



# Relationship between polyamines conjugated to mitochondrion membrane and mitochondrion conformation from developing wheat embryos under drought stress

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The mitochondrion conformation and the contents of conjugated polyamines were investigated using the embryos of developing wheat (*Triticum aestivum* L.) grains of two cultivars differing in drought tolerance as experiment materials. After drought stress treatment for 7 days, the relative water content of embryo and relative increase rate of embryo dry weight of the drought-sensitive Yangmai No. 9 cv. decreased more significantly than those of the drought-tolerant Yumai No. 18 cv. Furthermore, the changes in mitochondrion conformation of Yangmai No. 9 were more marked. Meanwhile, the increases of the contents of conjugated non-covalently spermidine (CNC-Spd) and conjugated covalently putrescine (CC-Put) of Yumai No. 18 were more obvious than those of Yangmai No. 9. Treatment with exogenous Spd not only alleviated the injury of drought stress to Yangmai No. 9, but also enhanced the increase of CNC-Spd content and inhibited the change in the mitochondrion conformation of this cultivar. The treatments of Yumai No. 18 with two inhibitors, methylglyoxyl-bis (guanylhydrazone) and phenanthroline, significantly inhibited the drought stress-induced increases of CNC-Spd and CC-Put contents of the cultivar, respectively. Meanwhile, the treatments with the two inhibitors aggravated the injury of drought stress to Yumai No. 18 and enhanced the change in the mitochondrion conformation of this cultivar. These results mentioned above suggested that the CNC-Spd and CC-Put in embryo mitochondrion membrane isolated from developing grains could enhance the wheat tolerance to drought stress by maintaining the mitochondrion conformation.

**Keywords.** Conjugated polyamines; Drought stress; Mitochondrion conformation; Wheat (*Triticum aestivum* L.)

## 1. Introduction

Due to an inability to mobilize so as to avoid environmental stress, plants have evolved unique adaptations to stress, some of which involve the mitochondria (Liu and Li 2019). Clearly, a plant mitochondrion is a highly unusual and complex organelle, and then, in light of its intricacy, progresses toward understanding its many unique features have been impressive over the past years (Machenzie and McIntosh 1999; Baker *et al.*

2019; Huang *et al.* 2020). In many cases, a mitochondrion is a key site of damage during environmental stress, especially mitochondrion electron transport (Downs and Meckathorn 1998). Drought stress is common stress that affects food supplies in natural and agricultural systems and the response of a plant to drought stress have been widely studied (Du *et al.* 2015, 2019, 2020). Usually, an embryo is the most important organ during grain filling and the stress-related impairment of mitochondrion function is likely to

be particularly important in an embryo. The changes in mitochondrion conformation under various abiotic stresses have been documented over the past decade (Liu and Li 2019; Machenzie and McIntosh 1999; Huang et al. 2020).

Polyamines (PAs) are biologically ubiquitous aliphatic amines that are implicated in growth and development in a wide range of organisms (microorganism, animals, and plants). PAs are related closely with many abiotic stresses such as water stress, salt stress, temperature and heavy metal stresses, etc. (Do et al. 2013; Du et al. 2017; Goyal and Asthir 2010; Tang and Newton 2005; Taie et al. 2019). In plants, the common PAs include mainly putrescine (Put), spermidine (Spd), spermine (Spm). Put converts into Spd via linking up an aminopropyl moiety at either end and into Spm via linking up two aminopropyl moiety at both ends. The key enzyme which catalyzes the conversion is S-adenosylmethionine decarboxylase, which is inhibited potently by methylglyoxyl bis (guanyl hydrazone) (MGBG) (Slocum 1991). Due to the virtue of their poly-cationic nature at physiological pH, by ionic bonding, PAs could interact with anionic macromolecules and form conjugated non-covalently PAs (CNC-PAs) in the membrane (Feuerstein and Marton 1989). Via these interactions, PAs play important roles in stabilizing the conformation and function of the bio-membrane (Galston and Kaur-Sawhney 1995; Du et al. 2015). Besides CNC-PAs, PAs could covalently link to endo-glutamines of proteins to transform into covalently conjugated PAs (CC-PAs) by action of transglutaminase, which is inhibited by phenanthroline (o-Phen) (Del-Duca et al. 1995). CC-PAs in plasma membrane play crucial roles in the process of protein modifications and enhance the tolerance of wheat to drought stress (Du et al. 2015). Also, the results of Del-Duca showed that CC-PAs have an important role in chloroplasts (Del-Duca et al. 1995).

However, to our knowledge, the relationship between mitochondrion conformation and the conjugated PAs in mitochondrion membrane remains to be elucidated. In this experiment, the research aimed to investigate the significance of conjugated PAs in mitochondrion membrane of the developing wheat embryos under drought stress, including the following items: the effects of drought stress on the contents of CNC-PAs and CC-PAs, the effects of drought stress on the mitochondrion conformation and the content of the -SH group in mitochondrion membrane, and the relationship between the two conjugated PAs and the mitochondrion conformation.

