INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0190/2019/22–1–155–160
DOI: 10.17957/IJAB/15.1045
http://www.fspublishers.org

Full Length Article

Polyamine are Involved in ABA Signaling by Enhancing NADPH Oxidase Activity in Maize Leaves under Osmotic Stress

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Abstract

Polyamines in plants are closely related to osmotic stress; however, the relationship between putrescine (Put) and signaling in plant cell still remains to be answered. In this research, the roots of maize seedlings were treated for 12 h with PEG-6000 and other reagents, including Put, D-arginine (D-Arg), tungstate (T), diphenylene iodonium (DPI), imidazole (I), pyridine (P), EGTA, $LaCl_3$ and verapamil (V) in different combinations. Put and ABA contents in leaves were detected and the activity of plasma membrane NADPH oxidase (PM-NOX), the main element involved in ABA signaling induced by osmotic stress, was also investigated. After rhizosphere treatment of seedlings with osmotic stress for 12 h, the contents of ABA and Put increased and the PM-NOX activity and the rate of $O_2^{\cdot-}$ production also increased. The inhibitor T of ABA bio-synthesis reduced the increases in ABA and Put contents induced by osmotic stress. The inhibitor D-Arg of Put bio-synthesis didn't reduce osmotic-induced increase of ABA. These findings suggested that ABA might regulate Put biosynthesis and ABA contents were not affected by Put. D-Arg inhibited the increases induced by osmotic stress in the PM-NOX activity, the rate of $O_2^{\cdot-}$ production and Put content and the treatment with exogenous Put weakened the inhibitory effects of D-Arg. These findings suggested that Put might regulate PM-NOX activity. Treatments with three inhibitors I, DPI and P of PM-NOX reduced significantly not only the $O_2^{\cdot-}$ production, but also the stress-induced increase of Put content, which indicated that $O_2^{\cdot-}$ production might regulate Put biosynthesis. Treatments with EGTA (Ca^{2+} chelator), $LaCl_3$ and V (Ca^{2+} channel blockers) reduced significantly the stress-induced increase of Put contents, which suggested that Ca^{2+} might regulate Put biosynthesis. In conclusion, Put was involved in ABA signaling induced by osmotic stress by regulating PM-NOX activity.

Keywords: Maize; Osmotic stress; Putrescine; Signaling; PM-NADPH oxidase

Introduction

Water deficit has a significant inhibitory effect on the cell division and expansion of maize (*Zea mays* L.) seedlings (Avramova *et al*., 2015). Cell membrane receptors sense the water deficit signal and transmit the signal to the cell inner via signal transduction pathway, triggering a series of biochemical reactions (Zhu, 2016). Plasma membrane NADPH oxidase (PM-NOX) is involved in abscisic acid (ABA) signal transduction in maize seedlings under osmotic stress (Jiang and Zhang, 2002, 2003) and heavy metals stress (Hao *et al*., 2006). In the past research, it has been extensively researched that ABA triggers a series of reactions of antioxidant defense system (Zhu, 2002; Wang *et al*., 2006; Zhu, 2016). For example, ABA could accelerate the increase of reactive oxygen originated from PM-NOX (Guan *et al*., 2000; Pei *et al*., 2000) and ABA signaling is involved in the expression of up-regulation genes of antioxidant enzymes under osmotic stress (Guan *et al*., 2000). Calcium (Ca^{2+}) has also been involved in ABA signal transduction in plant cells (Pei *et al*., 2000; Muruta *et al*., 2001). ABA promoted cytosolic Ca^{2+} increase induced by both Ca^{2+} release from intracellular and Ca^{2+} influx from the extracellular (Pei *et al*., 2000). The study of Jiang and Zhang (2003) suggested that a cross-talk between Ca^{2+} and reactive oxygen originated form PM-NOX was involved in the ABA signal transduction pathway (ABA→PM-NOX→$O_2^{\cdot-}$→H_2O_2→Ca^{2+}).

