Polyamines conjugated to deoxyribonucleic acid-protein in cell nucleus from filling grain embryos were involved in tolerance of wheat to drought

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Deoxyribonucleic acid-protein (DNAP) of the cell nucleus was purified from developing wheat (Triticum aestivum L.) embryo cells under drought stress, with two cultivars differing in drought tolerance as experimental materials – Longmai No. 079 (drought-tolerant) and Wanmai No. 52 (drought-sensitive). Levels of polyamines (PAs) non-covalently conjugated to the DNA and covalently conjugated to the proteins of DNAP were detected. After soil drought treatment for 10 days, in drought-tolerant Longmai No. 079, the increases in the levels of spermine and spermidine non-covalently conjugated to DNA of DNAP were more statistically significant ($P<0.05$) than in drought-sensitive Wanmai No. 52. Treatment of Wanmai No. 52 with exogenous Spm could not only enhance the tolerance of the cultivar to drought stress, as judged by flag leaf water content, plasma membrane permeability and grain growth, but also elevate the levels of spermine and spermidine non-covalently conjugated to the DNA of the cultivar. On the contrary, treatment of Longmai No. 079 with methylglyoxyl-bis guanylhydrazone, an inhibitor of S-adenosylmethionine decarboxylase, could significantly ($P<0.05$) aggravate the drought stress to this cultivar, accompanied by a marked decreases in the levels of spermine and spermidine non-covalently conjugated to the DNA of the cultivar. On the other hand, the content of putrescine covalently conjugated to the proteins of DNAP rose more markedly ($P<0.05$) in Longmai No. 079 than in Wanmai No. 52. The transglutaminase inhibitor, o-phenanthroline, could markedly reduce the drought-induced increase in the level of putrescine covalently conjugated to the proteins of DNAP and aggravate drought stress to the two cultivars. Collectively, it could be inferred that spermine and spermidine non-covalently conjugated to the DNA and putrescine covalently conjugated to the proteins of DNAP in the developing grain embryo cell nucleus might enhance the tolerance of wheat plants to soil drought.

Keywords. Abiotic stress; conjugated polyamines; deoxyribonucleic acid-protein (DNAP); drought stress; wheat (Triticum aestivum L.)

1. Introduction

Drought, salt, high and low temperatures, and heavy metal stress, greatly affect plant growth and development (Beshamgan et al. 2019; Zhong et al. 2020; Ebmeyer et al. 2021; Rakić et al. 2021), and thereby pose a severe threat to agricultural productivity and sustainability (Lechowska et al. 2022). Areas under soil drought are spreading rapidly worldwide and the tolerance of crops to drought stress (DS) is of particular importance. Therefore, it is increasingly important to analyze the mechanisms underlying crop tolerance to DS. Wheat is the world’s main food crop and easily subjected to soil drought and dry-hot wind stresses during grain filling and embryo maturing. So, it is vital to explore wheat drought tolerance mechanisms at the
Polyamines (PAs) are plant growth regulators with positive charges and strong biological activity, which are found in various organisms (Sobieszczuk-Nowicka et al. 2019). Three PAs, putrescine (Put), spermidine (Spd), and spermine (Spm), are the most common members of the PA family. In plants, Put derives from ornithine or arginine through decarboxylation and becomes an obligate precursor for the formation of higher PAs, Spd and Spm, by the sequential addition of amimo-propyl groups donated by decarboxylated S-adenosylmethionine (SAM), which is formed from SAM by SAM decarboxylase (SAMDC). Many factors and processes are involved in Put conversion to Spd (Spm). Among these, SAMDC is the most important enzyme and is exclusively and potently inhibited by methylglyoxal-bis guanylylhydrazone (MGBG) (Tiburcio et al. 1993; Lutts et al. 2013).

