ROLE OF SPECIFIC ISOFORMS OF PHOSPHOLIPASES IN JASMONIC ACID ACTION IN PLANTS

Kolesnikov Ya.S., Kretynin S.V., Kravets V.S.
V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry
of the National Academy of Sciences of Ukraine, kolesnikov@bpci.kiev.ua

Jasmonic acid (JA) is a plant hormone that plays a crucial role in various aspects of growth and developmental, and especially in stress tolerance. Role of lipid signaling in jasmonic acid action is actively studied in plants. Participation of phospholipase D (PLD) and phosphoinositide-specific phospholipase C (PI-PLC) as lipid signaling enzymes was proposed forjasmonic acid action.Rapid activation of PLD by JA was found in potato stolons [1], whereas its activation during the longer time points in other plants was shown to be independent on its protein level [2-4]. Rapid activation of PI-PLC [1-4] was also found in plants. However, roles of specific isoforms of the mentioned enzymes in different JA responses in plants have not been investigated.

In order tostudy the role of specific PLD and PI-PLC in jasmonic acid action, the effect of the hormone in regulation of growth responses was assessed in various single and double knockout mutants of their isoforms (*pld* and *plc*). It was found that the reduction of hypocotyl and root growth induced by high concentration of jasmonic acid in single PLD mutantswas the same as in wild-type plants. However, the reduction of hypocotyls growth was insensitive to high levels of jasmonic acid in *pldα3*/*pldγ1*, *pldζ1*/*pldζ2b*, and *plc9* mutants.Weak reduction of JA effect was found in hypocotyls of *plc1* and *plc8*, and in roots of *plc2* mutants. In order to investigate the role of PLD isoforms in cellular sensitivity to JA, JA-insensitive mutants (*pldα3*/*pldγ1*, *pldζ1*/*pldζ2b*) were tested for hypocotyl growth inhibition induced by different JA concentrations. It was found that hypocotyls sensitivity to JA was partially reduced in the mutants at all JA levels tested;therestoration of inhibitory action of JA in the mutants needed higher concentrations of JA and the level of hypocotyls growth reductionwas different in the mutants (Fig. 1). This suggests that simultaneous activity of PLDα3 and PLDγ1 isoforms, and, especially, PLDζ1 and PLDζ2 isoforms is the regulator of JA sensitivity in plant cells. Analysis of partial hypocotyl growth inhibition of single and double mutants of PLD genes under low jasmonicacid concentrations indicates that this response is insensitive to JA in mutants of PLDα1, PLDα3, PLDβ1, PLDβ2, PLDε, and PLDζ2 isoforms suggesting their roles in these JA-regulated growth responses.

Role of specific isoforms of phospholipases was studied in the regulation of plant tolerance to heavy metal stress. In wild-type plants, low concentrations of JA partially restored hypocotyl growth inhibition induced by heavy metal stress (copper and cadmium excess). However, mutants of PLDβ1 and PLDγ1 isoforms were insensitive to this JA response. At high concentrations of the hormone, the development of heavy metal tolerance was not observed. Analysis of reactive oxygen species production suggests that JA-induced reduction of superoxide radical formation initially stimulated by copper and cadmium excess is blocked in mutants of PLDβ1 and PLDβ2 isoforms, in comparison to other PLD mutants. In *plc9* mutants, the accumulation of superoxide radical was significantly increased in response to low level of JA in copper/cadmium stress conditions suggesting the negative role of PI-PLC9 in heavy metal stress tolerance. Hyposensitivity to JA in restoration of root growth observed in this mutant supports this conclusion.Analysis of hypocotyl growth restoration induced by low concentrations of JA suggests that PLDα1, PLDα2, PLDβ1, PLDβ2 and PLDγ1 play a positive role in JA-induced copper and cadmium stress tolerance.

For deeper investigations of the role of phospholipases in JA-induced heavy metal stress tolerance, endogenous levels of heavy metals and their natural chelators (free proline and phenolics) were analyzed in intact and mutated plants. It was found that in comparison to high concentrations, low levels of JA in leaves of wild-type plants induced an additional increase of free proline levels stimulated by heavy metals, and restored free phenolics levels that were reduced by the stressors. However, this response was not observed in PLDβ1 and PLDγ1 mutants. Results of histochemical localization of heavy metals in *Arabidopsis* hypocotyls indicates that JA-induced reduction in copper and cadmium localization was not observed in mutants of PLDβ1 and PLDγ1.

In contrast, the development ofJA-induced heavy metal stress tolerance was weakenedin*plc9* mutant. Therefore, PLDβ1, PLDγ1, and PI-PLC9 can be involved in most studied jasmonic acid responses in *Arabidopsis*.

