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Full Length Article

Relationship between the Conjugated Polyamines and the Activities of the Vacuolar Membrane Proteins in Embryo of Wheat Grains under Drought Stress

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Abstract

The effects of drought stress on the contents of conjugated covalently (C-), non-covalently (CN-) polyamines and the activities of vacuole membrane H^+-PPase (VM-PPase) and H^+-ATPase (VM-ATPase) were elucidated in vacuole membrane isolated from the embryos of developing wheat (*Triticum aestivum* L.) grains of two cultivars differing in drought-resistance. The results indicated that after drought stress treatment for 5 days, relative water content of embryo (RWC), relative increase rate of embryo dry weight (RIREDW) and the activities of VM-PPase and VM-ATPase of the drought susceptive Wenmai 10 decreased more significantly than the drought tolerant Zhoumai 26. Meanwhile, the increases of the contents of CN-spermidine (CN-Spd) and C-putrescine (C-Put) in embryo vacuole membrane from Zhoumai 26 were higher than Wenmai 10. Exogenous Spd not only alleviated drought stress injury to Wenmai 10, but also enhanced the increases of vacuole membrane CN-Spd content and the activities of VM-PPase and VM-ATPase. Under drought stress, the treatments of Zhoumai 26 with two inhibitors methylglyoxyl-bis (guanylhydrazone) and phenanthrolin significantly inhibited the drought stress-induced increases of CN-Spd and C-Put contents in the embryo vacuole membrane of the cultivar, respectively. At the same time, the treatments with the two inhibitors significantly aggravated the drought stress-induced decreases in activities of VM-PPase and VM-ATPase in embryo vacuole membrane of Zhoumai 26 and the drought stress injury to the cultivar. These results suggest that the CN-Spd and C-Put in embryo vacuole membrane isolated from developing grains could enhance the tolerance of wheat to drought stress by maintaining the activities of VM-PPase and VM-ATPase in vacuole membrane. © 2020 Friends Science Publishers

Keywords: Conjugated polyamines; Drought stress; VM-PPase, VM-ATPase; Vacuole membrane; Wheat (*Triticum aestivum* L.)

Introduction

In plants, vacuoles play key roles in the plant responses to any stress (Dietz *et al.* 2001). The vacuole membrane H^+-PPase (VM-PPase) and H^+-ATPase (VM-ATPase) are two major vacuole membrane proteins which have been extensively researched. Drought stress affects food yield in the world, and the plant response to drought stress has been widely studied (Farooq *et al*. 2014; Avramova *et al*. 2015; Zhu 2016). The relation between drought stress and the activities of VM-PPase and VM-ATPase in vacuole membrane have also been documented, however, the results have revealed variable notions (Colombo and Cerana 1993; Shantha *et al.* 2001; Wang *et al.* 2001). It is reported (Shantha *et al.* 2001) that osmotic stress treatment resulted in vacuolar alkalinization and decreased the pH gradient across vacuole membrane in the root cells of a maize cultivar relatively susceptive to osmotic stress, whereas the root cells of a pear millet cultivar relatively tolerant to osmotic stress, were able to keep the pH gradient across vacuole membrane and to inhibit the vacuolar alkalinization. It was suggested that the tolerance of this cultivar to stress should be attributed to maintaining the VM-ATPase activity. However, Wang *et al.* (2001) indicated that the VM-ATPase activity in vacuole membrane isolated from *Suaeda salsa* plants under PEG osmotic stress was almost same as the vacuole membrane isolated from controls. The VM-PPase activity was unaffected in the vacuole membrane isolated from the cell line of *Dacus carota* under sorbitol osmotic stress (Colombo and Cerana 1993). Wang *et al.* (2001) also showed that the activity of VM-PPase decreased 3-fold in the vacuole membrane isolated from *Suaeda salsa* plants under PEG-osmotic stress for 8 d. These results on the activities of VM-PPase and VM-ATPase in vacuole

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membrane under water stress might be attributed to their playing various physiological roles in various tissues at various growing and developing stages. Therefore, the effects of water stress on the activities of VM-PPase and VM-ATPase need to be further elucidated.