Polyamines (PAs) are aliphatic nitrogenous amines and are ubiquitously existed in plants. They are closely related to growth and development of plants (Du *et al*., 2017; Guo *et al*., 2018). In plants, PAs mainly include putrescine (Put) with two aminos, spermidine (Spd) with three aminos, spermine (Spm) with four aminos. D-arginine (D-Arg) is an inhibitor of Put biosynthesis. A lot of studies indicated that PAs in plants are closely related with osmotic stress (Liu *et al*., 2005a; Du *et al*., 2015; Pál *et al*., 2018). However, the mechanism by which PAs enhance the tolerance of plants to

To cite this paper: Du, H., D. Liu, G. Liu, H. Liu and R. Kurtenbach, 2019. Polyamine are involved in ABA signaling by enhancing NADPH oxidase activity in maize leaves under osmotic stress. *Intl. J. Agric. Biol*., 22: 155–160

stress is not yet clear. Researchers suggested that PAs in cell wall are oxidized by polyamine oxidase (PAO) to form H_2O_2, which trigger the hypersensitive response (Cona *et al*., 2003). Liu *et al*. (2000) reported that PAs target KATI-like inward K^+ channels in guard cells and modulate stomatal movement. Recently, PAs being mediated phosphorylation (Gupta *et al*., 2012) and interaction between PAs and nitric oxide signaling (Montilla-Bascón *et al*., 2017) have been documented. However, relationship between PA and osmotic-induced ABA signaling pathway remains to be investigated.

The study aimed to elucidate the significant of Put in maize seedling leaves in osmotic stress-induced ABA signaling, including the following items: relationship between PA and ABA, between PA and PM-NOX dependent $O_2^{\cdot-}$ production, and between PA and Ca^{2+}.

Materials and Methods

Maize Cultivation and Treatments

Maize (*Zea mays* L., Nongda No. 108 cultivar, from China Agricultural University) seeds were selected and sterilized in 0.2% (w/v) $HgCl_2$ solution for 10 min, then washed with distill water for three times. After that seeds were planted in plastic pot (diameter × high: 10 × 15 cm) with little pores on the bottom containing 3/4 sands of the pot volume. After the seeds germinated, the pots were put into mildly bigger pots with Hoagland 's solution for seedling. They were put into a controlled greenhouse at temperature of 28/22°C (day/night), photosynthetic active radiation (PAR) of 600 μmol m^{-2} s^{-1} and photoperiod of 14/10 h (day/night) and the solution was renewed per 2 d. When the second leaves of the maize seedlings were fully expanded, the roots of maize seedlings were treated for 12 h with Hoagland's solution containing (i) PEG-6000 (20%, -0.55 MPa), (ii) PEG-6000 (20%, -0.55 MPa) + D-Arg (1 m*M*), (iii) PEG-6000 (20%, -0.55 MPa) + D-Arg (1 m*M*) + Put (1 m*M*), (iv) PEG-6000 (20%, -0.55 MPa) + tungstate (T), (v) PEG-6000 (20%, -0.55 MPa) + diphenylene iodonium (DPI; 50 μM) + imidazole (I; 20 m*M*) + pyridine (P; 20 m*M*) and (vi) PEG-6000 (20%, -0.55 MPa) + EGTA (5 m*M*) + $LaCl_3$ (5 m*M*) + verapamil (V; 1 m*M*). Whereas seedlings in control pots remained well watered (normal moisture conditions) without the reagents mentioned above. After 12 h treatment, the second leaves of the treated and control seedlings were clipped and tested for the subsequent experiments. Put, D-Arg, T, I, P, DPI, EGTA, $LaCl_3$, and V were purchased from Sigma Chemical Co. (USA).

Detection of Leaf Relative Water Content (LRWC) of Maize Seedlings

The second leaves of the treated and control seedlings were sampled and clipped from the base of the leaves. LRWC of maize seedlings was measured from a formula:

$$LRWC\ (\%) = (FW - DW) / (TW - DW) \times 100$$

Where FW, DW and TW represents the leaf fresh weight, dry weight and saturation weight of maize seedlings, respectively.

Plasma Membrane Separation and Protein Determination

Leaf samples were ground and homogenized using extraction buffer. The homogenate was filtered using 0.45 μm filtering membrane and the filtrate was centrifuged at 11000 *g* for 30 min. Plasma membranes of maize seedling leaves were separated using the two-phase aqueous polymer partition system according to Du *et al.* (2015). The protein level from PM was quantified using the method described by Bradford (1976) with bull serum albumin (BSA) as standard.

Measure of PM-NOX Activity

Plasma membrane separated above was used to detect the PM-NOX activity according to Sagi and Fluhr (2001) and Jiang and Zhang (2003). The NOX activity was evaluated by the reduction of XTT.

Measure of PM-NADPH-dependent $O_2^{\cdot-}$ Production Rate

The production rate of PM-NADPH-dependent $O_2^{\cdot-}$ was evaluated according to the method described by Sagi and Fluhr (2001) and Jiang and Zhang (2003).

Measure of Put Content

Put in maize seedling was extracted according to the method described by Liu *et al.* (2005a) and quantified by HPLC (Waters 2696, U.S.) at 254 nm.

