Relationship between ATPase activity and conjugated polyamines in mitochondrial membrane from wheat seedling roots under osmotic stress

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Abstract: The effects of osmotic stress on the ATPase activity, the contents of —SH group and conjugated polyamines in mitochondrial membrane from wheat seedling [*Triticum aestivum* L. cv. Yumai No. 18 (drought-tolerant) and cv. Yumai No.9(drought-sensitive)] roots were investigated. The results showed that ATPase activity and —SH group content decreased with polyethylene glycol(PEG) 6000(- 0.55 MPa) treatment for 7 d, in concert with the decrease of the ratio of noncovalently conjugated spermidine (NCC-Spd)/noncovalently conjugated putrescine(NCC-Put) and increase of the covalently conjugated putrescine (CC-Put). Osmotic stress injury to Yangmai No.9 seedlings was alleviated greatly with 1 mmol/L exogenous spermidine(Spd), in concert with marked increases of the ratio of NCC-Spd/NCC-Put, —SH group contents and ATPase activity in mitochondrial membrane. Under osmotic stress, the concomitant treatment of Yumai No.18 seedlings with methylglyoxyl bis(guanylhydrazone) (MGBG), an inhibitor of S-adenosyl methionine decarboxylase(SAMDC), and phenanthrolin (*o*-Phen), an inhibitor of transglutaminase(TGase), caused a significant decrease of the ratio of NCC-Spd / NCC-Put, CC-Put contents, respectively, in concert with the marked decreases of ATPase activity, —SH group content and its tolerance to osmotic stress. All the results above suggested that osmotic stress tolerance of wheat seedlings was associated with the ATPase activity, the contents of —SH group, NCC-Spd and CC-Put in mitochondrial membrane.

Keywords: ATPase; conjugated polyamines; mitochondria; osmotic stress; wheat(*Triticum aestivum* L.)

Introduction

Due to an inability to mobilize so as to avoid environmental stress, plants have evolved unique adaptation to stress, some of which involve the mitochondria. Clearly, the plant mitochondria is a highly unusual and complex organelle, and then, in light of its intricacy, progress toward understanding its many unique features has been impressive over the past few years(Machenzie, 1999). In many cases, mitochondria is a key site of damage during environmental stress, especially mitochondria electron transport (Downs, 1998). Osmotic stress is a common stress in natural and agricultural systems. Roots are usually the first organ exposed to stress and stress-related impairment of mitochondria function is likely to be particularly important in roots. Mitochondria adaptation to water stress is usually relative to membrane permeability(Fratianni, 2001). ATPase is one of functional proteins in mitochondrial membrane. In addition to harnessing the flow of H^+ down an electronchemical proton gradient to make ATP, ATPase can work in reverse: it can use the energy of ATP hydrolysis to pump H^+ across the inner mitochondrial membrane (Buchanan, 2000; Alberts, 2002). It is documented that ATPase activity is relative to Tween detergent(Bi, 1981).

Polyamines (PAs) are biologically ubiquitous aliphatic amines that are implicated in growth and development in a wide range of organisms (microorganism, animals, and plants). The common PAs include putrescine(Put), spermidine(Spd), spermine(Spm), and so on. Due to the virtue of their poly-cationic nature at physiological pH, they can interact with macromolecules by hydrogen bound, ionic bound, electrostatic and hydrophobic actions and form noncovalently conjugated PAs (NCC-PAs) in the membrane (Feuerstein, 1989), which thereby play important roles in structure, replication and transcription of DNA and stabilizing the function of the biomembrane(Galston, 1995). Transglutaminase(TGase, EC 2.3.2.13) is a key enzyme, which converts free PAs into covalently conjugated PAs(CC-PAs). This enzyme covalently links PAs to glutamines of specific proteins, forming protein-Glu-PA and protein-Glu-PA-protein, which play important roles in the post-translational modifications of proteins (Serafini-Fracassini, 1995). However, to our knowledge, the relationships between ATPase activity and the levels of covalently and noncovalently conjugated PAs in mitochondria membrane from wheat seedling roots under osmotic stress remain to be elucidated.