Due to their poly-cationic nature, PAs, especially Spd and Spm, can be linked to anionic macromolecules (such as membrane phospholipid, acid proteins, and DNA) via ionic bonding, and form non-covalently conjugated PAs. Our previous research show that non-covalently conjugated PAs in bio-membranes play an important role in stabilizing membrane function and conformation (Du et al. 2020, 2021, 2022; Liu et al. 2021). In addition to the aforementioned form, PAs can be conjugated covalently to protein endo-Glu residues and converted into covalently conjugated PAs by the action of transglutaminase, which can be inhibited by o-phenanthrolin (Zhong et al. 2020; Du et al. 2022). The PAs can play important roles in the processing of post-translated protein modification (Del Duca et al. 2014). Our previous studies show that the form of PAs in plasma membranes can enhance chilling tolerance of post-harvest plum fruit (Du et al. 2021) and maize drought tolerance by maintaining membrane stability of maturing maize grain embryos (Du et al. 2022). Overall, as plant growth regulators, PAs regulate plant development, morphogenesis, and response to abiotic stress (Du et al. 2019; Gondor et al. 2021; Hashem et al. 2021; Pál et al. 2021). Especially, there is an increasing spate of interest in the functions of conjugated PAs. However, the relationship between DS and PAs conjugated to the DNAP from the cell nucleus of developing wheat embryos remains to be explored.

This research aimed to illuminate the significance of PAs conjugated to DNAP from embryo cells during wheat grain filling under DS, with two wheat cultivars, Longmai No. 079 (drought-tolerant) and Wanmai No. 52 (drought-sensitive), as experimental materials. To get more insight into PA biosynthesis, the activities of two key enzymes, SAMDC, which affects biosynthesis of higher PAs, and transglutaminase, which affects conversion of free PAs to bound PAs, were also determined. To further testify conjugated PA function, the exogenous Spm, which could elevate non-covalently conjugated PA contents, was applied to the experiment. Furthermore, treatments with inhibitors, MGBG and o-phenanthrolin, which could decrease the levels of non-covalently conjugated and covalently conjugated PAs, respectively, were also additionally implemented in the research, to show whether decreased in the two forms of PAs could affect wheat drought tolerance. The tolerance of wheat plants to DS was judged by flag leaf water content, plasma membrane permeability and grain growth.

2. Materials and methods

2.1 Plant material

Wheat (Triticum aestivum L.) cultivars Longmai No. 079 and Wanmai No. 52 were used in the present study. Longmai No. 079 (F1 generation of Loumai No. 1 and TW98-829-1) and Wanmai No. 52 (F1 generation of Zhengmai No. 8329 and Wanmai 19) are all hybrids and bred at the Pingliang Academy of Agricultural Sciences, Gansu province, China, and Suzhou Seed Company, Anhui Province, respectively. The wheat seeds were procured from Prof. Guozhang Kang of Henan Agricultural University. Permission to use them in this experiment was according to the legislation and guidelines of China. Longmai No. 079 is distributed in Northwest China, a drought ecotope, while Wanmai No. 52 is mainly planted in Central China, a rainy ecotope. From geographical distribution of the two cultivars, it was confirmed that Longmai No. 079 was drought-tolerant, and Wanmai No. 52 was drought-sensitive. The drought tolerance of the two cultivars would be further verified in the research.

This experiment was carried out at Zhoukou Normal University in Henan province from 2017 to 2020. On October 25, in 2017, 2018 and 2019, wheat seeds were
planted in plastic basins (33 seeds/basin) after the seed surface was sterilized for 5 min with 0.1% HgCl₂ (w/v). Each basin (height: 50 cm, bottom diameter: 35 cm, rim diameter: 40 cm) was filled with 15 kg screened soil, which was taken from the surface in wheat experimental field and contained 13.5 g organic matter kg⁻¹, 120 mg available nitrogen kg⁻¹, 30 mg available phosphorus kg⁻¹, 105 mg available potassium kg⁻¹, and the other macro-elements and micro-elements, meeting the crop’s demand for nutrients during the growth period. After the seedlings were vernalized, they were thinned to 10 seedlings/basin and then placed in the field. The wheat was grown under natural conditions. After fertilization, wheat plants were transferred to a greenhouse with 25°C/15°C (day/night) temperature, 35% relative air humidity, 16 h/8 h light/dark photoperiod, and 600 μmol m⁻² s⁻¹ photosynthetic quantum flux density.