## 2. Materials and methods

### 2.1 Plant materials and treatments

Two wheat (*Triticum aestivum* L.) cultivars (Yumai No. 18 and Yangmai No. 9) were used as materials. Yumai No. 18 grows well in drought ecotopes, whereas Yangmai No. 9 grows well in the rainy ecotopes. Yumai No. 18 cv. is drought-tolerant and Yangmai No. 9 cv. is drought-sensitive. The seeds of the two cv. were surface-sterilized in 5% NaClO (w/v) for 10 min, rinsed with tap water, and then germinated in plastic pots (100 seeds / pot) (rim diameter: bottom diameter: height: 50 cm: 40 cm: 60 cm), which contained water-normal and nutrient-rich topsoil. After the third leaf was expanded, the seedlings were thinned to 30 uniformly grown seedlings per pot. Then the pots with seedlings were moved into an environment greenhouse, in which a 25°C/15°C (day/night) temperature and a 70% air humidity were achieved and the cool-white fluorescent lamps were installed for supplying 16 h photoperiod at 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  quantum flux density. The wheat seedlings were cultivated up to flowering.

On the 10th day after fertilization, the developing wheat plants were treated as followings: (1) Control: the roots of the materials for the control groups grew in water-normal soil (soil water potential: -0.15 Mpa), and the ear and the flag leaf were sprayed with distilled water; (2) Drought: roots were treated with drought stress (soil water potential: -1.0 Mpa), and the ear and the flag leaf were sprayed with distilled water; (3) Drought+Spd: roots were treated with drought stress (soil water potential: -1.0 Mpa), and the ear and the flag leaf were sprayed with Spd solution (1 mM); (4) Drought+MGBG: roots were treated with drought stress (soil water potential: -1.0 Mpa) and the ear and the flag leaf were sprayed with MGBG solution (0.5 mM); (5) Drought+o-Phen: roots were treated with drought stress (soil water potential: -1.0 Mpa) and the ear and the flag leaf were sprayed with o-Phen solution (0.5 mM). Spd and inhibitors (MGBG and o-Phen) were obtained from Sigma Chemical Co. Water Potential Instrument (Model: TEN60) was used to detect soil water potential. All the wheat ears and the ear leaves mentioned above were sprayed with the test reagents by 50 ml/pot at 6:00 and 18:00 per day with 0.01 % (v/v) Tween 20 as a detergent. After treatment for 7 days, the embryos of the developing grains were sampled. In the middle of an ear, the grains were cut at the base from the ear with a scalpel and the embryos were stripped under a dissecting microscope.

## 2.2 Determination of relative water content of embryo (RWCE)

To counteract the diversity of different cultivar, we used RWCE rather than absolute water content as the index in the research. RWCE was calculated with the following formula:

$$\text{RWCE (\%)} = (\text{FW} - \text{DW}) / (\text{SW} - \text{DW}) \times 100$$

FW, DW and SW represent the fresh weight, dry weight and saturation weight of the embryo of the wheat materials treated for 7 days, respectively).

## 2.3 Determination of relative increase rate of embryo dry weight (RIREDW)

RIREDW was calculated with the following formula:

$$\text{RIREDW} = (\text{W7} - \text{W0}) / \text{W0}$$

RIREDW represents increase rate of embryo dry weight, W7 represents the embryo dry weight of all the material treated for 7 days and W0 represents the embryo dry weight of the material treated for 0 days). And then, to counteract the diversity of different cultivar, RIREDW of every cultivar was calculated with the following formula:

$$\text{RIREDW (\%)} = (\text{RIREDW of treatment} / \text{RIREDW of control}) \times 100$$

In the formula, the wheat materials of the treatment and the control were the same cultivar.

## 2.4 Purification and detection of mitochondrion membrane proteins

The part of purified mitochondrion membrane solution was added into with 10% (v/v) stock triton X-100 solution until the terminal triton concentration amounted to 1%. After the solution was treated three times for 30 s by means of an ultrasonic disintegrator (model 150-w), it was kept on ice bath for 30 min. Then it was centrifuged at 20,000g for 35 min at 4°C. The supernatant was taken as soluble membrane protein sample. Protein content was determined by the Bradford (Bradford 1976) method, with BSA as a standard.

## 2.5 Determination of PAs conjugated to mitochondrion membrane

The contents of two conjugated PAs were determined by the method of Sharma (1995) and Du (2015) with a few modifications. The mitochondrion membrane sample prepared above was added into with 10% (v/v) of perchloric acid (PCA) stock solution until the terminal PCA concentration amounted to 5%, and centrifuged at 30,000g for 30 min. The CNC-PAs in mitochondrion membrane were dissolved in the supernatant.

The pellet was re-suspended in 5% (v/v) of PCA, mixed with 12 N HCl in a same volume, hydrolyzed at 110°C for 1 days, filtered and desiccated at 70 °C. Then the pellet was dissolved in 5% (v/v) PCA. The solution contained the CC-PAs.

The CNC-PAs and CC-PAs in the two solutions above were derivatized by the method of Di-Tomaso (1989) and detected by HPLC.

## 2.6 Preparation of ultrathin section and observation of mitochondrion morphology

Blocks of wheat embryo tissue were fixed in 3% (w/v) glutaraldehyde (in 0.08 M phosphate buffer, pH 7.2) and post-fixed for 12 h in 1% (w/v) osmium tetroxide in the same buffer. Embedded samples (agar 100 resin) were cut into ultrathin sections (70 to 90 nm) and stained in a 3% (w/v) water solution of uranyl acetate and water solution of lead citrate. Samples were observed with a CM 100 transmission electron microscope (Philips) operating at 80 kV, and images were recorded with a Bioscan CCD camera using Digital Micrograph software (Gatan).