1 Materials and methods

1.1 Plant material and treatments

Wheat (*Triticum aestivum* L., cv. Yumai No. 18, drought-tolerant; cv. Yangmai No. 9, drought-sensitive) seeds were surface-sterilized in 0.1% $HgCl_2$ (w/v) for 5 min, rinsed with tap water, and germinated in plastic pots

containing sands. The pots with pores in bottom were put into half-strength Hoagland solution, and the solution was renewed every 2 d. They were placed in a controlled environment chamber with a temperature of 20℃/10℃ (day/night) and 14 h photoperiod at a quantum flux density of 200 μmol/($m^2 \cdot s$) from cool-white fluorescent lamps.

When the extension of the second leaf was completed, the seedlings were treated with Hoagland solution containing PEG(- 0.55 MPa) with 1 mmol/L Spd(or 1 mmol/L MGBG or 1 mmol/L o-Phen) or without Spd (or MGBG or o-Phen). Meanwhile, every set of plant leaves were sprayed with the same concentration Spd, MGBG or o-Phen to that in the root Hoagland solution, with 0.01% (v/v) Tween 20 as a detergent. Seedlings as control were kept in Hoagland solution without PEG, Spd, MGBG or o-Phen and their leaves were sprayed with 0.01% (v/v) Tween 20 solution. All the solutions above were renewed every 2 d. After treatment for 7 d, the wheat seedlings, seedling leaves and roots were sampled.

1.2 Determination of leaf relative water content (LRWC)

LRWC was calculated from the following formula:
$$LRWC = (W_f - W_d)/(W_t - W_d) \times 100\%,$$
where W_f, W_d, and W_t represents the fresh weight, dry weight, saturation weight, respectively, using the second fully expanded leaf of each seedling as sample.

1.3 Determination of the seedling relative dry weight increase rate (RDIR)

Growth rate (GR), on the base of dry weight, was calculated from the following formula:
$$GR = (W_a - W_b)/W_b,$$
where W_a and W_b represents the dry weight of seedlings after 7 d treatment and 0 d treatment, respectively. And then, RDIR of different treatment was calculated from the following formula:

RDIR = GR of treatment / GR of control $\times$ 100%.

1.4 Determination of relative electrolyte leakage (LREL)

The second fully expanded leaves(0.2 g) were placed in test containing 10 ml distilled deionized water. The tubes were incubated in a water bath at 25℃ for 3 h and the initial electrical conductivity of the medium(EC_1) was measured. And then, the samples were boiled at 100℃ for 30 min to release all electrolytes, cooled to 25℃ and the final electrical conductivity(EC_2) was measured. The relative electrolytic leakage(LREL) was calculated by using the formula:

LREL = EC_1 / EC_2 $\times$ 100%.

1.5 Preparation of mitochondrial membrane and determination of ATPase activity and —SH group content

Extraction of mitochondrial membrane from seedling roots, determination of ATPase activity and —SH group content was conducted according to the procedure described by Pomeroy(Pomeroy, 1974), Bi(Bi, 1981) and Ellman (Ellman, 1959), respectively.

1.6 Isolation of membrane proteins and determination

Triton x-100 was added to the partial purified

mitochondrial membrane vesicle extract solution until the terminal concentration amounted to 1%. The solution was sonicated twice for 30 s by means of an ultrasonic disintegrator(model 150-w), kept on an ice bath for 30 min, and then centrifugated at 20000 × g for 30 min at 4℃. The supernatant was taken as soluble membrane protein sample. Protein content was determined by the Bradford (Bradford, 1976) method, with BSA as a standard.