2.2 Plant material treatments

As soon as the basins with wheat plants had been placed in greenhouse, the soil water potentials of all basins were detected with a potential instrument (Zhejiang Top Yunong Technology Co., LTD, Hangzhou, China, Model: TRS-II) and each group was monitored every 3 h. If necessary, water was timely replenished to ensure the water potential of the control and treated groups were -0.15 and -1.0 MPa, respectively. On the 10th day after fertilization, the wheat plants were treated as follows:

Control: Flag leaf (FL) and spike were sprinkled with de-ionized water and root was in -0.15 MPa water potential soil.
Drought treatment: FL and spike were sprinkled with de-ionized water and root was in -1.0 MPa water potential soil.
Drought+Spm treatment: FL and spike were sprinkled with Spm (1 mM) and root was in -1.0 MPa water potential soil.
Drought+MGBG treatment: FL and spike were sprinkled with MGBG (0.5 mM) and root was in -1.0 MPa water potential soil.
Drought+o-phenanthrolin treatment: FL and spike were sprinkled with o-phenanthrolin (0.2 mM) and root was in -1.0 MPa water potential soil.

The PAs and inhibitors mentioned above were from Sigma Chemical Co. (USA). The dose determination of above-mentioned reagents was based on our preliminary experiments. Wheat FL and spikes of treated groups were sprayed with solution containing the above-mentioned reagents, 0.1% ethanol, and 0.01% (V/V) Tween 20, using a 25 mL/basin at 6:00 and 18:00 every day. The control group was sprayed with water containing 0.1% ethanol and 0.01% (V/V) Tween 20. The FL and seeds in the middle position of the spike were collected at 6:00 on the 10th day after treatment. The embryos were carefully sampled from the seeds with a scalpel and tested.

2.3 Assessment of flag leaf relative water content (FLRWC)

After fresh FL was weighed for fresh weight (LFW), the sample was immediately immersed in de-ionized water for about 5 h until the FL weight was constant. It was regarded as saturation weight (LSW). Then, the sample was placed in an oven and dried at 75°C for about 12 h until the sample weight was constant, i.e. the dry weight (LDW). FLRWC was assessed using the formula: FLRWC (%) = (LFW – LDW) / (LSW – LDW) x 100.

2.4 Assessment of flag leaf relative plasma membrane permeability (FLRPMP)

Wheat FLRPMP was assessed according to the method described by Du et al. (2021) with minor adjustments. 1 g of wheat FL was immersed into 10 mL de-ionized water in a test tube. Then, in the dark, the tube was put in water bath for 2 h at 25°C. Original electrical conductivity (OEC) of the water medium containing FL was determined with a conductivity meter (Guangzhou Ruibin Technology Co., Ltd, Guangzhou, China, Model: DDB-11A). Afterwards, the flag leaves were boiled for 20 min at 100°C, cooled to 25°C, and left to stand for 30 min. The terminal electrical conductivity (TEC) was determined. DEC represents the electrical conductivity of de-ionized water. FLRPMP was assessed by the formula FLRPMP (%) = (OEC – DEC) / (TEC – DEC) x 100.

2.5 Examination of 1000-grain weight and grain number per spike

The wheat plants from which no leaf and seeds were sampled were left growing in the greenhouse under normal growing conditions until the seeds were fully...
matured. Then, the seeds were sampled for examination of 1000-grain weight and grain number per spike.

2.6 Assay of activities of SAMDC and transglutaminase in filling grain embryos

The activity of SAMDC was assayed by examining the release of $^{14}$CO$_2$ using substrates labeled with isotope $^{14}$C by the method of Kaur-Sawhney and Shin (1982). One enzyme activity unit was expressed as 1 μL $^{14}$CO$_2$ g$^{-1}$ fresh weight (FW) min$^{-1}$.

Transglutaminase activity was determined by detecting the rate of incorporation of Put labeled with $^3$H into proteins according to the method of Ikekson and Apelbaum (1987). One enzyme activity unit was expressed as 1 nmol of $^3$H Put mg$^{-1}$ protein h$^{-1}$.