## 2.7 Preparation of mitochondrion membrane and determination of -SH group content

Extraction of mitochondrion membrane from wheat embryos and determination of -SH group content were conducted according to the procedure described by Pomeroy (Pomeroy 1974) and Ellman (1959), respectively.

## 2.8 Statistical analysis

The experiment was repeated 3 times and 3 samples were taken in every time. So the every value in this

paper was means ( $n=9$ )  $\pm$  stand error (S.E.) of 3 independent tests. Data were analyzed by software of SPSS 16.0 and Microsoft Excel software. The significant differences among the means were determined using Duncan's multiple range tests at a significance level of 0.05.

### 3. Results

#### 3.1 *Effects of drought stress, exogenous Spd, MGBG and o-Phen on RWCE and RIREDW*

Treatment of wheat with drought stress for 7 days induced decreases of RWCE (figure 1A) and RIREDW (figure 1B) of the two tested wheat cultivars, Yangmai No. 9 cv. (drought-sensitive) and Yumai No. 18 cv. (drought-tolerant), and the changes in the Yangmai No. 9 were more obvious than those in Yumai No. 18 (figure 1). Treatment of Yangmai No. 9 with exogenous Spd inhibited markedly the decreases of RWCE and RIREDW of this cultivar under drought stress, and the effects of the treatment on those of Yumai No. 18 were slightly. Treatments of Yumai No. 18 with MGBG and o-Phen aggravated obviously the decreases of RWCE and RIREDW of this cultivar under drought stress, and the effects of MGBG and o-Phen on those of Yangmai No. 9 were slightly.

#### 3.2 *Effects of drought stress, exogenous Spd and MGBG on the contents of CNC-PAs in mitochondrion membrane from developing wheat embryos*

In the experiment, the contents of CNC-Put (figure 2A) and CNC-Spd (figure 2B) in mitochondrion membrane could be detected, whereas the content of CNC-Spm could not be detected as the amount might be too little. Under drought stress, the contents of CNC-Put and CNC-Spd increased in mitochondrion membrane isolated from the two wheat cultivars. Meanwhile, CNC-Spd content in drought-tolerant Yumai No. 18 cv. increased much more significantly than that in drought-sensitive Yangmai No. 9 cv. (figure 2B). On the contrary, CNC-Put content in Yumai No. 18 cv. did not rise as much as that in Yangmai No. 9 cv. (figure 2A). With exogenous Spd treatment, the content of CNC-Spd increased obviously in the embryo mitochondrion membrane from drought-treated Yangmai No. 9 cv. However, the increase was negligible in drought-treated Yumai No. 18 cv. MGBG treatment brought about a

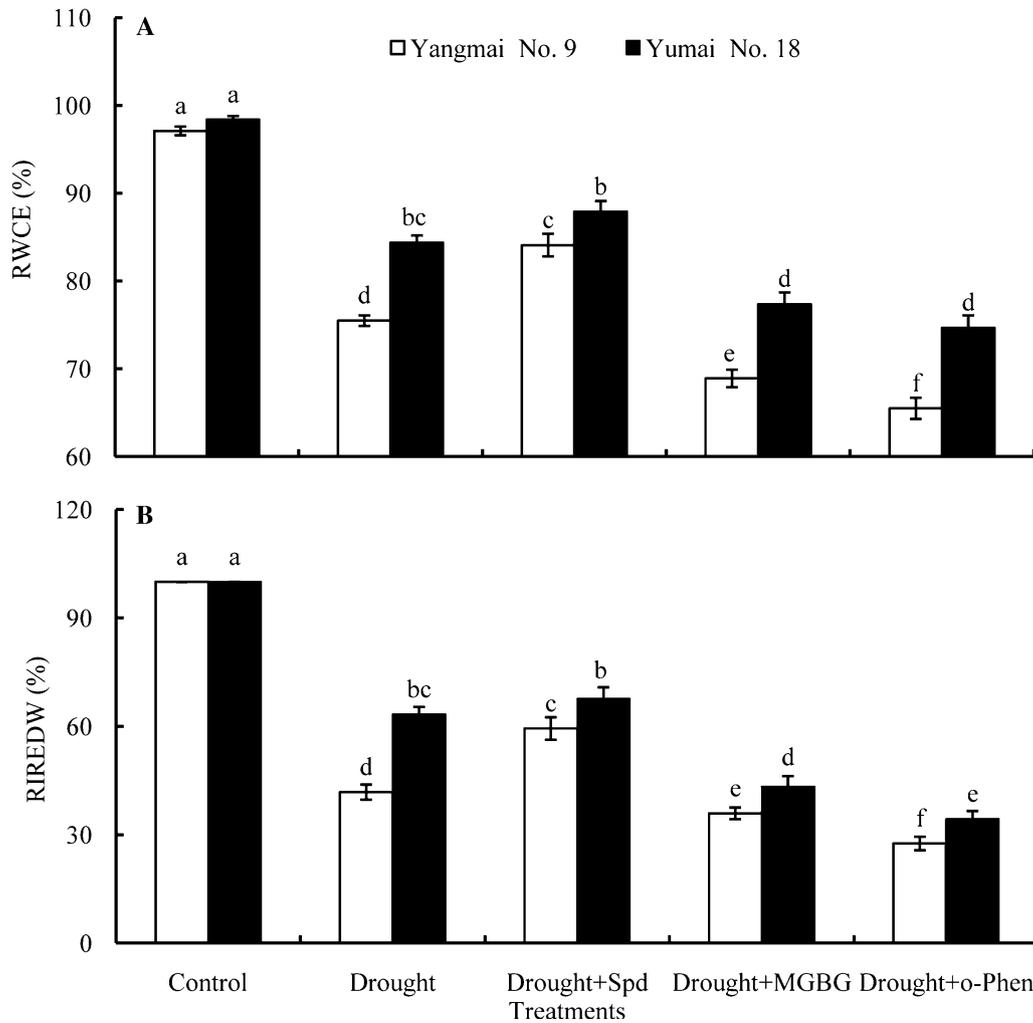
marked reduction in the content of CNC-Spd in drought-treated Yumai No. 18 cv. Exogenous Spd or MGBG treatment affected the CNC-Put level little in two drought-treated wheat cultivars.