Determination of ABA Content

Maize leaf leaves were washed with distilled water and weighted for 1 g. Leaves (1 g) was ground and homogenized in 80% methanol. Then the homogenate was centrifuged for 30 min at 11000 g. The supernatants were eluted by the Sep-Pak C18 cartridge (Waters, U.S.A.) to removal of polar compounds, then quantified by enzyme linked immune sorbent assay as described by Zhang *et al.* (2017).

Statistical Analysis

The experiments were repeated three times and three samples were taken for every experiment. Data were analyzed using S.P.S.S.16.0 and Microsoft Excel software. Every value in figures or tables reported in this paper is mean (n = 3 × 3) ± stand error (SE). The significant differences among different treatments were determined

using by Duncan's multiple range tests at $P<0.05$.

Results

Leaf LRWC

The LRWC of maize seedlings decreased from 98% to 80% under PEG osmotic stress. Treatment with PEG and D-Arg, the inhibitor of Put biosynthesis, aggravated the decrease to 65%. The treatment with T, the inhibitor of ABA biosynthesis, aggravated the decrease to 50%. DPI, I and P, three inhibitor of the PM-NOX activity, facilitated the decrease to 68, 66 and 67%, respectively. EGTA, $LaCl_3$ and V, the inhibitors which affect Ca^{2+} concentration of intracellular region, facilitated the decrease to 75%, 79% and 77%, respectively (Fig. 1).

Put and ABA Contents

Treatment with PEG for 12 h led to the increase of Put contents from 104 to 183 nmol g^{-1} FW in maize seedling leaves. D-Arg, an inhibitor of Put biosynthesis, reduced the PEG stress-induced increase from 183 to 132 nmol g^{-1} FW, and exogenous Put treatment reversed the effect of D-Arg (Fig. 2). These results verified that D-Arg inhibited the Put biosynthesis. Likewise, PEG treatment brought about the significant increase of ABA content from 76 to 397 ng g^{-1} FW; and the treatment with T, an inhibitor of ABA biosynthesis, markedly reduced the ABA content from 397 to 169 ng g^{-1} FW. In order to elucidate whether Put modulates ABA biosynthesis, exogenous Put and D-Arg (an inhibitor of Put biosynthesis) were used. The results showed that both of them scarcely affected the ABA contents (from 397 to 387 and to 401 ng g^{-1} FW) in maize seedling leaves subjected to osmotic stress (Fig. 3), which suggested that ABA biosynthesis was not modulated by Put.

PM-NOX Activity

Osmotic stress treatment promoted the increases of PM-NOX activity and PM-NADPH-dependent $O_2^{\cdot-}$ production from 2.0 to 6.8 nmol mg^{-1} protein min^{-1} and 0.65 to 2.01 nmol mg^{-1} protein min^{-1} in maize seedling leaves, respectively (Table 1). D-Arg treatment reduced the stress-induced increases from 6.8 to 3.2 nmol mg^{-1} protein min^{-1} and 2.01 to 1.12 nmol mg^{-1} protein min^{-1}, respectively, and exogenous Put reversed the inhibitory effects caused by D-Arg (Table 1), which indicated that the increases induced by osmotic stress in the PM-NOX activity and PM-NADPH-dependent $O_2^{\cdot-}$ production were partly attributed to the increase in Put content.

PM-NOX Activity and PM-NADPH Dependent $O_2^{\cdot-}$ Production

Under osmotic stress, the PM-NOX activity and PM-NADPH-dependent $O_2^{\cdot-}$ production increased by 340 and 309%, respectively (Table 2). However, the treatments with three inhibitors (DPI, I and P) of NOX significantly inhibited the increases in the PM-NOX activity from 6.8 to 3.5 nmol mg^{-1} protein min^{-1} or so and PM-NADPH-dependent $O_2^{\cdot-}$ production from 2.01 to 1.0 nmol mg^{-1} protein min^{-1} or so (Table 2), which verified that DPI, I and P actually inhibited the PM-NOX activity.