2.7 Preparation of DNAP

DNAP of the cell nucleus was purified from filling grain embryos by the method of Liu et al. (2005) with some modifications. First, 2 g of fresh embryos was immersed in cooled aether, and then washed 3 times with isolating-buffer solution, which contained Tris-HCl (10 mM, pH 7.6), MgCl$_2$ (5 mM), DTT (0.5 mM), and sucrose (1 mM). Then, the embryos were immediately homogenized in 5 mL of the aforementioned isolating-buffer solution. Seconds, the homogenate was carefully filtered with 3 layers of cheesecloth, and then centrifuged at 5000g for 10 min. The precipitation was re-suspended with 1 mL of the aforementioned isolating-solution, homogenized again, and then centrifuged at 5000g for 10 min. Third, the precipitation was re-suspended in 1 mL of buffer, which contained HEPES (10 mM, pH 7.9), KCl (10 mM), MgCl$_2$ (1.5 mM), and DTT (0.5 mM), and centrifuged at 2000g for 5 min. The sediment was re-suspended in the 1 mL HEPES buffer solution mentioned above, cooled by ice for 15 min, and homogenized and centrifuged at 4000g for 10 min. Finally, the sediment was re-suspended in 0.5 mL salt-buffer solution which contained HEPES (20 mM, pH 7.9), glycerol (25%, v/v), MgCl$_2$ (1.5 mM), DTT (0.5 mM), KCl (20 mM), and EDTA (0.2 mM), and was homogenized. The homogenate was then infused by 0.5 mL salt-buffer which contained HEPES (20 mM, pH 7.9), glycerol (25%, v/v), MgCl$_2$ (1.5 mM), DTT (0.5 mM), KCl (1.2 M), and EDTA (0.2 mM), and was mixed for 0.5 min and centrifuged at 30000g for 25 min. DNAP was contained in the supernatant and used for the detection of protein, DNA, and PAs conjugated to it.

2.8 Determination of protein and DNA contents

The content of protein in the aforementioned supernatant was determined using the classical way of Bradford (1976), with bovine serumalbumin as standard. The content of DNA in DNAP in the supernatant was determined with the method of Liu et al. (2005).

2.9 Extraction and quantification of PAs non-covalently conjugated to DNA

1 mL of the aforementioned supernatant (containing DNAP) was added with 1 mL 5% PCA and centrifuged at 25000g for 20 min. The PAs non-covalently conjugated to the DNA of DNAP were in the supernatant and the PAs covalently conjugated to the proteins of DNAP were in the precipitation. 0.5 mL supernatant was added with 1 mL 2 M NaOH and 7 μL benzoyl chloride, vortexed vigorously for 5 s three times, left to stand for 60 min at 25°C, and added with 2 mL saturated NaCl. Then, the mix was added with 2 mL diethyl ether, vortexed vigorously for 5 s three times, and centrifuged at 1500g for 5 min. 1 mL diethyl ether was collected and evaporated with warm air. The sediment was re-dissolved into 1 mL methanol. PAs were quantified by HPLC (Waters 2695, USA) with an ultraviolet detector (Waters 2487, USA). A C-18 reverse-phase separation column was used, with 1, 6-hexanediamine as the internal standard and 254 nm as the detecting wavelength. At 25°C, the PA sample was eluted from the separation column by a Perkin-Elmer Series 410 pump at 0.6 mL/min. PA contents in DNA were denoted as nmol μg$^{-1}$ DNA.

2.10 Extraction and quantification of PAs covalently conjugated to proteins in DNAP

The precipitation mentioned above contained PAs covalently conjugated to proteins of DNAP. PCA (1 mL of 5% solution) was added, vortexed vigorously, and centrifuged at 22000g for 40 min at 4°C. The sediment was re-suspended with 1 mL 1 M NaOH, vortexed, kept in an ice bath for 30 min, and centrifuged at 22000g for 40 min at 4°C. 1 mL of the supernatant was mixed with 1mL of 12 N HCl, sealed immediately in an ampere glass bottles, and acid-
hydrolyzed at 110°C for 15 h. The glass bottles were carefully crevassed to make HCl evaporate at 80°C. Finally, the residue was added with 10% PCA, benzoylated with benzoyl chloride, and quantified by HPLC as described as the PAs non-covalently conjugated to DNA. The content of PAs covalently conjugated to proteins was denoted as nmol mg⁻¹ protein.