1.7 PA analysis

Perchloric acid (PCA) was added to the soluble membrane protein extract to the terminal concentration of 5% (v/v), and the sample was centrifugated at 27000 × g for 40 min. The precipitate was resuspended in 5% PCA, then mixed with 12 mol/L HCl in equal volume, hydrolyzed at 110℃ for 24 h, and desiccated at 70℃ after being filtrated. The pellet was dissolved in an equal volume of 5% PCA to obtain the solution containing the covalently conjugated PAs. By adding directly 5% PCA to the prepared membrane vesicles and centrifugating at 27000 × g for 40 min, the noncovalently conjugated PAs in membrane vesicles were dissolved in the supernatant. The PAs in two solutions above were derived with benzyol chloride respectively by the method of DiTomaso(DiTomaso, 1989) and measured by HPLC. 10 μl of methanol(Merck KGaA, Germany)-redissolved samples were injected into a fixed 20 μl loop for loading onto a 3.9 mmol/L by 150 mmol/L, 4 μm particle size C_{18} reverse-phase column (Waters, USA). Samples were eluted from the column by a Perkin-Elmer Series 410 pump at room temperature with a flow rate of 0.5 ml/min. Polyamine peaks were detected by a Perkin-Elmer LC-95 absorbance detector at 254 nm. 1, 6-hexanediamine was used as an internal standard.

1.8 Statistical analysis

Data were analysed using the programs of SPSS 10.0 (the Statistical Package for the Social Science for Windows 10.0). All the values reported in this paper are the means of 9 replicates (three independent experiments). Significant difference among means were determined by Duncan's multiple range test, at $P < 0.05$.

2 Results

2.1 Effect of osmotic stress on the LRWG, LREL and RDIR of wheat seedlings

PEG osmotic stress caused the decreases of LRWC and RDIR and increase of LREL of two wheat cultivars, Yangmai No. 9 (drought-sensitive) and Yumai No. 18 (drought-tolerant), but the change in the former cv. was more apparent than that of the latter(Fig.1).

2.2 Effect of osmotic stress on the activity of ATPase and —SH group content in mitochondrial membrane from wheat seedling roots

Under PEG osmotic stress, ATPase activity and —SH group content in mitochondrial membrane from wheat seedling roots of cv. Yangmai No. 9 decreased more markedly than that of cv. Yumai No. 18 (Fig. 2, Fig. 3).

2.3 Effect of osmotic stress on the level of NCC-PAs in mitochondrial membrane of wheat seedling roots

Under PEG osmotic stress, the levels of NCC-Put and NCC-Spd in mitochondrial membrane isolated from the roots
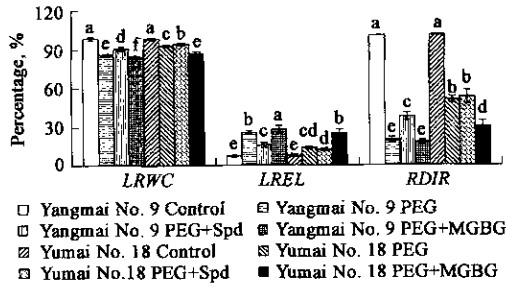
Fig.1 Effect of PEG, PEG + Spd and PEG + MGBG on *LRWC* , *LREL* and *RDIR* of wheat seedlings, wheat seedlings were treated with PEG(- 0.55 MPa), PEG(- 0.55 MPa) + Spd(1 mmol/L), or PEG(- 0.55 MPa) + MGBG(1 mmol/L) for 7 d. Error bars indicate *SE* (*n* = 9). Means with different letters are significantly different at *P* < 0.05 based on Duncan's multiple range test
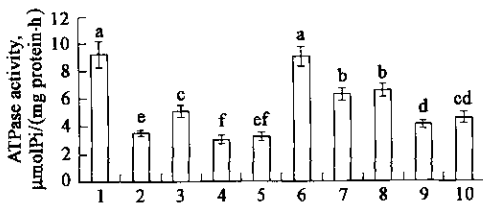

Fig.2 Effect of PEG, PEG + Spd, PEG + MGBG and PEG + *o*-Phen on the activity of ATPase in mitochondria membrane from wheat seedling roots. Wheat seedlings were treated with PEG (- 0.55 MPa), PEG(- 0.55 MPa) + Spd(1 mmol/L), PEG(- 0.55 MPa) + MGBG(1 mmol/L) or PEG(- 0.55 MPa) + *o*-Phen(1 mmol/L) for 7 d. Error bars indicate *SE*(*n* = 9). Means with different letters are significantly different at P < 0.05 based on Duncan's multiple range test

