Abstract

Phytohormones are small molecules that regulate almost all aspects of plant life including defence reactions. Plant defence and immunity are mainly regulated by two hormones – salicylic acid (SA) and jasmonic acid (JA). Other hormones such as auxins, cytokinins brassinosteroids or gibberellins modulate plant immunity to lesser extent. It has been described that plant pathogens are able to interfere with plant hormone signalling to overcome plant defence. Some pathogens are able to produce plant hormones themselves. This thesis is focused on plant hormone signalling involved in plant immunity both from the plant side and pathogen side and possible hormonal crosstalk in this interaction.

The first part is focused on salicylic acid signalling connected with plant actin cytoskeleton roles in plant immunity. It has been described that desintegration of actin cytoskeleton leads to increased plant susceptibility to bacteria. However, it has been also shown that pharmacological desintegration of actin filaments induces transcription of salicylic acid responsive genes *PR1* (*Pathogenesis related 1*) and *ICS1* (*Isochorismate synthase 1*). In this thesis we have investigated this inconsistency using actin depolymerizing drugs latrunculin B, cytochalasin E and jasplakinolide and two different pathosystems: *Arabidopsis thaliana* x *Pseudomonas syringae* pv. *tomato DC 3000* and *Brassica napus* x *Leptosphaeria maculans*. We treated the *A. thaliana* plants with the cytoskeletal drugs and first analyzed phytohormone profile and defence gene transcription. Specific induction of salicylic acid production and salicylic acid marker genes (*ICS1*, *ICS2* (*Isochorismate synthase 2*)*, PR1*) was observed. Subsequently we infected the drug-pretreated *A. thaliana* or *B. napus* plants with corresponding pathogens which eventually resulted in increased resistance in both pathosystems. This phenomenon is salicylic acid dependent. It also depends on treatment timing, infection duration and specific pathosystem. Since actin dynamics is vital for correct cellular trafficking and membrane formation, we investigated deeper into this mechanism and focused on the role of phospholipids. We used *A. thaliana* mutant in phosphatidylinositol-4-kinase β1 and β2 (PI4Kβ1β2), which is known to be an SA overaccumulator, and a set of mutants affected in salicylic acid signalling. First, we tested callose deposition which is a defence mechanism requiring functional trafficking machinery. We observed that treatment with cytoskeletal drugs triggers callose deposition via the activity of callose synthase 12 and is SA

independent since it was observed even in mutants with blocked SA accumulation. Defence gene transcription and SA accumulation were blocked in the SA-signalling impaired mutants and reverted or partly reverted in triple mutants impaired in SA-signalling and *pi4kβ1/β2*. Altogether the results show that relationship between the actin cytoskeleton and plant immunity is more complex than generally assumed. Salicylic acid seems to be a major regulator of the onset of actin-depolymerization- triggered defence. Correct phospholipid signalling also seems to be important in this process.

Since we have focused on the role of salicylic acid we have established a collection of *A. thaliana* mutants that are affected in SA production, accumulation or signalling. Several of these mutants show affected resistance to pathogens. We have extensively chracaterized this mutant collection in terms of growth, cultivation condition dependancy and SA production to create a tool for future studies dealing with plant immunity. Our characterization clearly shows correlation between SA overaccumulation and rosette growth retardation.

Second part of the thesis is focused on plant pathogens infection strategies affecting hormone signalling in plants. Pathogens secrete a variety of molecules that manipulate host hormone signalling. *Leptosphaeria maculans* is an important fungal pathogen of the brassica crops. We investigated the impact *of L. maculans* effector AvrLm4-7 on virulence and host defence. We performed inoculation assay with *L. maculans* isolates possesing functional and non-functional alelle of *AvrLm4-7* that revealed that effector AvrLm4-7 contributes significantly to *L. maculans* virulence. Further we analyzed host defence reactions – defence gene transcription, phytohormone profile and ROS burst. Infection with *AvrLm4-7* containing isolate reduced SA-dependent defence response in *B. napus* plants. ROS burst was also supressed. The results show that effector AvrLm4-7 increases virulence of *L. maculans* by suppressing SA related defence mechanisms.

Since there is increasing evidence that pathogens are able to produce phytohormones to manipulate host plant defence, we tested whether *L. maculans* posseses such activity. We tested phytohormone production in *L. maculans* and identified a variety of auxins, particularly the bioactive form indole-3-acetic acid (IAA). The IAA production can be stimulated by supplementing *L. maculans* culture with biosynthetic precursors tryptophan and tryptamine. There are orthologues of several known biosynthetic genes in *L. maculans* genome. The precursors induce transcription of several of those genes; mainly *LmTAM1*, *LmIPDC2* and *LmNIT1*. Transcription of *LmIPDC1*, *LmIaaM3* and

LmIaaM5 was only slightly induced. Exogenous addition of highly concentrated auxin inhibited growth of *L. maculans* while no stimulatory effect was observed even upon low concentration of IAA. Auxin profile of infected plant showed only minor changes; ednogenous concentration of indole-3-acetonitrile increased upon infection with *L. maculans*. The results show that *L. maculans* is able to produce high concentration of bioactive auxin but with no significant role in virulence. Auxin might function as a regulator in *L. maculans* itself.

This thesis focuses on particular aspects of plant signalling mainly connected with salicylic acid and other hormones to lesser extent and provides new insight into phytohormone signalling during infection process.