Polyamines (PAs) are long nitrogenous aliphatic amines extensively prevalent in microorganism, animals and plants. In plants, they are implicated closely in plant growth and development (Du *et al*. 2018; Guo *et al*. 2018; Cetinbas-Genc 2019). Furthermore, PAs are related to many abiotic stresses containing water stress, salt stress, heavy metal, and temperature stresses (Tang and Newton 2005; Farooq *et al*. 2009; Goyal and Asthir 2010; Do *et al*. 2013; Du *et al*. 2017; Taie *et al.* 2019). Spermine (Spm), spermidine (Spd) and putrescine (Put) are three main PAs. 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 these conversions is S-adenosylmethionine decarboxylase (SAMDC), and SAMDC is inhibited potently by methylglyoxyl bis (guanyl hydrazone) (MGBG) (Slocum 1991). At physiological pH, PAs are naturally cationic (Kumer *et al.* 1997), and by ionic bonding, PAs could interact with anionic macromolecules and form conjugated non-covalently PAs (CN-PAs) in the membrane (Feuerstein and Martin 1989). By this, PAs function in stabilizing the conformation of the bio-membrane (Galston and Kaur-Sawhney 1995; Du *et al.* 2015). Besides CN-PAs mentioned above, PAs could covalently link to endo-glutamines of proteins to transform into covalently conjugated PAs (C-PAs) by the enzyme transglutaminase (TGase). Phenanthrolin (o^-Phen) inhibits the TGase activity (Del-Duca *et al.* 1995; Serafini-Fracassini 1995). C-PAs in plasma membrane play crucial roles in modifying protein and enhancing the wheat tolerance to drought stress (Du *et al.* 2015). Del-Duca *et al.* (1995) showed that C-PAs play crucial roles in cell chloroplasts. However, to our knowledge, the relationship between the conjugated PAs (CN-PAs and C-PAs) and the activities of VM-PPase and VM-ATPase in wheat embryo vacuole membrane under drought stress remains to be elucidated.

In this study, the effects of drought stress on the contents of CN-PAs and C-PAs, the activities of VM-PPase and VM-ATPase, and their relationship were investigated.

Materials and Methods

Wheat cultivation

Two wheat (*Triticum aestivum* L.) cultivars (Zhoumai No. 26 and Wenmai No. 10) were used as materials. Zhoumai 26 is drought tolerant, whereas Wenmai 10 is drought susceptive. The seeds of the two cultivars were surface-sterilized in 5% NaClO (w/v) for 10 min, rinsed with distilled water, and then germinated in plastic pots (20 seeds/pot) (rim diameter: bottom diameter: height: 45 cm: 35 cm: 55 cm), which contained water-normal and nutrient-rich topsoil. After the seedlings vernalized, the pots with seedlings were moved into 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 μmol m^{-2} s^{-1} quantum flux density.

Experimental treatments

On the tenth 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 flag leaf for the control groups were sprayed with distilled water; ② Drought: Roots were treated with drought stress (soil water potential: -1.0 MPa) and the ear and flag leaf were sprayed with distilled water; ③ drought + Spd: Roots were treated with drought stress (soil water potential: -1.0 MPa), and the wheat ear and flag leaf were sprayed with Spd solution (1 m*M*); ④ drought + methylglyoxyl-bis (guanylhydrazone) (MGBG): Roots were treated with drought stress (soil water potential: -1.0 MPa) and the wheat ear and flag leaf were sprayed with MGBG solution (1 m*M*); ⑤ drought + phenanthrolin (o^-Phen): Roots were treated with drought stress (soil water potential: -1.0 MPa) and the wheat ear and flag leaf were sprayed with o^-Phen solution (1 m*M*). 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 flag leaves mentioned above were sprayed with the test reagents by 50 mL/pot at 7:00 and 19:00 per day. After treatment for 5 d, the embryos of the developing grains were sampled.

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

RIREDW was calculated with the following formula:

GREDW = ($W_5 - W_0$) / W_0 (GREDW represents grow rate of embryo dry weight, W_5 represents the embryo dry weight of all the material treated for 5 d and W_0 represents the embryo dry weight of the material treated for 0 d). And then, to counteract the diversity of different cultivar, RIREDW of every cultivar was calculated with the following formula:

RIREDW (%) = (GREDW of treatment/GREDW of control) × 100
(In the formula, the wheat materials of the treatment and the control were the same cultivar).