#### 3.3 *Effects of drought stress and o-Phen on the contents of CC-PAs in mitochondrion membrane from developing wheat embryos*

Same as CNC-PAs, the contents of the two CC-PAs (CC-Put and CC-Spd) could be detected in mitochondrion membrane from drought-treated wheat embryos, but the CC-Spm content could not be detected. Under drought stress, the CC-Put content in mitochondrion membrane from Yumai No. 18 cv. increased more markedly than that in Yangmai No. 9 cv. However, there was no marked difference in the CC-Spd content between the two wheat cultivars (data not shown), which indicated that CC-Spd level had little effect on drought tolerance. o-Phen treatment obviously inhibited the drought-induced increase of CC-Put content in embryo mitochondrion membrane from Yumai No. 18 cv. more markedly than it did in the mitochondrion membrane from Yangmai No. 9 cv. (figure 3).

#### 3.4 *Effects of drought stress, exogenous Spd, MGBG and o-Phen on mitochondrion morphology*

As could be seen from figure 4A and B, when the two wheat cultivars were not subjected to drought stress, the mitochondria were mostly spherical and ellipsoidal, with a large number, close arrangement and different sizes. It might be that the embryos of wheat grains were in the developing stage, and then the embryonic cells were exuberant, and the appearance of proliferating mitochondria were different at this stage. The mitochondrial membrane boundaries of the two cultivars were clear and the cristae of mitochondria were numerous, indicating that active respiration and metabolism were occurring in the mitochondria. No significant difference was observed between the two cultivars. From figure 4C and D, it could be observed that under drought stress, mitochondria of the drought-sensitive Yangmai 9 cv. were dispersed and the mitochondrion membrane was swollen and deformed. Mitochondrial integrity was damaged to some extent because the number of cristae was reduced and some of them were damaged by dissolution, and the inner chamber of the mitochondrial matrix was enlarged and began to show the vesiculation tendency. However,

