PS 1-1

THE ROLE OF PHOSPHOLIPID SIGNALLING IN THE DEFENCE OF PLANTS AGAINST PATHOGENS

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Plants are sessile organisms and need to respond quickly to environmental stresses. The innate immune system of plants allows sensing the presence of pathogens, resulting in a defence response that prevents infection. Elicitors produced by the pathogen are specifically recognized by host-encoded receptor-like resistance (R) proteins. The R_protein Cf-4 from tomato is responsible for the recognition of the Avr4 protein elicitor produced by the leaf mold fungus *Cladosporium fulvum*. The receptor-like part of the Cf-4 protein, consisting of leucine rich repeats, is on the outside of the cell (where it can mediate recognition of Avr4), whereas the remainder, a short C-terminal stretch of amino acids, is inside the cell (where it is thought to initiate a defence response). Our group studies the early signalling processes that are initiated by Cf-4 inside the cell after Avr4 recognition. It was found that a very early response of Cf-4-expressing cells (within five minutes after exposure to Avr4) is the activation of two enzymes involved in phospholipid signalling; phospholipase-C (PLC) and diacylglycerol kinase (DGK). As a result of this activation, phosphatidic acid (PA) rapidly accumulates which could act as a second messenger able to initiate responses further downstream. We revealed that PLC and DGK are required for an effective defence response to *C. fulvum.* Accordingly, we aim to identify and characterise the *PLC* and *DGK* gene families of tomato. The identified genes will be individually silenced or over-expressed and the effect of such manipulation on disease resistance will be studied. Once the key members of the *PLC* and *DGK* families playing a role in disease resistance have been identified, we will study both the mechanism by which the encoded enzymes are activated and their role in phospholipid signalling upon pathogen recognition.

PS 1-2

TOWARDS REGULATORY COMPONENTS OF FLS2 ENDOCYTOSIS AND SIGNALING

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The membrane-resident leucine-rich-repeat receptor kinase FLS2 is responsible for the perception of bacterial flagellin. Upon physical interaction with flg22, the elicitor active epitope of flagellin, FLS2 relocates from the membrane into vesicles *via* an endocytic process. Induced FLS2 endocytosis seems to play a role in flg22 signalling.

To identify regulatory components of FLS2 endocytosis, we performed a standard yeast-two-hybrid screen using the FLS2 cytoplasmic part, which revealed a PP2A-type phosphatase as interacting candidate. A role for phosphatases in flg22 signalling is supported by flg22-triggered transcriptional up-regulation of genes encoding various phosphatases. Single knock-out lines of selected phosphatases have been investigated in *Arabidopsis*. None of them exhibited alterations in flg22 responses, which implies redundancies. However, simultaneous inhibition of PP2A activities by chemical interference with Cantharidin affected FLS2 endocytosis as well as flg22-mediated response. Currently, we are extending our search for FLS2 endocytic regulators by a yeast split-ubiquitin based screen using full-length FLS2. First candidates have been isolated and are being investigated.

In a second approach we are using a high throughput genetic screen for mutant lines impaired in flg22-triggered sensitivity. First screens were performed with an *Arabidopsis thaliana* collection comprised of 172 ecotypes. We identified four ecotypes affected in flg22 responses. Their further characterization revealed two ecotypes with strong flg22 insensitivity, whereas the others showed intermediate phenotypes. Further analysis includes transformation with FLS2-GFP to monitor potential alterations in FLS2 endocytosis by confocal microscopy.

PS 1-3

MOLECULAR AND GENETIC ANALYSIS OF RESISTANCE IN *MEDICAGO TRUNCATULA* TO *RHIZOCTONIA SOLANI*: AN INTRACTABLE, BROAD HOST RANGE, ROOT PATHOGEN.

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Necrotrophic pathogens for which there is no usable source of natural resistance impose major constraints on agricultural production. For these pathogens, conventional breeding for resistance is difficult due to a number of loci contributing small quantitative differences in susceptibility. This work focuses on dissecting the molecular and genetic mechanisms involved in partial resistance to *Rhizoctonia solani*. *R. solani* is a soil borne fungus causing root and crown rot or bare patch of a wide range of legumes, cereals and brassicas with individual isolates causing disease on a wide range of hosts. This broad host range (polyphagous habit) suggests that pathogenesis mechanisms employed by *R. solani*, and the plant defences against them, are likely to be largely different to those employed during more specific (mono or oligophagous) biotrophic and necrotrophic (e.g. *Fusarium oxysporum*) interactions for which more information is available. To begin investigating the mechanisms involved in resistance to broad host range necrotrophs a collection of *Medicago truncatula* genotypes and mutants has been screened for resistance and susceptibility to several *R. solani* isolates. As in many other crops, no complete resistance to *R. solani* was found in *M. truncatula*. However, the identification of partially resistant and highly susceptible lines allows the study of mechanisms involved in increased performance in the presence of *R. solani*. The observation of increased susceptibility in the ethylene insensitive mutant, *sickle*, suggests the importance of this pathway in partial resistance. Progress on the genetic and molecular characterisation of other partially resistant and highly susceptible genotypes of *M. truncatula* will be presented.