2.11 Statistical analysis

The experiment was performed three times, i.e. three biological replicates, in 2017–2020, and three technical replicates were carried out in every biological replicate. Therefore, the data shown in the paper were averages of 9 values ± S.E. Average deviation was evaluated using two-way analysis of variance (ANOVA), and Duncan’s method was used to compare multiple group means at the P<0.05 level. The significant differences among multiple groups were indicated by different letters above the columns in the figures.

3. Results

3.1 Effects of DS, exogenous Spm and inhibitors on FLRWC and FLRPMP of wheat

Under DS for 10 d, FLRWC of Longmai No. 079 decreased to 85%, whereas it decreased to 68% in Wanmai No. 52 (figure 1A). The results of statistical analysis showed that FLRWC decreased more significantly (P<0.05) in drought-sensitive Wanmai No. 52 than that in drought-tolerant Longmai No. 079. Exogenous Spm treatment markedly (P<0.05)
alleviated the drought-induced decrease in FLRWC of the both cultivars, especially of the drought-sensitive Wanmai No. 52, while the treatment with inhibitor, MGBG or \( \sigma \)-phenanthrolin, markedly \((P<0.05)\) aggravated the drought-induced decrease in FLRWC of the both cultivars, especially of the drought-tolerant Longmai No. 079 (figure 1A). As shown in figure 1B, FLRPMP of the two wheat cultivars increased under DS treatment, and the parameter increased more significantly \((P<0.05)\) in drought-sensitive Wanmai No. 52 than in drought-tolerant Longmai No. 079. It rose up to 35% and 19% in Wanmai No. 52 and Longmai No. 079, respectively. Exogenous Spm treatment markedly \((P<0.05)\) alleviated the increase in FLRPMP of the two cultivars induced by DS, especially of the drought-sensitive Wanmai No. 52.

### 3.2 Effects of DS, exogenous Spm and inhibitors on wheat 1000-grain weight and grain number per spike

From figure 2, it could be seen that DS treatment distinctly decreased 1000-grain weight (figure 2A) and grain number per spike (figure 2B) of the two wheat cultivars, compared with the control, and the two parameters of Wanmai No. 52 decreased by 32.4% and 32.6%, respectively, whereas they decreased by 7.2% and 9.1% in Longmai No. 079, respectively, indicating they decreased more significantly in Wanmai No. 52 than those in Longmai No. 079. Application with MGBG or \( \sigma \)-phenanthrolin aggravated the DS-induced decreases in the two parameters of the two wheat cultivars, and the effects of the inhibitors were more marked \((P<0.05)\) on Longmai No. 079 than on Wanmai No. 52. Exogenous Spm could alleviate the effects of DS on the two parameters of both cultivars, especially of the drought-sensitive Wanmai No. 52.
3.3 Effects of DS, exogenous Spm, and MGBG on levels of PAs non-covalently conjugated to DNA of DNAP from embryo cells of filling wheat grains

Spm and Spd non-covalently conjugated to DNA could be detected in filling grain embryos and Spm was abundant, while non-covalently conjugated Put might be too trace to be detected in the study. Following DS treatment, the levels of Spm and Spd non-covalently conjugated to DNA rose more markedly ($P<0.05$) in drought-tolerant Longmai No. 079 cv. than those in Wanmai No. 52 cv. Treatment with exogenous Spm apparently enhanced the DS-induced increase in the levels of the two forms of PAs in Wanmai No. 52 cv., while treatment with MGBG obviously inhibited the DS-induced increase in the levels of the both PAs in Longmai No. 079 cv. greatly. However, the effects of Spm on Longmai No. 079 and MGBG on Wanmai No. 52 were all slight (figure 3).

3.4 Effects of DS and o-phenanthrolin on level of Put covalently conjugated to proteins of DNAP from embryo cells of filling wheat grains

The other PAs covalently conjugated to the proteins of DNAP could not be detected except Put. The level of Put covalently conjugated to DNAP increased by over 3 times in drought-tolerant Longmai No. 079 cv., compared with the control (figure 4). However, it increased only by 1.5 times in drought-sensitive Wanmai No. 52 cv. (figure 4). Application of o-phenanthrolin retarded the increases in the level of this form of Put of the two wheat cultivars induced by DS and the inhibition effect...
was more marked \((P < 0.05)\) on Longmai No. 079 cv. than that on Wanmai No. 52 cv. (figure 4).