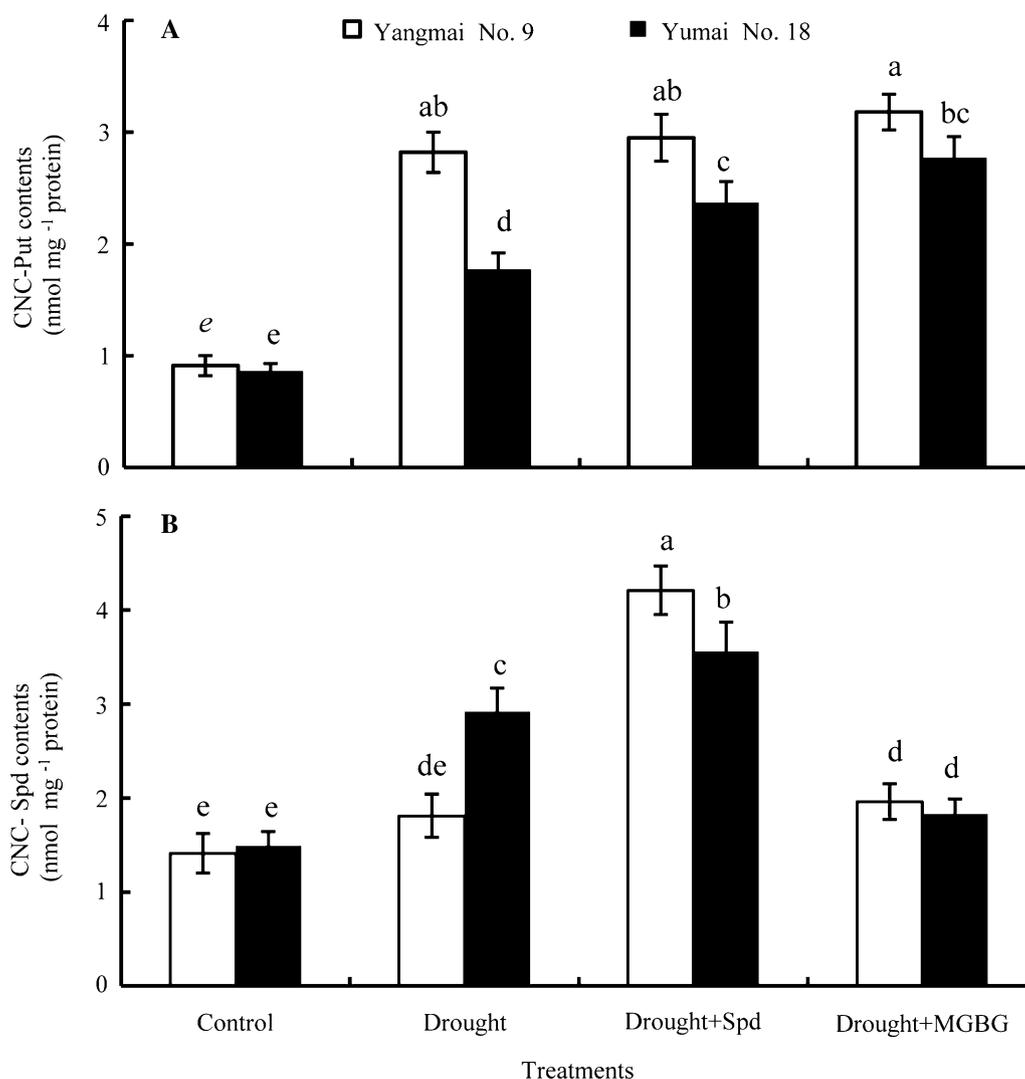


**Figure 1.** Effects of drought stress, exogenous Spd, MGBG and o-Phen on RWCE (A) and RIREDW (B) of the developing wheat embryos. Control, the roots of the control groups grew in water-normal soil (soil water potential:  $-0.15$  Mpa), and the ear and the flag leaf were sprayed with distilled water; Drought, roots were treated with drought stress (soil water potential:  $-1.0$  Mpa), and the ear and the flag leaf were sprayed with distilled water; Drought+Spd, roots were treated with drought stress (soil water potential:  $-1.0$  Mpa), and the ear and the flag leaf were sprayed with Spd solution (1 mM); Drought+MGBG, roots were treated with drought stress (soil water potential:  $-1.0$  Mpa) and the ear and the flag leaf were sprayed with MGBG solution (0.5 mM); Drought+o-Phen, roots were treated with drought stress (soil water potential:  $-1.0$  Mpa) and the ear and the flag leaf were sprayed with o-Phen solution (0.5 mM). Each value in the figure represents the mean of three experiments  $\pm$  SE. Error bars indicate SE ( $n = 9$ ), and different letters (a–f) above the columns are significantly different by Duncan’s multiple range tests ( $P < 0.05$ ).

under drought stress, the mitochondria of drought-tolerant Yumai No. 18 cv. were still closely arranged, with intact and undeformed membrane, and the membrane boundary was still clear. The cristae were still clearly obvious, although there was the phenomenon of slightly dissolving cristae in the center of mitochondria. Therefore, it only showed slight vesiculation in the center of mitochondrial stromal chamber.

The results of the treatments simultaneously with drought stress and exogenous Spd were showed from figure 4E and F. Under drought stress, exogenous

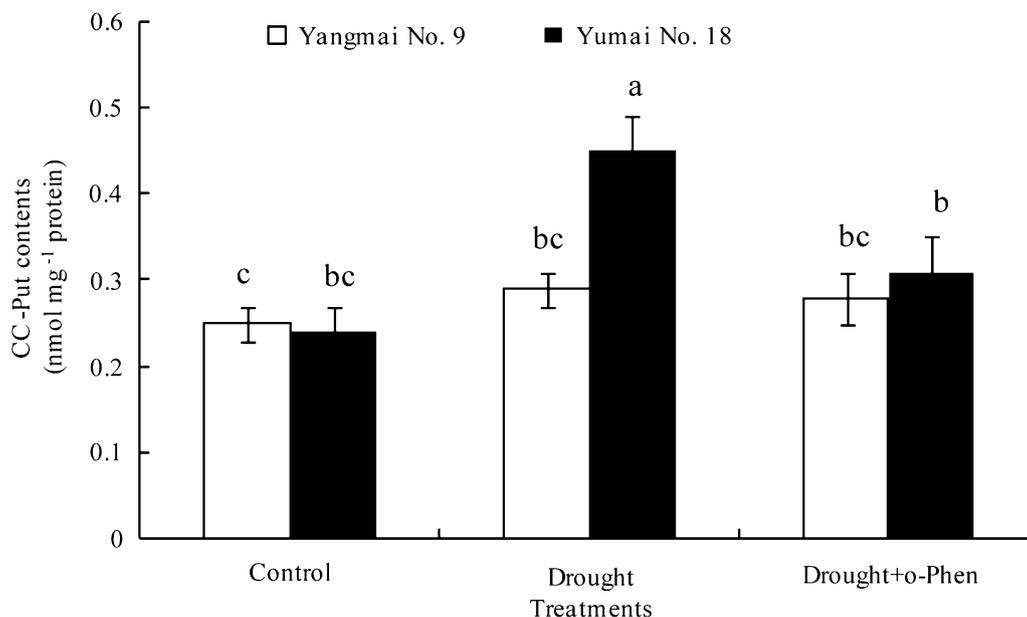
Spd treatment brought about a greater effect on drought-sensitive Yangmai 9 cv. The mitochondria of the cultivar were re-accumulated from the dispersed state and the integrity of the membrane was restored with the clear membrane boundaries and the abundant cristae, showing that slight dissolution damage was occurring in the mitochondria (figure 4E). Exogenous Spd had little effect on drought-tolerant Yumai No. 18 cv (figure 4F). To investigate the significance of Spd further, MGBG, inhibitor of Spd biosynthesis, was used in the experiment. The results



