ROLE OF PHOSPHOLIPASE D IN ABSCISIC ACID AND SALT STRESS SIGNALING IN REGULATION OF ANTIOXIDANT ENZYMES IN *ARABIDOPSIS THALIANA*

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Abscisic acid (ABA) is a plant hormone is involved in plant abiotic stress tolerance. ABA action in cells is mediated by various signaling events where signaling phospholipids are implicated. Phospholipase D (PLD), an enzyme that generates a signaling phospholipid phosphatidic acid (PA), plays an important role in ABA signaling. ABA was observed to stimulate rapidly the activity of PLDα1 isoform in epidermal protoplasts of Arabidopsis thaliana leaves leading to accumulation of phosphatidic acid during the short period of time. Activation of reactive oxygen species (ROS) accumulation by this system leads to stomatal closure [1] . It was also found that PLDα1 functions cooperatively with other PLD isoform, PLDδ, during stomatal closure and PA formation evoked by ABA [2] . PLD and PA are also involved in salt stress signaling in plants. Salt excess was shown to rapidly stimulate PA accumulation in Arabidopsis thaliana leaves. This response is significantly reduced in PLDα1 and PLDδ knockout mutants, especially in double mutants [3] . PLD activation by salt stress was blocked in PLDα1 mutants suggesting its unique role in salt stress signaling [4] . However, the role of PLDα1 and PLDδ in regulation of oxidative balance systems particularly in early stages of plant responses to salt stress and abscisic acid has not been investigated.

In order to analyze the contribution of PLDα1 and PLDδ in PA accumulation in response to abscisic acid and salt stress in vivo, leaf discs of intact four-weeks old Arabidopsis thaliana plants and knockout mutants of PLDα1 (pldα1) and PLDδ (pldδ) isoforms were incubated with fluorescent PLD substrate NBD-phosphatidylcholine and then subjected to physiological concentrations of ABA (50 μM) and salt stress (NaCl, 200 mM) for 40 min. Fluorescent PLD lipid product were extracted and separated by thin-layer chromatography and analyzed by fluorescent scanner. Activity of antioxidant enzymes after 20 h of salt stress and ABA action – catalases and guaiacol peroxidases – was determined biochemically and quantified spectrophotometrically according to standard methodology.

The results obtained indicate that ABA sharply elevates PA production in Arabidopsis leaf discs. Its level is reminiscent to that found in previous papers [5, 6] . However, in pldδ, and, specially, in pldα1 mutants, the PA accumulation induced by ABA was dramatically reduced (Fig.1). This supports previous data where PLDα1 [1] , in comparison to PLDδ [5] , contributes ostly to PA formation in response to ABA. In addition, partial reduction in PA accumulation observed in PLD mutants (Fig. 1) suggests a role of other PLD isoforms in PA production in response to ABA. The results of the present study indicate that salt stress also activates PA accumulation in Arabidopsis leaves that was reduced in above mentioned mutants supporting previously obtained data [3] .

Investigation of the association of PLD with antioxidant systems is necessary for the understanding of the full picture of modulation of ROS balance by phospholipases. It was observed that the activity of guaiacol peroxidase in response to salt stress and ABA was increased in PLDδ mutants but decreased in PLDα1 mutants, in comparison to intact plants. On the other hand, catalase activity was increased by ABA and, especially, in response to salt stress in wild-type plants, but decreased in pldδ and, particularly, in pldα1 mutants. The results suggest the important role of PLDα1 in modulation of ROS balance by catalases in plant responses to osmotic stress. PLDδs role in these responses is less important as is clearly seen with ABA treatment.