Determination of relative water content of embryo (RWCE)

RWCE was calculated with the following formula:
RWCE (%) = (FW - DW) / (SW - DW) × 100 (SW, FW and DW represents the saturation weight, fresh weight and dry weight of the embryo of the wheat materials treated for 5 d, respectively).

Purification of vacuole membrane

Vacuole membrane was purified by the methods of Chen *et al.* (1999) and Suzuki and Kanayama (1999) with minor modifications.

Determination of the activities of VM-PPase and VM-ATPase

The purity of the vacuole membrane was estimated by the method of Widell and Larsson (1990), with three inhibitors nitrate, vanadate and azide. The activity of vacuole membrane H^+-ATPase is inhibited by nitrate. In the present experiment, the activity of isolated enzyme was inhibited by nitrate more than 75%, showing that the vacuole membrane band at the down-layer/up-layer interface was vacuole membrane-enriched. The activities of VM-PPase and VM-ATPase were detected by the method of Zhang and Liu (2002) with a little modification.

Conjugated PA determination

The vacuole membrane sample prepared above was added into with 10% (v/v) of perchloric acid (PCA) stock solution until 5% terminal PCA concentration. Then, CN-PAs and C-PAs were detected according to the method of Du *et al*. (2015) by HPLC.

Statistical analysis

The experiments were repeated 3 times and 3 samples were taken in every experiment. Every value was means (n=9) ± stand error (S.E.) of 3 independent tests. Data were analyzed by software of SPSS16.0 and Microsoft Excel software.

Results

Changes in RIREDW and RWC

Treatment of wheat with drought stress for 5 d induced decreases of RIREDW (Fig. 1A) and RWC (Fig. 1B) of wheat cultivars, Wenmai 10 (drought susceptive) and Zhoumai 26 (drought tolerant), and the changes in the Wenmai 10 were more obvious than in Zhoumai 26 (Fig. 1). Exogenous Spd inhibited markedly the decreases of RIREDW and RWCE of the cultivar Wemai 10 under drought stress and the effects on Zhoumai 26 were slight. Treatments of Zhoumai 26 with MGBG and o-Phen aggravated obviously the decreases of RIREDW and RWCE of the cultivar under drought stress, and the effects of MGBG and o-Phen on those of Wenmai 10 were slight.

Changes in the activities of VM-PPase and VM-ATPase

Under drought stress, the activities of VM-PPase (Fig. 2A) and VM-ATPase (Fig. 2B) in Wenmai 10 decreased by half approximately. However, in vacuole membrane of the

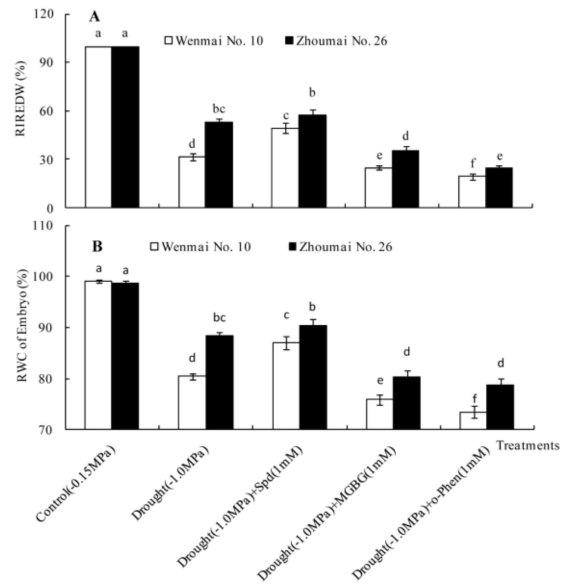

Fig. 1: The effects of drought, Spd, MGBG and o-Phen on RIREDW and RWC of embryos. Control–roots of the materials for the control groups grew in water-normal soil (soil water potential: -0.15 MPa), and the ear and flag leaf for the control groups were sprayed with distilled water; Drought–roots were treated with drought stress (soil water potential: -1.0 MPa), and the ear and flag leaf were sprayed with distilled water; Drought+Spd–roots were treated with drought stress (soil water potential: -1.0 MPa), and the wheat ear and flag leaf were sprayed with Spd solution (1 m*M*); Drought+MGBG–roots were treated with drought stress (soil water potential: -1.0 MPa) and the wheat ear and flag leaf were sprayed with MGBG solution (1 m*M*); Drought + o-Phen–roots were treated with drought stress (soil water potential: -1.0 MPa) and the wheat ear and flag leaf were sprayed with o-Phen solution (1 m*M*)