**Figure 2.** Effects of drought stress, exogenous Spd and MGBG on the contents of CNC-PAs in mitochondrion membrane from the developing wheat embryos. Control, Drought, Drought+Spd and Drought+MGBG are the same as these in figure 1. Each value in the figure represents the mean of three experiments  $\pm$  SE. Error bars indicate SE (n = 9), and different letters (a–e) above the columns are significantly different by Duncan's multiple range tests ( $P < 0.05$ ).

of the treatments simultaneously with drought stress and MGBG could be observed from figure 4G and H. Under drought stress, MGBG treatment aggravated the effects of drought stress on the two wheat cultivars. The mitochondrial boundaries of Yangmai 9 were unclear and the number of mitochondria decreased. The cristae became fuzzy and stromal chambers were significantly enlarged, indicating that they were damaged by dissolution (figure 4G). The mitochondria of Yumai No. 18 showed different manifestations, some of which showed obvious vesiculation with the inner mitochondrial membrane disappearing and the cristae being dissolved (figure 4H).

To determine the function of CC-PAs, o-Phen, inhibitor of CC-PAs, was also used in the experiment. The results of treatments with drought stress and o-Phen were shown in figure 4I and J. The mitochondrial membrane boundaries of the two cultivars were not clear, cristae were badly dissolved and mitochondria showed obvious vesiculation, indicating the integrity of mitochondrial structure was severely destroyed. Furthermore, the phenomenon of mitochondrial fusion was observed and Yumai No. 18 appeared even more severely. These results implied that o-Phen treatment aggravated the injury of drought stress to mitochondria.



**Figure 3.** Effects of drought stress and o-Phen on the contents of CC-PAs in mitochondrion membrane from the developing wheat embryos. Control, Drought and Drought+o-Phen are the same as these in figure 1. Each value in the figure represents the mean of three experiments  $\pm$  SE. Error bars indicate SE ( $n = 9$ ), and different letters (a–c) above the columns are significantly different by Duncan's multiple range tests ( $P < 0.05$ ).

### 3.5 Effects of drought stress, exogenous Spd, MGBG and o-Phen on the content of –SH group in mitochondrion membrane

Under drought stress, –SH group content in mitochondrion membrane from wheat embryos of Yangmai No. 9 cv. decreased more markedly than that of Yumai No. 18 cv. (figure 5). Treatment of Yangmai No. 9 cv. with 1 mM exogenous Spd inhibited more markedly the drought-induced decreases of –SH group content. While treatment of Yumai No. 18 cv. with 0.5 mM MGBG promoted more significantly the drought-induced decreases of –SH group content in mitochondrion membrane of the wheat embryos (figure 5). Drought-induced decreases of –SH group content in mitochondrion membrane from wheat embryos were promoted with 0.5 mM o-Phen treatment and the effect was more significantly on Yumai No. 18 cv. than that on Yangmai No. 9 cv. (figure 5).

## 4. Discussion

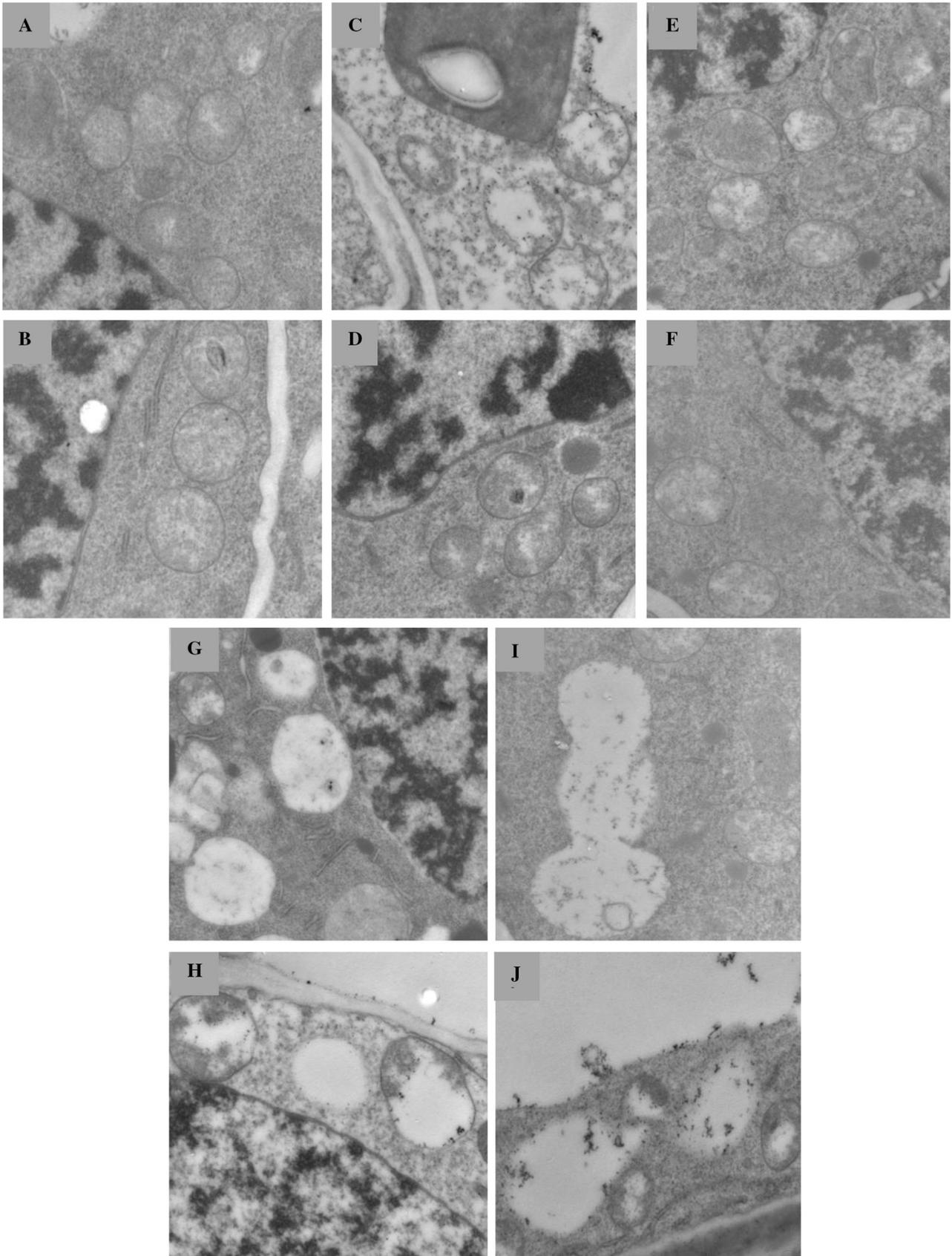
### 4.1 Relationship between drought stress and conjugated PAs