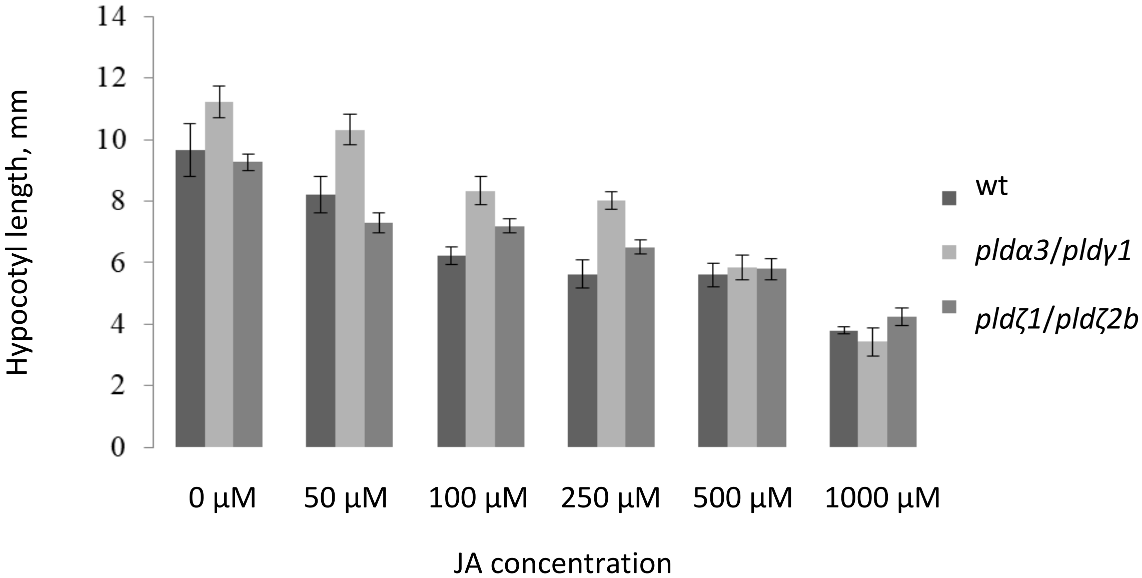

Fig. 1 Role of PLD in hypocotyl growth sensitivity to JA with application of wild-type (wt) and transgenic plants, and different hormone concentrations

Analysis *in silico* and literature data support the role of PLD isoforms in jasmonic acid action in plants. Prediction of protein-protein interaction by "AraPPINet" *Arabidopsis* database suggests the possible interaction of PLDα1 with JAZ1, JAZ4, JAZ5, and JAZ10 proteins – negative transcription regulators of JA signaling. Moreover, rice database "RicePPINet" predicts the interaction of rice PLDα1 with COI – a jasmonic acid receptor. Activation of oleate-dependent PLD and phosphoinositide-dependent PLD in *B. napus* leaves [2], and also reduction of JA-sensitive gene expression in knockout mutants of *Arabidopsis* PLDβ1 [5] support the role of PLDδ and PLDβ/γ isoforms in primary stages of jasmonic acid action in plants.

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

1. Cenzano A., Cantoro R., Racagni G., Los Santos-Briones C., Hernández-Sotomayor T., Abdala G. Phospholipid and phospholipase changes by jasmonic acid during stolon to tuber transition of potato // Plant Growth Regul. – 2008. – 56, №3. – P. 307–316.

2. Profotová B., Burketová L., Novotná Z., Martinec J., Valentová O. Involvement of phospholipases C and D in early response to SAR and ISR inducers in *Brassica napus* plants // Plant Physiol. Biochem. – 2006. – 44, №2–3. – P. 143–151.

3. Altúzar-Molina A.R., Muñoz-Sánchez J.A., Vázquez-Flota F., Monforte-González M., Racagni-Di Palma G., Hernández-Sotomayor S.M.T. Phospholipidic signaling and vanillin production in response to salicylic acid and methyl jasmonate in *Capsicum chinense* J. cells // Plant Physiol. Biochem.– 2011. – 49, №2. – P. 151–158.

4. Muñoz-Sánchez J.A., Altúzar-Molina A., Hérnandez-Sotomayor S.M.T. Phospholipase signaling is modified differentially by phytoregulators in *Capsicum chinense* J. cells // Plant Signal. Behav. – 2012. – 7, №9. – P. 1103–1105.

5. Zhao J., Devaiah S.P., Wang C., Li M., Welti R., Wang X. Arabidopsis phospholipase Dβ1 modulates defense responses to bacterial and fungal pathogens // New Phytol. – 2013. – 199, №1. – P. 228–240.