drought resistant cv. Zhoumai 26, both of the activities of VM-PPase and VM-ATPase decreased less. Exogenous Spd retarded markedly decreases the activities of VM-PPase and VM-ATPase of Wenmai 10 under drought stress, and the Spd treatment effects on Zhoumai 26 were slight. Treatments of Zhoumai 26 with MGBG and o-Phen aggravated obviously the decreases of the activities of VM-PPase and VM-ATPase of this cultivar under drought stress, and the effects of MGBG and o-Phen on Wenmai 10 were slight (Fig. 2).

Changes in the contents of CN-PAs

The contents of CN-Put and CN-Spd could be detected, but the content of CN-Spm was not detected as the amount might be too little. Under drought stress, the contents of CN-Put and CN-Spd rose in vacuole membrane of the two cultivars. However, CN-Spd content in drought stress-treated Zhoumai 26 rose much more significantly than in drought stress-treated Wenmai 10. On the contrary, CN-Put content in drought treated Zhoumai 26 did not rise as much as in drought treated Wenmai 10 (Fig. 3). With Spd treatment, the content of CN-Spd rose obviously in the embryo vacuole membrane from

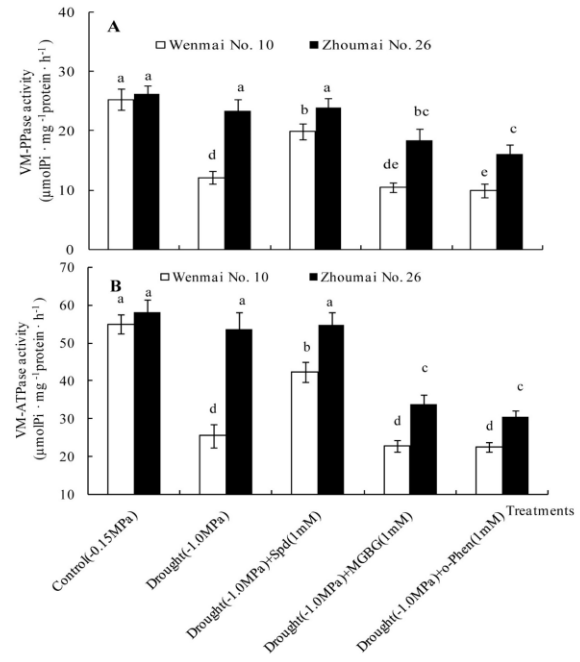

Fig. 2: The effects of drought, Spd, MGBG and o-Phen on the activities of VM-PPase and VM-ATPase in embryo cells

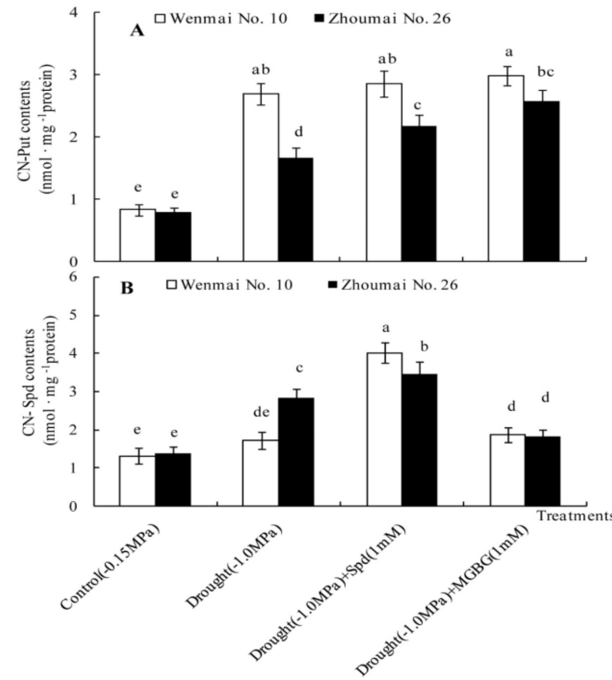

Fig. 3: The effects of drought, Spd and MGBG on the contents of CN-PAs in vacuole membrane of embryo cells