Table 1: Effects of PEG, D-Arg and exogenous Put on PM-NOX activity and PM-NADPH dependent $O_2^{\cdot-}$ production rate in leaves of maize seedlings

Treatments	PM-NOX activity (nmol mg^{-1} protein min^{-1})	$O_2^{\cdot-}$ production rate (nmol mg^{-1} protein min^{-1})
Control	2.0 ± 0.21 c	0.65 ± 0.08 c
PEG	6.8 ± 0.72 a	2.01 ± 0.13 a
PEG+D-Arg	3.2 ± 0.39 b	1.12 ± 0.08 b
PEG+D-Arg+Put	6.3 ± 0.59 a	1.95 ± 0.15 a

PEG–osmotic stress treatment; D-Arg–D-Arginine (an inhibitor of Put biosynthesis); Control–seedlings remained well-watered (normal moisture conditions) without PEG, D-Arg, or Put. Each value in the table represents the mean of three experiments ± SE. Error bars indicate SE (n = 9), and the different letters (a-c) in the column are significantly different as determined by Duncan's multiple range tests ($P < 0.05$)

Table 2: Effects of PEG, DPI, I and P on activity of PM-NOX and PM-NADPH dependent $O_2^{\cdot-}$ production rate in leaves of maize seedlings under osmotic stress

Treatments	NOX activity (nmol mg^{-1} protein min^{-1})	$O_2^{\cdot-}$ production rate (nmol mg^{-1} protein min^{-1})
Control	2.0 ± 0.21 d	0.65 ± 0.08 d
PEG	6.8 ± 0.72 a	2.01 ± 0.13 a
PEG+DPI	3.1 ± 0.19 c	0.95 ± 0.06 c
PEG+I	3.8 ± 0.28 b	1.32 ± 0.09 b
PEG+P	3.6 ± 0.35 b	1.26 ± 0.08 b

PEG–osmotic stress treatment; DPI–diphenylene iodonium (an inhibitor of PM-NOX); I–imidazole (an inhibitor of PM-NOX); P–pyridine (an inhibitor of PM-NOX); Control–seedlings remained well-watered (normal moisture conditions) without PEG, DPI, I, or P. Each value in the table represents the mean of three experiments ± SE. Error bars indicate SE (n = 9), and different letters (a-d) in the column are significantly different as determined by Duncan's multiple range tests ($P < 0.05$)

Put Contents in Maize Seedling Leaves under Osmotic Stress

In order to analyze further whether Put level is related to ABA, NADPH-dependent $O_2^{\cdot-}$ and Ca^{2+}, we investigated the effects of T (an inhibitor of ABA biosynthesis), three inhibitors (DPI, I and P) of NOX, EGTA (Ca^{2+} chelator) and $LaCl_3$ and verapamil (Ca^{2+} channel blockers) on Put content in maize seedling leaves under osmotic stress. From Fig. 4, it could be showed that Put content increased markedly under osmotic stress. However, the increase induced by osmotic stress was inhibited by T treatment (from 183 to 125 nmol g^{-1} FW), which suggested that ABA might modulate Put biosynthesis. All of the treatments with three inhibitors (DPI, I and P) of PM-NOX activity inhibited the osmotic stress-induced increase from 183 to 145 nmol g^{-1} FW or so, which indicated that Put biosynthesis might be modulated by PM-NADPH dependent $O_2^{\cdot-}$ production. The treatments with EGTA,

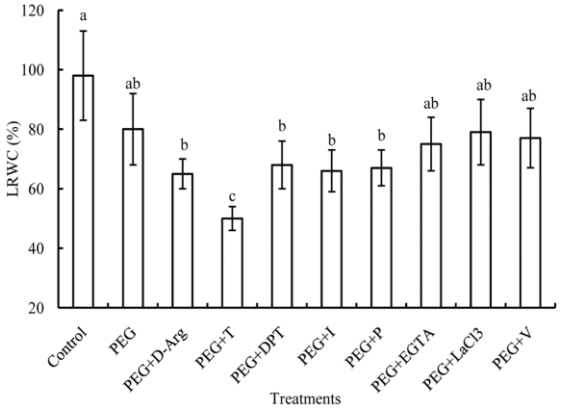

Fig. 1: Effect of PEG, D-Arg, T, DPI, I, P, EGTA, $LaCl_3$ and V on LRWC of Maize Seedlings. Control–seedlings remained well-watered (normal moisture conditions) without PEG, DPI, I, P, EGTA, $LaCl_3$, or V; PEG–osmotic stress treatment; D-Arg–D-Arginine (an inhibitor of Put biosynthesis); T–tungstate (an inhibitor of ABA biosynthesis); DPI–diphenylene iodonium (an inhibitor of PM-NOX); I–imidazole (an inhibitor of PM-NOX); P–pyridine (an inhibitor of PM-NOX); EGTA–ethylene glycol-bis (2-aminoethyl ether)- N,N,N',N'- tetraacetic acid (Ca^{2+} chelator); $LaCl_3$–$LaCl_3$ (Ca^{2+} channel blockers); V–verapamil (Ca^{2+} channel blockers). Each value in the figure represents the mean of three experiments ± SE. Error bars indicate SE (n = 9), and different letters (a-c) above the column are significantly different as determined by Duncan's multiple range tests ($P < 0.05$)

