ABSTRACT

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Title of Doctoral Thesis **The role of cytoskeleton and phospholipids in signaling during plant defense response**

Upon abiotic and biotic cues, plant surveillance system simultaneously triggers a variety of signaling events to develop an effective defense response within a few hours. On the molecular level, signaling period is the chain of many consecutive and fast biochemical reactions. Tracking of one particular signaling event e.g. during plant-pathogen interactions is quite difficult task.
Presented work is focused on several components of this intricate defense network; namely on the role of salicylic acid, phospholipid signaling and cytoskeleton dynamics and their mutual interaction coupled with downstream changes of plant metabolom.
Live imaging can help to elucidate localization and dynamics of particular, fluorescently labeled proteins during defense response. Plenty of important events occurring in plant-
-pathogen interactions take place in plasma membrane: where pathogen perception, exocytosis, endocytosis occure and ion channels and carriers are located. Also phosphatidic acid (PA) emerges in plasma membrane by the action of phospholipase D. Phospholipase Dδ (PLDδ) is supposed to be directly connected with cytoskeleton. Signaling pathway triggered by second messenger, PA participates in the changes of cytoskeleton dynamics through cytoskeleton binding proteins and interacts with many others pathways, leading to successful defense.
This work tries to address localization of PLDδ beneath plasma membrane and its connection with microtubules employing live imaging methods. Expression of reporter gene GFP-PLDδ in Arabidopsis thaliana, revealed very dynamic localization of PLDδ on plasma membrane in dot patterns as well as rare, steady filamentous structures, sometimes following trajectory of microtubules and probably occupying sites underneath lipid rafts in plasma membrane. Lipid rafts are well known for their signaling potencial.
Importance of PLDδ for stabilization of microtubules was revealed by monitoring of microtubules regeneration in plants lacking PLDδ during salt stress. PLDδ stabilizes microtubules on the plasma membrane through direct or indirect link.
Phospholipid signaling obviously influences all levels of defense, 1-butanol eliminates PA from cells and influences downstream reaction during phospholipid signaling leading to the decrease of secondary metabolites production.
Plant hormone salicylic acid (SA) is involved in the course of defense against both biotic and abiotic stresses. Live imaging uncovered rapid decrease of actin filament amount after treatment with salicylic acid, but phosphatidic acid was able to prevent this effect. This phenomenon could be explained by correct succession of changes in actin dynamics or other signals in general during defense response. SA or short, depolymerized actin filaments could activate PLD which, in turn could cause polymerization of filaments by production of PA during defense response. Based on our results and other published data we can speculate that accurate alternation of depolymerization and polymerization of actin filaments could be necessary for successful defense response of plants