1. Yangmai No. 9 control; 2. Yangmai No. 9 PEG; 3. Yangmai No. 9 PEG + Spd; 4. Yangmai No. 9 PEG + MGBG; 5. Yangmai No. 9 PEG + *o*-Phen; 6. Yumai No. 18 control; 7. Yumai No. 18 PEG; 8. Yumai No. 18 PEG + Spd; 9. Yumai No. 18 PEG + MGBG; 10. Yumai No. 18 PEG + *o*-Phen
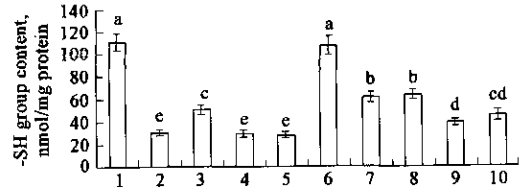

Fig.3 Effect of PEG, PEG + Spd, PEG + MGBG and PEG + *o*-Phen on the —SH group content in mitochondria membrane from wheat seedling roots. Wheat seedlings were treated with PEG(- 0.55 MPa), PEG(- 0.55 MPa) + Spd(1 mmol/L), PEG(- 0.55 MPa) + MGBG(1 mmol/L) or PEG(- 0.55 MPa) + *o*-Phen(1 mmol/L) for 7 d. Error bars indicate *SE*(*n* = 9). Means with different letters are significantly different at *P* < 0.05 based on Duncan's multiple range test

1. Yangmai No. 9 control; 2. Yangmai No. 9 PEG; 3. Yangmai No. 9 PEG + Spd; 4. Yangmai No. 9 PEG + MGBG; 5. Yangmai No. 9 PEG + *o*-Phen; 6. Yumai No. 18 control; 7. Yumai No. 18 PEG; 8. Yumai No. 18 PEG + Spd; 9. Yumai No. 18 PEG + MGBG; 10. Yumai No. 18 PEG + MGBG

2.4 Effect of exogenous Spd and MGBG on the *LRWC* , *LREL* and *RDIR* of wheat seedlings under osmotic stress

The PEG osmotic stress injury to Yangmai No.9 cv. was alleviated apparently with exogenous Spd (1 mmol/L), as judged by a reduction of *LREL* and increases of *LRWC* and *RDIR*. Osmotic stress injury to Yumai No. 18 cv. was aggravated more significantly with 1 mmol/L MGBG treatment than that to Yangmai No. 9 cv., as judged by a bigger increased rang of *LREL* and larger decreased rangs of *LRWC* and *RDIR*(Fig.1).

2.5 Effect of exogenous Spd and MGBG on the ATPase activity and —SH group content in mitochondrial membrane of wheat seedlings under osmotic stress

Treatment of Yangmai No.9 cv. with exogenous Spd at 1 mmol/L inhibited more markedly the PEG-induced decreases of ATPase activity and —SH group content. While treatment of Yumai No.18 cv. with exogenous MGBG at 1 mmol/L promoted more significantly the PEG-induced decreases of ATPase activity and —SH group content in mitochondrial membrane of wheat seedling roots (Fig. 2, Fig. 3).

2.6 Effect of exogenous Spd and MGBG on the levels of NCC-PAs in mitochondrial membrane of wheat seedling roots under osmotic stress

With the exogenous Spd treatment, the content of NCC-Spd increased substantially in mitochondrial membrane from PEG-treated Yangmai No. 9 cv. seedling roots and it did slightly in PEG-treated Yumai No. 18 cv., while MGBG treatment induced a significant reduction in content of NCC-Spd in PEG-treated Yumai No. 18 cv. Whether exogenous Spd or MGBG affected NCC-Put content slightly in two PEG-treated wheat cv. seedlings (Table 1). With regard to the ratio of NCC-Spd/NCC-Put presented in Table 1, we found that exogenous Spd treatment elevated greatly the ratio in PEG-treated Yangmai No.9 cv., and it affected the ratio in PEG-treated Yumai No. 18 cv. slightly, while MGBG treatment reduced the ratio more significantly in PEG-treated Yumai No.18 cv.