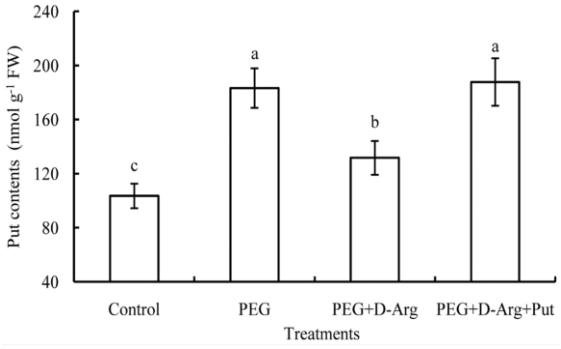

Fig. 2: Effects of PEG, D-Arg and exogenous Put on Put content in leaves of maize seedlings. Control–seedlings remained well-watered (normal moisture conditions) without PEG, D-Arg, or Put; PEG–osmotic stress treatment; D-Arg–D-Arginine (an inhibitor of Put biosynthesis). Each value in the figure represents the mean of three experiments ± SE. Error bars indicate SE (n = 9), and different letters (a-c) above the column are significantly different by Duncan's multiple range tests ($P < 0.05$)

La^{3+} and verapamil inhibited the increase of Put content from 183 to 130 nmol g^{-1} FW or so (Fig. 4), which suggested that Ca^{2+} might modulate Put biosynthesis.

Discussion

The response of plant to water stress has been extensively investigated (Butt *et al.*, 2015; Du *et al.*, 2018). PEG

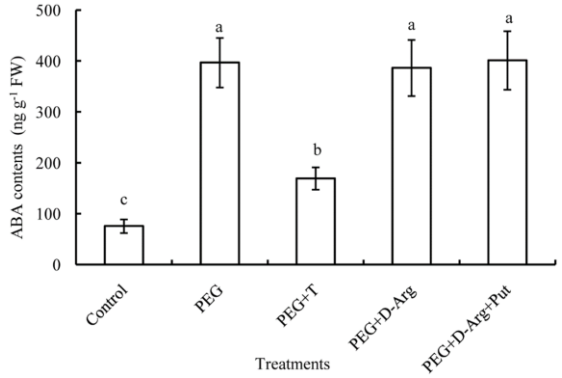

Fig. 3: Effects of PEG, D-Arg, exogenous Put and T on ABA content in leaves of maize seedlings. Control–seedlings remained well-watered (normal moisture conditions) without PEG, T, D-Arg, or Put; PEG–osmotic stress treatment; T–tungstate (an inhibitor of ABA biosynthesis); D-Arg–D-Arginine (an inhibitor of Put biosynthesis). Each value in the figure represents the mean of three experiments ± SE. Error bars indicate SE (n = 9), and different letters (a-c) above the column are significantly different as determined by Duncan's multiple range tests ($P < 0.05$)

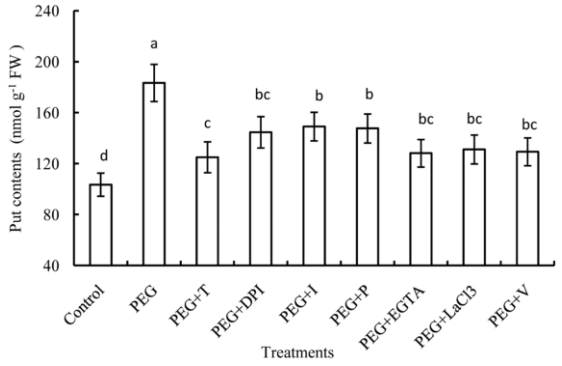

Fig. 4: Effects of T, DPI, I, P, EGTA, $LaCl_3$ and V on Put content in leaves of maize seedlings under osmotic stress. Control–seedlings remained well-watered (normal moisture conditions) without PEG, DPI, I, P, EGTA, $LaCl_3$, or V; PEG–osmotic stress treatment; T–tungstate (an inhibitor of ABA biosynthesis); DPI–diphenylene iodonium (an inhibitor of PM-NOX); I–imidazole (an inhibitor of PM-NOX); P–pyridine (an inhibitor of PM-NOX); EGTA–ethylene glycol-bis (2-aminoethyl ether)- N,N,N',N'-tetraacetic acid (Ca^{2+} chelator); $LaCl_3$–$LaCl_3$ (Ca^{2+} channel blockers); V–verapamil (Ca^{2+} channel blockers). Each value in the figure represents the mean of three experiments ± SE. Error bars indicate SE (n = 9) and different letters (a-d) above the column are significantly different as determined by Duncan's multiple range tests ($P < 0.05$)