drought treated Wenmai 10. However, the increase was negligible in drought treated Zhoumai 26. MGBG treatment brought about a marked reduction in the content of CN-Spd in drought treated Zhoumai 26. Exogenous Spd or MGBG treatment affected the CN-Put level little in drought treated wheat cultivars. With respect to the ratio of CN-Spd to CN-Put showed clearly that exogenous Spd obviously raised the ratio in drought treated Wenmai 10, but to a lesser extent, the treatment affected the ratio in drought treated Zhoumai 26 (Fig. 3). Treatment with MGBG lowered the ratio in drought treated Zhoumai 26 more significantly than in Wenmai 10.

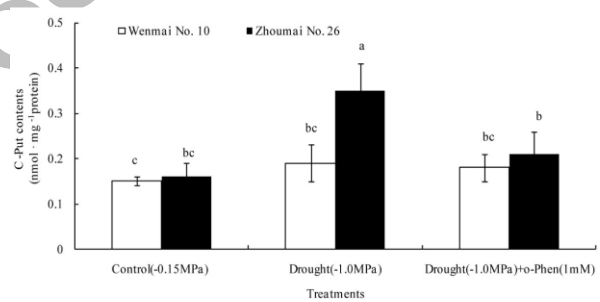

Fig. 4: The effects of drought and o-Phen on the C-Put content in vacuole membrane of embryo cells

Changes in the contents of C-PAs

Likely CN-PAs, the contents of the two C-PAs (C-Spd and C-Put) were detected in vacuole membrane from drought treated wheat embryos. However, the C-Spm content was not detected. Under drought stress, the C-Put content in vacuole membrane from Zhoumai 26 increased more markedly than from Wenmai 10. However, as to C-Spd, no obvious difference between the two cultivars was detected. O-Phen treatment obviously inhibited the drought induced increase of C-Put content in embryo vacuole membrane from Zhoumai 26 more markedly than Wenmai 10 (Fig. 4).

Discussion

Growth inhibiting is the most susceptive 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, wheat Zhoumai 26 was drought tolerant and Wenmai 10 was drought susceptive (Fig. 1). Using the two diverse wheat cultivars elucidated the significance of the activities of VM-PPase and VM-ATPase in embryo vacuole membrane under drought stress.

Under drought stress, the activities of VM-PPase and VM-ATPase in drought tolerant Zhoumai 26 could be maintained in a higher level, whereas in drought susceptive Wenmai 10 the activities decreased obviously (Fig. 2). Thus, it could be concluded that the maintenance of the activities of VM-PPase and VM-ATPase might enhance the wheat tolerance to drought stress. Shantha *et al.* (2001) on the roots of pear millet found that the cells of the cultivar relatively tolerant to osmotic stress were able to keep the pH gradient across vacuole membrane and to inhibit the vacuolar alkalinization, suggesting that the tolerance of this cultivar to stress should be attributed to maintaining the VM-ATPase activity. It is obvious that vacuolar water content and turgor are closely associated with ion accumulation in vacuole, and ion accumulation might be attributed to pH gradient across vacuole membrane and vacuolar acidification (Dietz *et al.* 2001). Maintenance of pH gradient across vacuole membrane in turn is attributed to the activities of VM-PPase and VM-ATPase. Hence, under drought stress, maintaining activities of VM-PPase and VM-ATPase in vacuole membrane is of crucial importance for plant tolerance to the stress.