2.7 Effect of osmotic stress and *o*-Phen on the levels of CC-PAs in mitochondrial membrane of wheat seedling roots

of two wheat cultivar seedlings increased. However, NCC-Spd content in PEG-treated Yumai No. 18 cv. (drought-tolerant) increased much more markedly than that in PEG-treated Yangmai No.9 cv. (drought-sensitive), while NCC-Put content in PEG-treated Yumai No. 18 cv. did not increase as much as that in PEG-treated Yangmai No.9 cv. (Table 1).

Table 1 Effect of PEG, Spd and MGBG on the levels of noncovalently conjugated PAs(NCC-PAs) in mitochondria membrane prepared from wheat seedling roots

Cultivars	Treatments	NCC-PA contents		Spd/Put
		Put, nmol/mg protein	Spd, nmol/mg protein	
Yangmai No. 9	Control	3.67 ± 0.35[c]	6.42 ± 0.57[d]	1.75
	PEG	11.66 ± 1.12[a]	8.15 ± 0.85[c]	0.70
	PEG + Spd	12.87 ± 1.21[a]	18.02 ± 1.49[a]	1.40
	PEG + MGBG	13.13 ± 1.34[a]	8.08 ± 0.77[c]	0.62
Yumai No. 18	Control	3.16 ± 0.38[c]	5.97 ± 0.70[d]	1.89
	PEG	7.75 ± 0.74[b]	11.91 ± 1.18[b]	1.54
	PEG + Spd	8.01 ± 0.76[b]	17.58 ± 1.59[a]	2.19
	PEG + MGBG	8.46 ± 0.83[b]	8.87 ± 0.73[c]	1.05

Notes: Wheat seedlings were treated with PEG(- 0.55 MPa), PEG(- 0.55 MPa) + Spd(1 mmol/L) or PEG(- 0.55 MPa) + MGBG(1 mmol/L) for 7 d. Means with different letters within a column are significantly different at *P* < 0.05 based on Duncan's multiple range test. The values are the mean ± *SE* (*n* = 9) of three experiments

The levels of the two CC-PAs (CC-Put and CC-Spd) could be detected in root mitochondrial membrane vesicle from the seedlings of two PEG-treated wheat cultivars, while the CC-Spm level could not be statistically detected. Under PEG stress, CC-Put level in Yumai No. 18 cv. seedlings increased much more greatly than that in Yangmai No. 9 seedlings (Table 2), however, there was no significant difference in CC-Spd level between the two wheat cv. (data not shown). Exogenous o-Phen treatment inhibited more significantly the PEG-induced increase of CC-Put level in mitochondrial membrane from Yumai No. 18 cv. seedling roots than from Yangmai No.9 cv. (Table 2).

Table 2   **Effect of PEG and o-Phen on the levels of covalently conjugated PAs(CC-PAs) in mitochondria membrane prepared from wheat seedling roots**

Cultivars	Treatments	CC-Put content, nmol/mg protein
Yangmai No. 9	Control	1.53 ± 0.14^d
	PEG	1.84 ± 0.14^c
	PEG + o-Phen	1.66 ± 0.17^{cd}
Yumai No. 18	Control	1.60 ± 0.15^d
	PEG	3.25 ± 0.32^a
	PEG + o-Phen	2.27 ± 0.24^b

Notes: Wheat seedlings were treated with PEG(-0.55 MPa) or PEG(-0.55 MPa) and o-Phen(1 mmol/L) for 7 d. Means with different letters within a column are significantly different at $P < 0.05$ based on Duncan's multiple range test. The values are the mean $\pm SE(n = 9)$ of three experiments

2.8 Effect of o-Phen on the LRWC, LREL, RDIR of wheat seedlings under osmotic stress

Osmotic stress injury to Yumai No. 18 cv. was aggravated significantly with 1 mmol/L o-Phen treatment, as judged by a bigger increased range of *LREL* and larger decreased ranges of *LRWC* and *RDIR*. However, the o-Phen aggravation effect seemed more slightly on the osmotic stress injury to Yangmai No.9 cv. (Fig. 4).
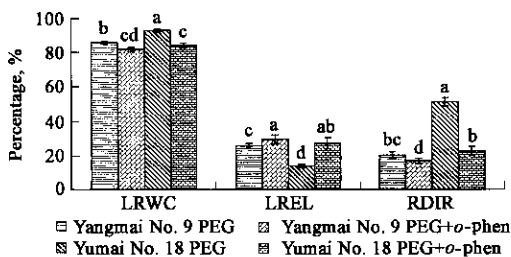