osmotic stress led to the decrease of LRWC of maize seedlings (Fig. 1). ABA is a significant regulator of plant responses to water stress (Larkindale and knight, 2002). Although it has also been well verified that water stress can facilitate the accumulations of PAs (Liu *et al*., 2005a; Pál *et al*., 2018), it is not clear whether the changes in ABA contents are physiologically relevant to PAs. The study of Liu *et al*. (2005b) shows the production of PAs is enhanced

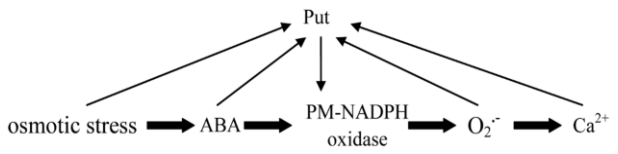

Fig. 5: Implying the involvement of Put in osmotic stress-induced ABA signalling pathway and the cross-talks between Put, $O_2^{\cdot-}$ and Ca^{2+}. The arrow indicates the positive regulation. The coarse arrows present the results that have been already documented, and the thin arrows present the results in the present research

by ABA in maize seedlings subjected to salt stress. In the present study, osmotic stress brought about marked increases in Put (Fig. 2) and ABA contents (Fig. 3) in maize seedling leaves. The treatment with T, an inhibitor of ABA biosynthesis (Hansen and Grossmann, 2000), reduced the osmotic-induced the increases not only in ABA (Fig. 3), but also in Put contents (Fig. 4), which suggested that Put content is regulated by ABA. However, D-Arg treatment reduced the osmotic stress-induced increase in Put content (Fig. 2), but the ABA content was hardly affected by D-Arg (Fig. 3), which suggested that ABA is not regulated by Put in maize seedling leaves under osmotic stress. Taking the notions above together, it could be concluded that Put might function downstream of ABA in the osmotic stress-induced ABA signal transduction event in plants.

In the osmotic stress-induced ABA signaling, ABA could activate the NOX to form $O_2^{\cdot-}$, then $O_2^{\cdot-}$ is turned into H_2O_2 by dismutation (Pei *et al*., 2000; Muruta *et al*., 2001). So, it is obvious that PM-NADPH dependent $O_2^{\cdot-}$ is downstream of ABA in the ABA signaling. Then, whether Put accumulation induced by osmotic stress was associated to PM-NADPH dependent $O_2^{\cdot-}$. In the present research, PEG treatment led to the increases not only in Put content (Fig. 2), but also in the PM-NOX activity and the rate of PM-NADPH dependent $O_2^{\cdot-}$ production (Table 1). D-Arg treatment reduced the osmotic stress-induced increases in Put content (Fig. 2) and with the decrease of Put content, the PM-NOX activity and the rate of PM-NADPH dependent $O_2^{\cdot-}$ production were also reduced accordingly (Table 1). Exogenous Put reversed the inhibitory effects of D-Arg on Put content (Fig. 2), PM-NOX activity and the rate of PM-NADPH dependent $O_2^{\cdot-}$ production (Table 1). These findings suggested that Put might modulate the of PM-NOX activity and the rate of PM-NADPH dependent $O_2^{\cdot-}$ production. Put might up-regulate the activity of PM-NOX and function upstream of oxidation production in osmotic stress-induced ABA signaling. Then, whether PM-NADPH dependent $O_2^{\cdot-}$ could regulate Put content?

To explore the problem, three treatments with inhibitors (DPI, I and P) of PM-NOX activity (Murphy and Auh, 1996) were carried out in the research. The results showed that three inhibitors significantly reduced not only the activity of PM-NOX and the rate of PM-NADPH dependent $O_2^{\cdot-}$ production (Table 2), but also the Put content (Fig. 4), which indicated that PM-NADPH dependent $O_2^{\cdot-}$ production modulated Put biosynthesis. Taking together the notion mentioned above that Put might modulate the PM-NADPH dependent $O_2^{\cdot-}$ production. It could be concluded that a cross-drtalk between Put and PM-NADPH dependent $O_2^{\cdot-}$ was involved in the ABA signaling pathway induced by PEG osmotic stress.