Growth inhibition is the most sensitive physiological reaction of plants to various stresses, and plant

tolerance to drought stress has been closely associated with water content and growth rate (Hsiao 1973; Schonfeld *et al.* 1988). Therefore, from the results presented in figure 1, it could be confirmed that wheat Yumai No. 18 cv. was drought-tolerant and Yangmai No. 9 cv. was drought-sensitive. Using the two wheat cultivars as experimental materials, it would be easy to elucidate the significance of conjugated PAs in mitochondrion membrane of the developing wheat embryos under drought stress.

Under drought stress, in mitochondrion membrane of drought-tolerant Yumai No. 18 cv., the level of CNC-Spd increased more significantly than that of drought-sensitive Yangmai No. 9 cv. (figure 3). The result seems to imply that the wheat tolerance might partly be attributed to CNC-Spd level. Two further studies by using exogenous Spd and inhibitor MGBG supported the hypothesis. Exogenous Spd treatment obviously elevated not only the CNC-Spd level in mitochondrion membrane of drought-sensitive Yangmai No. 9 cv. (figure 2), but also the tolerance of the cultivar to drought stress as judged by RWCE and RIREDW (figure 1). Inhibitor MGBG treatment significantly reduced not only the CNC-Spd content in mitochondrion membrane of drought-tolerant Yumai No. 18 cv. (figure 2), but also the tolerance of the cultivar (figure 1).

As regard to the CC-PAs in mitochondrion membrane, under drought stress, the CC-Put content of



◀ **Figure 4.** Effects of drought stress, exogenous Spd, MGBG and o-Phen on mitochondrion morphology from the developing wheat embryos (20000 $\times$ ). (A) Yangmai No. 9 Control; (B) Yumai No. 18 Control; (C) Yangmai No. 9 Drought; (D) Yumai No. 18 Drought; (E) Yangmai No. 9 Drought+Spd; (F) Yumai No. 18 Drought+Spd; (G) Yangmai No. 9 Drought+MGBG; (H) Yumai No. 18 Drought+MGBG; (I) Yangmai No. 9 Drought+o-Phen; (J) Yumai No. 18 Drought+o-Phen (Control, Drought, Drought+Spd, Drought+MGBG and Drought+o-Phen are the same as these in figure 1).

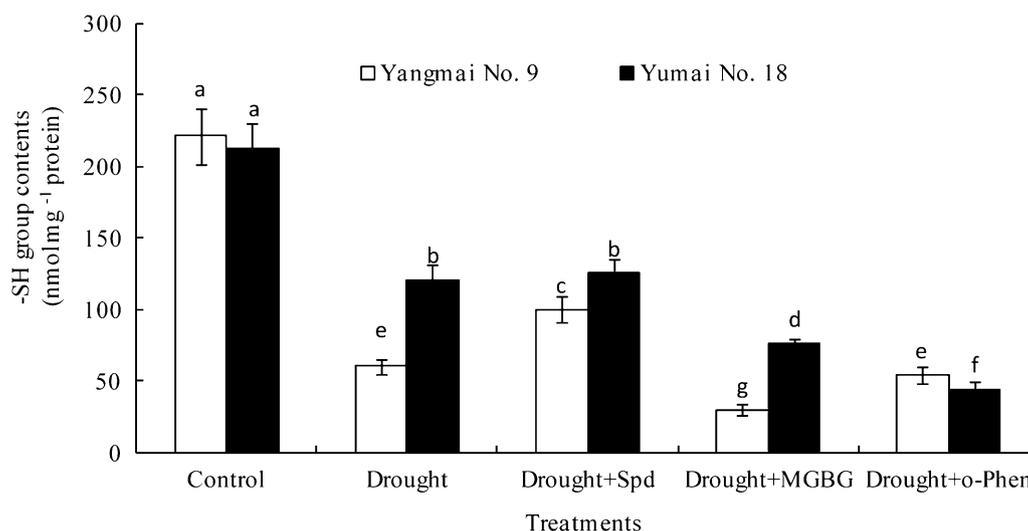
drought-tolerant Yumai No. 18 cv. increased substantially (figure 3). The result seems to imply that wheat tolerance might partly attribute to CC-Put. The experiment by using inhibitor o-Phen supported the notion. O-Phen treatment obviously reduced not only the CC-Put content in the membrane of drought-tolerant Yumai No. 18 cv. (figure 3), but also the tolerance of the wheat cultivar to drought stress, as judged by RWCE and RIREDW (figure 1).

