

101 The role of phospholipid signaling in plant defence

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Plants are sessile organisms and need to respond quickly to attacking pathogens. The innate immune system of plants allows recognition of pathogens, resulting in a defense response that prevents infection. The receptor-like protein (RLP) Cf-4 of tomato mediates recognition of the Avr4 effector of the leaf mold fungus *Cladosporium fulvum*, thereby inducing immunity. We study the early signaling processes that are initiated by Cf-4 after Avr4 perception. One of the very early responses is the activation of two enzymes involved in phospholipid signaling; phospholipase-C (PLC) and diacylglycerol kinase (DGK). As a result of this, phosphatidic acid (PA) rapidly accumulates. This phospholipid could act as a second messenger able to trigger downstream responses.

We have identified the PLC and DGK gene families of tomato and we showed that PLCs and DGKs are required for an effective defense response to *C. fulvum*. We are currently silencing and over-expressing the individual genes and studying the effect on effector recognition and disease resistance. We found that resistance mediated by the RLP Ve1 (to *Verticillium dahliae*) and by Pto/Prf (kinase/NB-LRR; to *Pseudomonas syringae*) is also affected by silencing PLC genes in tomato. In addition, we are studying the mechanism by which the enzymes become activated and are characterizing their substrate specificity. For two PLC family members we have been able to show that they are able to hydrolyze phosphatidylinositol (PI) into diacyl glycerol (DAG). It is anticipated that the generated DAG is subsequently phosphorylated by DGKs, resulting in the accumulation of PA.

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103 Functional Analysis of the Symbiosis Receptor Kinase (SYMRK)

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The plant Symbiosis Receptor Kinase (SYMRK) is required at the early stages of symbiosis between plant roots and arbuscular mycorrhizal fungi, between actinorhiza hosts and *Frankia* bacteria and between legumes and rhizobia (Stracke et al., 2002; Markmann et al., 2008). To understand SYMRK mediated signalling, the SYMRK localization and SYMRK interactome are under investigation. In tobacco epidermal as well as in Lotus root cells, SYMRK fused to fluorescent proteins localized to the plasma membrane. Tagged versions of SYMRK, which complement *symrk L. japonicus* mutants, are used to study the spatiotemporal dynamics of SYMRK. An antibody raised against the extracellular domain of SYMRK recognizes a band corresponding to the full-length protein and a second band representing the N-glycosylated extracellular domain suggesting targeted cleavage of the SYMRK ectodomain in roots. Moreover, several SYMRK interactors were isolated through a yeast split-ubiquitin screen. The confirmation of SYMRK-protein interactions as well as their functional significance is evaluated in planta. The analysis of the SYMRK complex and SYMRK dynamics will provide insight into symbiotic signal transduction and plant receptor kinase function.

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102 Identification and Development of Sex Specific Markers in Date Palm

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Date palm is a dioecious plant which means that male and female plants are separated. However, no morphological method is known at the present for sex identification in date palm at an early stage. A molecular technique to find sex specific DNA markers could be the best way to eliminate non-productive male trees in the nursery plantation on a field scale. The objective of this study was to segregate analysis of ISSR and RAPD products to identify markers for sex determination in Qatari Date palm cultivars at an early stage nursery.

Sex-pooled DNA samples were prepared for bulk segregate analysis by mixing equal quantities of the DNA of three male individuals (male bulk) and three female individuals (female bulk) separately. Twenty primers and thirty RAPD primers were screened using sex bulk (Male and female). ISSR and RAPD primers that showed polymorphism between male and female bulk DNA samples were repeated in individual male and female DNA samples and specific bands were observed either for male and female to confirm the screening.

Among the twenty eight ISSR and thirty RAPD primers tested, only one ISSR and two RAPD primers successfully produced clear bands. The ISSR markers linked with sex in date palm and could be a starting point for further analysis to design specific PCR primers that can be used for sex identification.

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104 Comparative Analysis of Early Events in Arabidopsis Brassinosteroid Signal Transduction

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Brassinosteroids (BRs) are essential plant hormones that regulate multiple aspects of plant growth and development. BRs are perceived at the cell surface by *BRASSINOLIDE INSENSITIVE 1 (BRI1)*, a leucine-rich repeat receptor. The cytoplasmic kinase domains of tomato and Arabidopsis BRI1 are highly conserved (82% identical) and many Ser and Thr residues in Arabidopsis BRI1 occur in the same relative position in tomato BRI1, based on sequence alignment. We used liquid chromatography-tandem mass spectrometry (LC/MS/MS) to identify 8 *in vitro* phosphorylation sites in tomato BRI1 compared to the 11 sites we previously identified in Arabidopsis. Interestingly, five of the tomato sites were conserved in Arabidopsis, but three were not, suggesting significant conservation but also possible differences in BRI1 downstream signaling between the two species. Identification of tomato BRI1 phosphorylation sites has allowed us to initiate a comparative functional analysis of signaling in tomato and Arabidopsis in order to determine inter-species conservation and divergence of receptor mechanisms involved in BR signaling.