Since that a cross-talk between PM-NADPH dependent $O_2^{\cdot-}$ and Ca^{2+} is involved in osmotic stress-induced ABA signaling pathway (Chen and Li, 2001; Yang and Poovaiah, 2002; Jiang and Zhang, 2003; Kreslavski *et al*., 2012) and $O_2^{\cdot-}$ production triggers Ca^{2+} influx and the increase in cytosolic Ca^{2+}, taking together the above notion that Put modulated PM-NADPH dependent $O_2^{\cdot-}$ production. It could be concluded that Put might up-regulated cytosolic Ca^{2+} level in the signaling pathway.

However, whether Ca^{2+} modulates Put level remains unknown. Therefore, three treatments with Ca^{2+} chelator EGTA (Blume *et al*., 2000), Ca^{2+} channel blockers, $LaCl_3$ and V (Pei *et al*., 2000) were carried out in the research. The previous results verified that EGTA, $LaCl_3$ and V could reduce cytosolic Ca^{2+} level (Jiang and Zhang, 2003). Our present study showed that EGTA, $LaCl_3$ and V significantly reduced the Put content (Fig. 4). The same results obtained with the three different inhibitors suggested that cytosolic Ca^{2+} might up-regulate Put biosynthesis in the signaling pathway. From the suggestion and the notion mentioned above that Put up-regulated the cytosolic Ca^{2+} level, it could be concluded that a cross-talk between Put and cytosolic Ca^{2+} was also involved in osmotic stress-induced ABA signaling pathway.

Conclusion

The research first indicated that PEG osmotic stress-induced Put was involved in the stress-induced ABA signaling pathway (osmotic stress→ABA→PM-NOX→$O_2^{\cdot-}$ →H_2O_2→Ca^{2+}) in maize seedling leaves and there were cross-talks between Put, $O_2^{\cdot-}$ originated from PM-NOX and Ca^{2+} in the transduction pathway **(**Fig. 5).

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No.31271627) and Science and Technology Project of Henan Province (Grant No.192102110135).

References

Avramova, V., H. AbdElgawad, Z. Zhang, B. Fotschki, R. Casadevall, L. Vergauwen, D. Knapen, E. Taleisnik, Y. Guisez, H. Asard and G.T.S. Beemster, 2015. Drought induces distinct growth response, protection, and recovery mechanisms in the maize leaf growth zone. *Plant Physiol*., 169: 1382–1396

Blume, B., T. Nürnberger, N. Nass and D. Scheel, 2000. Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. *Plant Cell*, 12: 1425–1440

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binging. *Anal. Biochem*., 72: 248–254

Butt, A., N. Ahmed, M. Mubin, I. Khaliq and D.A. Lighfoot, 2015. Effect of PEG and manniyol induced water stress on regeneration wheat (*Triticum aestivum* L.). *Pak. J. Agric. Sci*., 52: 1025–1033

Chen, W.P. and P.H. Li, 2001. Chilling-induced Ca^{2+} overload enhances production of active oxygen species in maize (*Zea mays* L.) cultured cells: the effects of abscisic acid treatment. *Plant Cell Environ*., 24: 791–800 apprised

Cona, A., F. Cenci, M. Cervelli, R. Federico, P. Mariottini, S. Moreno and R. Angelini, 2003. Polymine oxidase, a hydrogen peroxide-producing enzyme, is up-regulated by light and down- regulated by auxin in the outer tissues of the maize mesocotyl. *Plant Physiol*., 131: 803–813

Du, H.Y., D.X. Liu, G.T. Liu, H.P. Liu and R. Kurtenbach, 2018. Relationship between polyamines and anaerobic respiration of wheat seedling root under water-logging stress. *Russ. J. Plant Physiol*., 65: 874–881

Du, H.Y., X.G. Zhou, G.T. Liu, H.P. Liu and R. Kurtenbach, 2017. Relationship between seed quality and changes in conjugated polyamine in plasma membrane purified from wheat embryos during grain ripening. *Russ. J. Plant Physiol*., 64: 325–332

Du, H.Y., X.G. Zhou, Q.H. Yang, H.P. Liu and R. Kurtenbach, 2015. Changes in H^+- ATPase activity and conjugated polyamine contents in plasma membrane purified from developing wheat embryos under short-time drought stress. *Plant Growth Regul*., 75: 1–10

Guan, L.M., J. Zhao and J.G. Scandalios, 2000. Cis-elements and trans-factors that regulate expression of the maize Cat I antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. *Plant J*., 22: 87–95

Guo, J., S. Wang, X. Yu, R. Dong, Y. Li, X. Mei and Y. Shen, 2018. Polyamines regulate strawberry fruit ripening by ABA, IAA and Ethylene. *Plant Physiol*., 177: 339–351