Fig.4   Effect of o-Phen on *LRWC*, *LREL* and *RDIR* of wheat seedlings under osmotic stress. Wheat seedlings were treated with PEG(-0.55 MPa), PEG(-0.55 MPa) + o-Phen(1 mmol/L) for 7 d. The second expanded leaves were sampled for *LRWC* and *LREL* determination and whole seedlings for *RDIR*. Error bars indicate $SE(n = 9)$. Means with different letters are significantly different at $P < 0.05$ based on Duncan's multiple range test

2.9 Effect of o-Phen on the ATPase activity and —SH group content in mitochondrial membrane from wheat seedling roots under osmotic stress

PEG-induced decreases of ATPase activity and —SH group content in mitochondrial membrane from wheat seedling roots were promoted with o-Phen treatment and the effect was more significantly on Yumai No. 18 seedlings than that on Yangmai No. 9 seedlings(Fig. 2, Fig. 3).

3 Discussion

3.1 Effect of osmotic stress on the ATPase activity and —SH content in mitochondrial membrane from wheat seedling roots

Plants tolerance to water stress has been associated with lower *LREL* and higher *LRWC* and *RDIR* of plants subjected to stress(Hsiao, 1973; Schonfeld, 1988). Therefore, from the results presented in Fig. 1, it is confirmed that cv. Yumai No.18 is osmotic tolerant and cv. Yangmai No.9 is osmotic sensitive.

The results that PEG treatment induced the much more significant decrease of ATPase activity and —SH content in mitochondrial membrane from drought-sensitive Yangmai No. 9 cv. seedling than that from drought-tolerant Yumai No. 18 cv. (Fig. 2, Fig. 3) indicated that ATPase and —SH content were possibly involved in the water stress tolerance of wheat seedlings. The two following further experimental results confirmed this hypothesis from different aspect: (1) exogenous Spd treatment alleviated significantly PEG osmotic stress injury to drought-sensitive Yangmai No.9 cv. seedlings (Fig.1) in concert with inhibiting greatly the PEG-induced decrease of ATPase activity and —SH content in Yangmai No.9 seedling roots (Fig.2, Fig.3), and (2) exogenous MGBG and o-Phen treatments aggravated apparently the PEG-induced injury to drought-tolerant Yumai No. 18 cv. seedlings(Fig.1, Fig.4) with significant promotion of PEG-induced decrease of ATPase activity and —SH content in Yumai No. 18(Fig. 2, Fig. 3). It is documented that the direction of ATPase action at any instant depends on the balance between the steepness of electrochemical proton gradient and the local ΔG for ATP hydrolysis. Suppose under osmotic stress, a large amount of ATP is synthesized to adapt the stress, causing sudden drop of proton-motive force across mitochondrial inner membrane. As a result, ATPase will start hydrolyzing some of the ATP in the matrix until a new balance of ATP to ADP and Pi is reached(Alberts, 2002), which is important for wheat seedlings to tolerate osmotic stress.

3.2 Relationship between ATPase activity, —SH content and contents of NCC-PAs in mitochondrial membrane from wheat seedling roots

There were extensive reports concerning effects of exogenous PAs on the activities of membrane-associated enzymes(Lester, 2000). PAs were considered to be able to enhance membrane associated enzyme activities. However, PAs associated to bio-membrane vesicles were very seldom detected directly to investigate relationship between PA levels and activities of membrane-associated enzymes(Sun, 2002). In present study, we detected noncovalently conjugated PAs (NCC-PAs) in mitochondrial membrane. The results showed that PEG treatment induced much more significant increases of NCC-Spd content in root mitochondria membrane vesicle from drought-tolerant Yumai No. 18 cv. seedlings than that from drought-sensitive Yangmai No.9 cv. (Table 1). These findings, together with the result presented in Fig.2 and Fig. 3 that PEG-treated Yumai No. 18 seedlings could maintain higher ATPase activity and —SH content in root mitochondrial membrane than PEG-treated Yangmai No. 9