It has been reported that CN-Spd and C-Put in vacuole membrane purified from barley seedlings were associated with the tolerance of the seedlings to salt stress (Zhao *et al*. 2000; Sun *et al.* 2002). Our previous study showed that conjugated polyamine contents in plasma membrane purified from developing wheat embryos under short-time drought stress enhanced the tolerance of wheat plants to the stress (Du *et al.* 2015). Under drought stress, in vacuole membrane of drought tolerant Zhoumai 26, the content of CN-Spd was much more than in drought susceptive Wenmai 10 (Fig. 3), which seems to imply that the wheat tolerance might partly attribute to CN-Spd. Two further studies by using exogenous Spd and inhibitor MGBG supported the hypothesis. Exogenous Spd treatment obviously elevated not only the CN-Spd content in vacuole membrane of drought susceptive Wenmai 10 (Fig. 3), but also the tolerance of the cultivar (Fig. 1). Inhibitor MGBG treatment significantly reduced not only the CN-Spd content in vacuole membrane of drought tolerant Zhoumai 26 (Fig. 3), but also the tolerance of the cultivar (Fig. 1). As regard to the C-PAs in vacuole membrane, under drought stress, the C-Put content of drought tolerant Zhoumai 26 increased substantially (Fig. 4), which imply that the wheat tolerance might partly attribute to C-Put. The experiment by using inhibitor o-Phen supported the notion. Exogenous o-Phen treatment obviously reduced not only the C-Put content in vacuole membrane of drought tolerant Zhoumai 26 (Fig. 4), but also the tolerance of the cultivar (Fig. 1). In one word, the contents of CN-Spd and C-Put in embryo vacuole membrane were associated with the wheat tolerance.

The relationship between polyamines and ion channels has been reported (Williams 1997). The Dobrovinskaya *et al.* (1999) indicated that the ion channels of vacuolar were inhibited by polyamines. The Liu *et al.* (2000) showed that polyamines could target the channels of KAT_1-like in guard cells to modulate stomatal movements. These studies provided a link among polyamines, membrane proteins and stress conditions. In the presented study, an interesting fact found was that under drought stress contents of CN-Spd (Fig. 3) and C-Put (Fig. 4) increased markedly in drought tolerant Zhoumai 26, and simultaneously the activities of VM-PPase and VM-ATPase of the cultivar were maintained in a higher level. These results were indicative of possible involvement of the two conjugated PAs in the relationship with the activities of VM-PPase and VM-ATPase in vacuole membrane. This hypothesis was supported by using exogenous Spd, inhibitors MGBG and o-Phen (Fig. 2, 3 and 4). Furthermore, statistical analysis showed that there was positive correlation between VM-PPase activity and the ratio CN-Spd/CN-Put ($r_{0.05}$ = 0.95, n = 8), between VM-ATPase activity and the ratio CN-Spd/CN-Put ($r_{0.05}$ = 0.96, n = 8), between VM-PPase activity and C-Put levels ($r_{0.05}$ = 0.94, n = 4), and between VM-ATPase activity and C-Put levels ($r_{0.05}$ = 0.91, n = 4).

The reason why CN-Spd, but not CN-Put could promote the activities of VM-PPase and VM-ATPase in vacuole membrane might be attributed to cationic nature (Sood and Nagar 2003). With more positive charges, CN-Spd might modulate the enzyme activities by binding to protein non-covalently to affect their conformations and functions more easily than CN-Put. Besides that, CN-Spd might be associated with the enzyme activities by conjugating non-covalently to membrane phospholipids and affecting vacuole membrane physical state. Of course, the changes in physical state of vacuole membrane are associated with the activities of the enzymes. As to C-PAs, Del-Duca *et al.* (1995) 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. It could be inferred that under drought stress, C-Put might stabilize conformation and function of VM-PPase and VM-ATPase by preventing the enzyme from denaturing, and thus maintain the activities of these enzymes. Obviously, the effects of conjugated-PAs on the activities of VM-PPase and VM-ATPase in vacuole membrane are interesting and complex, which deserves further elucidation.

Conclusion

The study elucidated that the induced CN-Spd and C-Put might enhance the wheat resistance to drought stress via maintaining the activities of VM-PPase and VM-ATPase in developing embryo vacuole membrane.

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Author Contributions

HY Du and HP Liu, conceived and designed the experiments; HY Du and HL Liu, performed the experiments; DX Liu, analyzed the data; HY Du, HP Liu and R Kurtenbach, rote the paper

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