Gupta, B., K. Gupta and D.N. Sengupta, 2012. Spermidine-mediated in vitro phosphorylation of transcriptional regulator OSBZ8 by SNF1-type serine/threonine protein kinase SAPK4 homolog in indica rice. *Acta Physiol. Plantarum*, 34: 1321–1336

Hansen, H. and K. Grossmann, 2000. Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. *Plant Physiol*., 124: 1437–1448

Hao, F., X. Wang and J. Chen, 2006. Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. *Plant Sci*., 170: 151–158

Jiang, M. and J. Zhang, 2003. Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisis acid-induced antioxidant defence in leaves of maize seedlings. *Plant Cell Environ*., 26: 929–939

Jiang, M. and J. Zhang, 2002. Involvement of plasma membrane NADPH oxidase in abscisic acid and water stress-induced antioxidant defense in leaves of maize seedlings. *Planta*, 215: 1022–1030

Kreslavski, V.D., D.A. Los, S.I. Allakhverdiev and VI.V. Kuznetsov, 2012. Signaling role of reactive oxygen species in plants under stress. *Russ. J. Plant Physiol*., 59: 141–154

Larkindale, J. and M.R. Knight, 2002. Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene and salicylic acid. *Plant Physiol*., 128: 682–695

Liu, H.P., B.J. Yu, W.H. Zhang and Y.L. Liu, 2005a. Effect of osmotic stress on the activity of H^+-ATPase and the levels of covalently and noncovalently conjugated polyamines in plasma membrane from wheat seedling roots. *Plant Sci*., 168: 1599–1607

Liu, J., M.Y. Jiang, Y.F. Zhou and Y.L. Liu, 2005b. Production of polyamines is enhanced by endogenous abscisic acid in maize seedlings subjected to salt stress. *J. Integr. Plant Biol*., 47: 1326–1334

Liu, K., H.H. Fu, Q.X. Bei and S. Luan, 2000. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. *Plant Physiol*., 124: 1315–1325

Montilla-Bascón, G., D. Rubiales, K.H. Hebelstrup, J. Mandon, F.J.M. Harren, S.M. Cristescu, L.A.J. Mur and E. Prats, 2017. Reduced nitric oxide levels during drought stress promote drought tolerance in barley and is associated with elevated polyamine biosynthesis. *Sci. Rep.*, 7: 1–15

Murphy, T.M. and C.K. Auh, 1996. The superoxide synthases of plasma membrane preparations from cultured rose cells. *Plant Physiol*., 110: 621–629

Muruta, Y., Z.M. Pei, I.C. Mori and J. Schroeder, 2001. Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires cytosolic NAD (P) H and is differentially disrupted upstream and dounstream of reactive oxygen species production in abi 1-1 and abi 2-1 protein phosphatase 2C mutants. *Plant Cell*, 13: 2513–2523

Pál, M., I. Majláth, E. Németh, K.Á. Hamow, G. Szalai, S. Rudnóy, G. Balassa and T. Janda, 2018. The effects of putrescine are partly overlapping with osmotic stress processes in wheat. *Plant Sci*., 268: 67–76

Pei, Z.M., Y. Murata, G. Benning, S. Thomine, B. Klüsener, G.J. Allen, E. Grill and J.I. Schroeder, 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. *Nature*, 406: 731–734

Sagi, M. and R. Fluhr, 2001. Superoxide production by plant homo-logues of the gp91 phox NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. *Plant Physiol*., 126: 1281–1290

Wang, P.C., Y.Y. Du, G.Y. An, Y. Zhou, C. Miao and C.P. Song, 2006. Analysis of global expression profiles of *Arabidopsis* genes under abscisic acid and H_2O_2 applications. *J. Integr. Plant Biol*., 48: 62–74

Yang, T. and B.W. Poovaiah, 2002. Hydrogen peroxide homeostasis activation of plant catalase by calcium/calmoduin. *Proc. Natl. Acad. Sci. U.S.A.*, 99: 4097–4102

Zhang, S.H., B.Z. Hou, L. Chai, A.Z. Yang, X.Y. Yu and Y.Y. Shen, 2017. Sigma factor FaSigE positively regulates strawberry fruit ripening by ABA. *Plant Growth Regul*., 83: 417–427

Zhu, J.K., 2016. Abiotic stress signaling and responses in plants. *Cell*, 167: 313–324

Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol*., 53: 247–273

[Received 29 Jan 2019; Accepted 20 Feb 2019; Published (online) 26 Apr 2019]