduces oxidative damage to the photosynthetic apparatus caused by salinity stress, while protecting chlorophyll-carrying organelles from damage[42,43]. More importantly, we found that the increase in photosynthetic pigment content and photosynthesis rate was more pronounced when TU and Arg were combined (Figs. 2 and 3). These findings suggest that TU and Arg, when used together, can more effectively reduce the damage caused by salinity stress on wheat photosynthesis. It is speculated that TU and Arg together could better mitigate the oxidative damage caused by salinity stress.

One of the most significant detrimental effects of salinity stress is oxidative damage caused by the accumulation of ROS[44]. Our study revealed that salinity stress significantly increased the ROS levels within the plants. This excessive accumulation of ROS inevitably led to membrane lipid peroxidation and disruption of the cell

membrane structure, as evidenced by increased MDA content and REL (Fig. 5). However, plants can mitigate this damage through their enzymatic and non-enzymatic antioxidant systems[34]. Among these, the enzymatic antioxidant systems are particularly efficient and sensitive. Previous research has demonstrated that antioxidant enzyme activities significantly increase in plants under salinity stress[6,10,35]. Consistent with these findings, our study also observed a significant increase in the activities of SOD, CAT, POD and APX under salinity stress (Fig. 6). Despite these increases, the ROS levels in the plants remained significantly elevated, both in previous studies and in our current research. This suggests that the observed increase in antioxidant enzyme activity was not sufficient to effectively scavenge the excessively produced ROS. Interestingly, the introduction of TU and Arg significantly enhanced the activities of these antioxidant enzymes and reduced the accumulation of ROS and MDA (Figs. 4, 5, and 6). TU and Arg have been demonstrated to activate the plant's antioxidant enzyme defense system and increase the activity of these enzymes[20–22,30]. TU, known as a ROS scavenger, predictably maintains plant redox homeostasis. For example, Pandey, et al. observed that TU modified the redox state in rice seedlings, enhancing their growth under salinity stress[20]. Additionally, Hussein reported that TU could mitigate the impact of salinity on the growth and yield traits of flax plants by enhancing their antioxidant defense mechanisms[21]. In our study, the beneficial effects of TU were accompanied by an increase in antioxidant enzyme activity and a decrease in ROS levels (Figs. 4 and 6). Meanwhile, the beneficial effect of Arg on plant antioxidant enzymes may be related to the protective effect of the released NO[45]. Arg acts as an exogenous NO donor that induces endogenous NO production[46]. NO functions as a signaling molecule that enhances salinity tolerance by increasing the production rate of various antioxidant enzymes in mitochondria[47]. NO helps prevent oxidative damage in stressed plants by regulating redox homeostasis and boosting the activity of H_2O_2 scavenging enzymes[48]. Additionally, NO triggers the expression of antioxidant genes, leading to higher enzyme activities that protect plants from stress[49,50]. More excitingly, the combination of TU and Arg triggered even higher antioxidant enzyme activity and a more effective reduction of ROS levels (Figs. 4 and 6). This suggests that the two plant regulators, each with its distinct mode of action, modulate the salinity tolerance of the wheat seedlings collaboratively, ultimately producing a synergistic effect.

Plants exposed to salinity stress often face concurrent osmotic and ionic stresses. Early signals that initiate plant responses to salinity stress include an excess of Na^+, an elevated intracellular Ca^{2+} level, and ROS accumulation[51]. An increase in Na^+ activates NHX-type Na^+/H^+ antiporters (NHXs) located at the plasma membrane, which facilitates the efflux of excess Na^+ from the cytoplasm or its sequestration into vesicles. This action also allows the efflux of K^+ from the vesicles, thus maintaining Na^+/K^+ balance in the cytoplasm[52]. TU and Arg have been shown to promote K^+ uptake in plants under salinity stress, thereby maintaining Na^+/K^+ homeostasis[35,53]. In this study, we found that the Na^+ content in wheat leaves increased significantly under salinity stress (Fig. 7a). Meanwhile, the K^+ content in wheat decreased significantly under the same conditions, indicating that salinity stress had severely disrupted the Na^+/K^+ balance. However, the application of TU and Arg reduced the increase in Na^ + and the decrease in K^+ (Fig. 7a,b). This result suggests that introducing TU and Arg can decrease Na^+ uptake and increase K^+ uptake in wheat under salinity stress to maintain Na^+/K^+ balance. Surprisingly, the combination of TU and Arg even exhibited a synergistic effect on achieving this balance. Furthermore, the high concentration of Na^+ in cells under salinity stress can activate calcium channels in the plasma membrane, leading to an increase in cytoplasmic Ca^{2+} content. Our study revealed a significant increase in Ca^{2+} content in wheat seedlings under salinity stress (Fig. 7d), suggesting that the rise in Na^+ content in the cells due to salinity stress induced an increase in Ca^{2+} content. Moreover, the abundant Ca^{2+} in the cytoplasm can stimulate the activity of antioxidant enzymes, thus reducing the H_2O_2 content in the cells[54,55]. However, this self-protection mechanism is insufficient in the face of severe salinity stress, as evidenced by the increased H_2O_2 content in wheat under such stress in this study (Fig. 4b). Interestingly, TU and Arg compensated for this effectively. Although Ca^{2+} was slightly reduced in their presence, the content of H_2O_2 was also reduced.

Conclusions

In conclusion, this study highlights that despite their differing modes of action and physicochemical properties, both TU and Arg can impart a considerable level of NaCl stress tolerance to wheat seedlings, particularly when used in combination. The application of TU and Arg can activate the antioxidant enzyme defense system in wheat seedlings, reduce ROS content, and maintain the stability of membrane lipids, thus supporting photosynthesis and the Na^+/K^+ balance in the seedlings. However, this study has limitations; although the synergistic effect of TU and Arg has been demonstrated and its physiological mechanism explained to some degree, the more complex molecular mechanisms remain unknown. Therefore, further investigation into the mechanisms of the combined action of TU and Arg using transcriptomics, metabolomics, and other methods is necessary.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Conceptualization, R.L. and L.X.; investigation, J.L. and Z.T.; writing—original draft preparation, J.L.; writing—review and editing, J.L. and L.X.; supervision, P.X; project administration, P.X.; funding acquisition, R.L. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare no competing interests.

Ethical approval

We confirm that all the experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation. All of the material is owned by the authors and/or no permissions are required.

Additional information

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