### 3.5 Effects of DS and inhibitors on activities of SAMDC and transglutaminase in wheat filling grain embryos

Under DS for 10 d, SAMDC activity rose in wheat filling grain cells (figure 5A). More interestingly, the DS-induced increase was much more significantly \((P<0.05)\) in drought-tolerant Longmai No. 079 than that in drought-sensitive Wanmai No. 52. MGBG treatment could inhibit significantly \((P<0.05)\) the DS-induced increase in SAMDC activity. From the results shown in figure 5B, it could be seen that TGase activity in grain embryo cells increased in both Longmai No. 079 and Wanmai No. 52 under DS, and the increase in the former was more significant \((P<0.05)\) than that in the latter. Compared with the control, in Longmai No. 079, the activity rose by 2 times, while in Wanmai No. 52, it rose merely by 50%. Application with inhibitor, o-phenanthroline could inhibit markedly \((P<0.05)\) the DS-induced increase in TGase activity.

### 4. Discussion

#### 4.1 Selection of wheat cultivars with different drought tolerance

Based on the following two reasons, two wheat cultivars, Longmai No. 079 and Wanmai No. 52 were selected as experimental materials in the study. First, Longmai No. 079 was planted in a drought ecotope of Northwest China, such as Gansu and Ningxia province, while Wanmai No. 52 was distributed in a rain-rich ecotope of Central China, such as Anhui, Jiangsu, and Hubei province. Second, growth inhibiting is regarded as one of obvious reactions of crops to DS and quantified by biomass and production accumulation. So, by analyzing the differences in important agronomic traits, such as 1000-grain weight (figure 2A) and grain number per spike (figure 2B), between the two cultivars under DS, it could be confirmed that Longmai No. 079 and Wanmai No. 52 were drought-tolerant and drought-sensitive, respectively. To further verify the difference between the two cultivars in drought tolerance, besides agronomic parameters above, FLRWC and FLRPMP, which were closely associated with DS and membrane injury, respectively, were also assessed. From the results, we could securely conclude that Wanmai No. 52 was drought-sensitive and Longmai No. 079 was drought-tolerant.

#### 4.2 Relationship between DS and PAs non-covalently conjugated to DNA of DNAP from embryo cells of filling wheat grains

By carrying more positive charges at physiological pH, Spm and Spd, especially tetra-amine Spm, can be non-covalently conjugated to DNA, which carried negative charges, more easily than Put (Dutra et al.)
So, in this research, Spm non-covalently conjugated to DNAP of embryo cells of wheat filling grains was the most abundant, followed by Spd (figure 3). From the results in figure 3, it was suggested that the two forms of PAs might play important roles in enhancing the tolerance of wheat plants to DS. The finding was further testified with application of exogenous Spm and inhibitor MGBG. Exogenous treatment with Spm not only enhanced markedly ($P<0.05$) the increases induced by DS in the contents of Spm and Spd non-covalently conjugated to DNAP of Wanmai No. 52 (figure 3), but also alleviated markedly drought injury to this cultivar simultaneously, as judged by the decrease in FLPMP (figure 1B) and increases in FRWC (figure 1A), 1000-grain weight (figure 2A), and grain number per spike (figure 2B). The results of inhibitor MGBG treatment also verified the finding. The finding was further supported by correlation analysis. Under DS for 10 days, the total content of Spd+Spm non-covalently conjugated to DNAP in grain embryos was positively significantly correlated to 1000-grain weight ($r_{0.05}=0.99$, $n=6$) (figure 6A) and grain number per spike ($r_{0.05}=0.90$, $n=6$) (figure 6B).