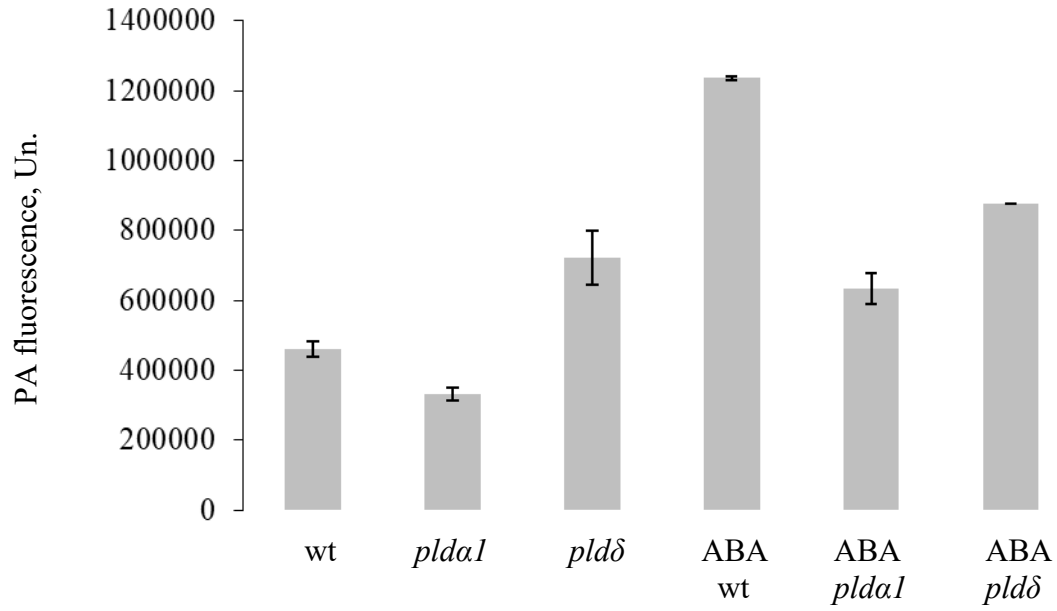

Fig. 1 Role of phospholipase D in PA accumulation in *Arabidopsis thaliana* leaves in response to abscisic acid and salt stress (wt – wild-type plants)

Taken together, the data obtained indicate that modulation of PLD by the above-mentioned stimuli represents an important mechanism of restoration and maintenance of ROS balance inprimarystagesofABAandsaltstressresponses. ROS accumulation stimulated by PLDα1-produced PA could further promote the activation of antioxidant enzymes by substrate activation for further suppression of the ABA-induced ROS burst. Alternatively, PLDα1-produced PA in response to ABA could be a second messenger of regulatory pathways that lead to catalase activation playing a role in metabolic adaptation to future stress-induced ROS accumulation. On the other hand, the role of PLDδ isoformin these responses is minor since this enzyme was found to be a ROS target in plants.

This work was supported by the grants №2.1.10.32-21, 2.1.10.32-22.

1. Zhang Y., Zhu H., Zhang Q., Li M., Yan M., Wang R., Wang L., Welti R., Zhang W., Wang X. Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis* // Plant Cell.–2009. – 21, №8. – P. 2357–2377.

2. Uraji M., Katagiri T., Okuma E., Ye W., Hossain M.A., Masuda C., Miura A., Nakamura Y., Mori I.C., Shinozaki K., Murata Y. Cooperative function of PLDδ and PLDα1 in abscisic acid-induced stomatal closure in Arabidopsis // Plant Physiol. – 2012. – 159, №1. – P. 450–460.

3. Bargmann B.O.R., Laxalt A.M., Riet B.t., van Schooten B., Merquiol E., Testerink C., Haring M.A., Bartels D., Munnik T. Multiple PLDs required for high salinity and water deficit tolerance in plants // Plant Cell Physiol. – 2009. – 50, №1. – P. 78–89.

4. Yu L., Nie J., Cao C., Jin Y., Yan M., Wang F., Liu J., Xiao Y., Liang Y., Zhang W. Phosphatidic acid mediates salt stress response by regulation of MPK6 in Arabidopsis thaliana // New Phytol. – 2010. – 188, №3. – P. 762–773.

5. Guo L., Devaiah S.P., Narasimhan R., Pan X., Zhang Y., Zhang W., Wang X. Cytosolic Glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the Arabidopsis response to stress // Plant Cell. – 2012. – 24, №5. – P. 2200–2212.

6. Guo L., Mishra G., Markham J.E., Li M., Tawfall A., Welti R., Wang X. Connections between sphingosine kinase and phospholipase D in the abscisic acid signaling pathway in *Arabidopsis* // JBC. – 2012. – 287, №11. – P. 8286–8296.