Summarily, the levels of CNC-Spd and CC-Put in embryo mitochondrion membrane were associated with the tolerance of wheat plants to drought stress. Why the conjugated PAs were associated with plant tolerance might be attributed to many factors. For example, the conjugated PAs might play crucial roles in the process of protein modifications (Del-Duca *et al.* 1995). The relationship between polyamines and ion channels has also been reported (Williams 1997). It has been

documented that the ion channels of vacuolar were inhibited by polyamines (Dobrovinskaya *et al.* 1999). The research of Liu (2000) showed that polyamines could target the channels of KAT<sub>1</sub>-like in guard cells to modulate stomatal movements. These studies provided a link among conjugated polyamines and stress conditions. In the presented research, the relationship between the PAs conjugated to mitochondrion membrane and mitochondrion morphology was elucidated.

#### 4.2 Relationship between conjugated PAs and the mitochondrion morphology

In the presented research, we found an interesting fact that the levels of CNC-Spd and CC-Put were closely associated with the mitochondrion morphology under drought stress. Under normal water condition, mitochondrion morphology performed normal conformation, such as spherical and ellipsoidal mitochondrion with a large number, close arrangement and different sizes (figure 4A and B). Under drought stress, changes in the mitochondrion morphology of the two cultivars were visualized and the changes in the morphology of drought-tolerant Yumai No. 18 cv. were not obvious (figure 4D), whereas the injury of the stress to the mitochondrion of drought-sensitive Yangmai No. 9 cv. was worse (figure 4C). Interestingly, under drought stress, in drought-tolerant Yumai No. 18 cv., CNC-Spd (figure 2) and CC-Put (figure 3) in mitochondrion



**Figure 5.** Effects of drought stress, exogenous Spd, MGBG and o-Phen on the content of -SH group in mitochondrion membrane from the developing wheat embryos. Control, Drought, Drought+Spd, Drought+MGBG and Drought+o-Phen are the same as these in figure 1. Each value in the figure represents the mean of three experiments  $\pm$  SE. Error bars indicate SE ( $n = 9$ ), and different letters (a–f) above the columns are significantly different by Duncan's multiple range tests ( $P < 0.05$ ).

membrane increased much more markedly than those in sensitive Yangmai No. 9 cv. Therefore, it could be inferred that CNC-Spd and CC-Put in membrane might function in maintaining the morphology integrity of the embryo mitochondria of developing wheat under drought stress. The notion was supported by the following additional experiments. First, the treatment with exogenous Spd elevated the CNC-Spd level in membrane (figure 2) and simultaneously it mitigated the changes in mitochondrion morphology induced by drought stress (figure 4E and F). Second, the treatment with inhibitor MGBG decreased the CNC-Spd level in membrane (figure 2) and simultaneously it aggravated the changes in mitochondrion morphology induced by drought stress (figure 4G and H). Third, the treatment with inhibitor o-Phen decreased the CC-Put level in membrane (figure 3), and coupled with it, the o-Phen treatment aggravated obviously the changes in mitochondrion morphology induced by drought stress (figure 4I and J). So, from the results mentioned above, it could be concluded that there were positive relationships between the levels of the polyamines conjugated to the mitochondrion membrane and the morphology integrity of the embryo mitochondrion of the developing wheat plants under drought stress. The morphology integrity was involved in many factors such as bacterial pathogens (Creasey and Isberg 2014), brassinosteroids (Yamagami *et al.* 2018), and so on.

Why could CNC-Spd and CC-Put in mitochondrion membrane promote the integrity of mitochondrion morphology? With positive charges, Spd might affect membrane physical state and maintain mitochondrion conformation by binding non-covalently to negatively charged phospholipids and acidic proteins. Furthermore, the changes in the contents of –SH group in mitochondrion membrane (figure 5) were consistent with the changes in the contents of CNC-Spd in membrane (figure 2) under drought stress, exogenous Spd, MGBG. It could be inferred that drought-induced Spd might maintain the level of –SH group in membrane proteins by binding non-covalently to the acidic proteins in mitochondrion membrane and forming CNC-Spd. The maintaining of the –SH group level enhanced the increasing of antioxidant ability of the proteins, which was in favor of integrity of mitochondrion morphology. As to CC-PAs, Del-Duca reported that PAs played an important role in chloroplasts by being conjugated covalently to CP24, CP26 and large subunit of Rubisco, forming protein–Glu-PAs-protein and protein–Glu-PAs (Del-Duca *et al.* 1995). It could be inferred that under drought stress, CC-Put might stabilize conformation and function of the proteins in

mitochondrion membrane by preventing the enzyme from denaturing, and thus maintain the integrity of mitochondrion morphology. Obviously, the relationship between the polyamines conjugated to the membrane and the mitochondrion morphology under drought stress is interesting and complex, so it deserves further elucidation. The studies underling the mitochondrion conformation modulation by conjugated-PAs might shade light on the PA mechanism of action in plants under drought stress.

## 5. Conclusion

In summary, the present work is the first to elucidate that the drought-induced CNC-Spd and CC-Put might enhance the tolerance of wheat plants to drought stress via maintaining the integrity of mitochondrion morphology.

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