The present results are in agreement with those of our previous research on the relationship between water stress and non-covalently conjugated PAs in plasma membranes (Du et al. 2022), tonoplast vesicles (Liu et al. 2004; Du et al. 2020), and mitochondrion membranes (Liu et al. 2021). It has been well documented that Spm (Spd) could be non-covalently conjugated to bio-macromolecules, such as acidic protein and membrane phospholipids, to play important roles in DS (Farooq et al. 2009; Dutra et al. 2013). For instance, Farooq et al. (2009) argue that Spm is the most effective in enhancing drought
resistance among the PAs. Additionally, data recently provided testimony for PA significance in regulating cell membrane proteins, such as two major vacuolar cation channels and plasma membrane H⁺-ATPase (Pottosin et al. 2021; Du et al. 2022). Therefore, it could be inferred that by being non-covalently conjugated to DNAP under DS, Spm (Spd) maintained the DNA double helix conformation and thereby regulated gene expression (Ha et al. 1998). Additionally, these forms of PAs might function in a highly compressed chromosome structure (Antony et al. 1999).

Exogenous PA applications in agriculture have been well documented (Dutra et al. 2013). However, the effects of different exogenous PAs on different plants were inconsistent. For example, the results of Jing et al. (2020) suggested Spm could play a better function in alleviating the injury of heat stress to wheat plants than Spd, which was different from our results. Our results indicated that exogenous Spm had the same effect as exogenous Spd (data not shown) in enhancing the tolerance of wheat plants to DS. This disagreement might be attributed to different abiotic stress and differently tolerant wheat cultivars.

### 4.3 Relationship between DS and Put covalently conjugated to proteins of DNAP from embryo cells of filling wheat grains

From the results in figure 4, it could be concluded that Put covalently conjugated to the proteins of DNAP was also involved in wheat DS tolerance. This notion was further supported by the following experimental

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**Figure 6.** The results of statistical analysis for correlation (A) between total content of non-covalently conjugated Spd+Spm and 1000-grain weight; (B) between total content of non-covalently conjugated Spd+Spm and grain number per spike; (C) between covalently conjugated Put and 1000-grain weight; D: between covalently conjugated Put and grain number per spike.
results. Treatment with o-phenanthroline, by inhibiting transglutaminase activity (figure 5B), markedly ($P<0.05$) inhibited the DS-induced increase in the level of Put covalently conjugated to DNAP in Longmai No. 079 (figure 4), and accordingly, the tolerance of this cultivar to DS decreased markedly (figures 1 and 2). The present finding was further supported by correlation analysis. Under DS for 10 days, the level of Put covalently conjugated to proteins of DNAP in grain embryos were positively significantly correlated to 1000-grain weight ($r_{0.05}=0.99$, $n=4$) (figure 6C) and grain number per spike ($r_{0.05}=0.96$, $n=4$) (figure 6D).

This was also supported by previous research (Del Duca et al. 2014; Zhong et al. 2020). For example, Del Duca et al. (2014) reported that PAs covalently conjugated to the proteins could function in post-translational modification of chlorophyll proteins and stabilization of the protein conformation by preventing the proteins from denaturing and were associated with senescence and programmed cell death. Yang et al. (2007) also reported that in flag leaves of rice plants, the drought-induced increase in acid-insoluble conjugated Put has a role in rice grain yield formation. Recently, it was reported that PAs covalently conjugated to proteins might enhance salt stress tolerance in tomato and tobacco (Zhong et al. 2020) and chilling tolerance in plum fruit (Du et al. 2021). Especially, our recent research suggested that PAs covalently conjugated to the proteins in plasma membranes mitigated cell plasmolysis and thereby maintained membrane conformation of maize embryos under DS (Du et al. 2022). So, it could be inferred that Put covalently conjugated to histone might maintain nucleosome conformation and function.

5. Conclusion

In summary, this study was the first to examine the conjugated PAs in DNAP from embryo cells of filling wheat grains. Furthermore, it was suggested that Spm/Spd non-covalently conjugated to the DNA and Put covalently conjugated to the proteins in DNAP could elevate the tolerance of wheat plants to DS. The mechanism underlying conjugated PAs-mediated tolerance deserve further study. Additionally, our findings also elucidated one of reasons why exogenous Spm could enhance the tolerance of wheat plants to DS. Although there were important discoveries revealed by this study, there were also limitations.

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