seedlings, were indicative of possible involvement of NCC-Spd in the association with the enzyme activity and —SH content in root mitochondrial membrane. The hypothesis was further confirmed by the following two experiment results: (1) PEG-induced increases of NCC-Spd level in Yumai No. 18 cv. seedling root mitochondrial membrane were inhibited with the concomitant exogenous MGBG treatment(Table 1), in concert with the promotion of PEG-induced decreases of ATPase activity and —SH content(Fig.2, Fig.3); and (2) exogenous Spd treatment increased markedly the NCC-Spd level (Table 1) and inhibited PEG-induced decreases of ATPase activity and —SH content in Yangmai No.9 seedling root mitochondrial membrane (Fig. 2, Fig. 3). Statistical analysis also indicated that under osmotic stress, there was significant positively correlation between the ratio NCC-Spd/NCC-Put and ATPase activity ($r = 0.9874$, $r_{0.01} = 0.9172$, $n = 6$) and —SH content($r = 0.9786$, $r_{0.05} = 0.9500$, $n = 6$). One reason why NCC-Spd could stimulate the ATPase activiy was attributed to the following statement: Due to the virtue of their cationic nature at physiological pH, higher value PAs, such as Spd, affect mitochondrial membrane physical state by noncovalently binding to negative charges of membrane phospholipids more easily, and also affect protein configuration and function by noncovalently binding to negative charges of membrane acidic protein (including ATPase in mitochondrial membrane) more easily.

3.3 Relationship between ATPase activity, —SH content and contents of CC-PAs in mitochondrial membrane from wheat seedling roots

In present study, we also detected the covalently conjugated polyamines(CC-PAs) in PEG-treated mitochondrial membrane. The result presented in Table 2 showed that PEG-treatment increased CC-Put content more markedly in drought-tolerant Yumai No.18 cv. seedling root mitochondrial membrane than in drought-sensitive Yangmai No.9 cv. seedlings. Putting the results together with the study on ATPase activity and —SH content(Fig.2, Fig.3), we could suggest that ATPase activity and —SH content were associated with the level of CC-Put, while CC-Spd was irrelative to enzyme activities because of its minor content and disordered content changes under stress (date not shown). This suggestion was also supported by the following experiment result that the concomitant treatment with o-Phen, an inhibitor of TGase, promoted the PEG-induced decrease effect on ATPase activity and —SH content(Fig.2, Fig.3) in concert with inhibition of CC-Put biosynthesis in mitochondrial membrane Yumai No. 18 cv. seedling roots (Table 2). This suggestion was also corroborated by statistical analysis result, indicating that under osmotic stress, there was a significantly positive correlation between CC-Put level and ATPase activity ($r = 0.9572$, $r_{0.05} = 0.9500$, $n = 4$) and —SH content ($r = 9660$, $r_{0.05} = 0.9500$, $n = 4$) in mitochondrial membrane from wheat seedling roots. It is hypothesized that Put might mediate membrane wounding(DiTomaso, 1989), so the conversion of free Put to CC-Put could alleviate free Put wounding effect. Furthermore, the conversion of free Put to CC-Put, forming protein-Gln-Put and protein -Gln-Put-protein, could stabilize the configuration and function of mitochondrial membrane

proteins(including ATPase) by preventing the proteins from denaturing under PEG osmotic stress and then facilitate the enzyme activity(Serafini-Fracassini, 1995).

4 Conclusions

In summary, to our knowledge, the present report is the first to demonstrate that under osmotic stress, NCC-Spd and CC-Put were involved in maintenance of the ATPase activity and —SH content in mitochondrial membrane, and involved in osmotic stress tolerance of wheat seedlings. PAs are localized in various cellular organelles (Kumer, 1997). Thus, further study is needed to investigate the subcellular distribution of PAs in leave and root cells under osmotic stress, exogenous PAs and inhibitor effect. Instead of the free PAs, the importance of the collective analysis of the three fractions of PAs should be emphasized. This investigation may help understand better the roles of various forms of PAs in the plant tolerance to osmotic stress.

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