

Ascorbate oxidation primes the rice plants for enhanced defence against root-knot nematode *Meloidogyne graminicola* through jasmonate/ethylene activation

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Ascorbic acid (AA) is known to play a vital role in plant growth and detoxification of reactive oxygen species, however little is known about the significance of AA oxidation in plant defence against pathogens. The role of ascorbate oxidation in rice defence against root-knot nematodes, *Meloidogyne graminicola*, was tested with application of AA, ascorbate oxidase (AO), dehydroascorbic acid (DHA), biosynthesis inhibitors and use of mutants. Transcriptome analysis was done on AO treated plants, and hormone measurements were executed to confirm the results. Biochemical analyses were used to study oxidative stress markers, including accumulation of hydrogen peroxide (H₂O₂), malondialdehyde (MDA) and AA/DHA. AO and DHA treated plants are significantly less susceptible to *M. graminicola*, while AA mutants are significantly more susceptible. Transcriptome data, corroborated by hormone measurements, show that ethylene (ET) and jasmonic acid (JA), are activated in rice roots upon ascorbate oxidation, while Methyl-JA, ethephon or AO can complement the susceptibility phenotype of the vitamin C (*vtc*) mutant. Additionally, oxidative stress markers are accumulating in nematode-induced root galls. Our data further demonstrate a novel pathway showing that induced defence by ascorbate oxidation is dependent on a functional JA and ET pathway and leads to primed accumulation of H₂O₂.

The role of ncRNAs in the interaction between root-knot nematode and rice

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In this study, the involvement of non-coding RNAs (ncRNAs) in the infection of rice (*Oryza sativa*) by the root-knot nematode (*Meloidogyne graminicola*) is being investigated. *Meloidogyne graminicola*, is a major economic threat to worldwide rice production. By performing small and total RNA sequencing on healthy roots and galls at 3 days postinfection, we identified 15 differentially expressed miRNAs and thousands of lncRNAs likely to play a role in the infection process. Putative targets for the miRNAs were predicted and cross referenced with the total RNA dataset. The expression profile of differentially expressed targets was validated by RT-qPCR. To ensure the quality of the validation, several candidate reference miRNAs were tested for expression stability and the two stablest transcripts were chosen. Among the predicted targets is a gene encoding ascorbate oxidase, which could play a role in the plant defense response. Furthermore, two miRNAs are putatively involved in a miRNA-decoy-target system. Clustering analysis revealed that hundreds of differentially expressed lncRNAs are clustered together with differentially expressed protein coding genes, suggesting potential *in cis* expression regulation. Furthermore, overexpression lines of four differentially expressed miRNAs are currently tested in infection assays. This research will contribute to a deeper understanding of ncRNA involvement in rice immunity which, in turn, may contribute to new nematode control strategies.

DNA hypomethylation confers enhanced immunity against parasitic nematodes in rice

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Epigenetics plays an important role in the plant response to adverse environmental conditions. A role for DNA hypomethylation has recently been suggested in the pathogenic interaction between bacteria and plants, yet it remains unclear whether this phenomenon reflects a conserved and general plant immunity response. We therefore investigated the role of DNA methylation in the interaction between rice and one of its most damaging pathogens, *Meloidogyne graminicola* (Mg). Global DNA methylation analysis of nematode induced gall tissue at 3 days postinoculation demonstrated general hypomethylation in galls. Treatment of uninfected roots by a pathogen-associated molecular pattern (PAMP) revealed similar hypomethylation, suggesting causal impact on immunity. This was confirmed by a reduced plant susceptibility upon azacytidine treatment. Whole genome bisulfite sequencing of gall tissue 3 dpi revealed that hypomethylation was massively present in the CHH context, while absent for CpG or CHG nucleotide contexts. CHH hypomethylated regions were significantly enriched for gene promoter regions, leading to gene overexpression at 7 dpi, but not at 3 dpi. Finally, the relevance of CHH hypomethylation in plant defence was confirmed in mutants of the RNA-directed DNA methylation pathway (RdDM). We demonstrated that DNA hypomethylation confers enhanced defence in rice towards root-parasitic nematodes and is likely to be part of the basal PAMP-triggered immunity response in plants.

The potato cyst nematode effector RHA1B is a ubiquitin ligase and uses two distinct mechanisms to suppress plant immune signaling

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Plant pathogens, such as bacteria, fungi, oomycetes and nematodes, rely on wide range of virulent effectors delivered into host cells to suppress plant immunity. Although phyto-bacterial effectors have been intensively investigated, little is known about the function of effectors of plant-parasitic nematodes, such as *Globodera pallida*, a cyst nematode responsible for vast losses in the potato and tomato industries. Here, we demonstrate using *in vivo* and *in vitro* ubiquitination assays the potato cyst nematode (*G. pallida*) effector RHA1B is an E3 ubiquitin ligase that employs multiple host plant E2 ubiquitin conjugation enzymes to catalyze ubiquitination. RHA1B was able to suppress effector-triggered immunity (ETI), as manifested by suppression of hypersensitive response (HR) mediated by a broad range of nucleotide-binding leucine-rich repeat (NB-LRR) immune receptors, presumably via E3-dependent degradation of the NB-LRR receptors. RHA1B also blocked the flg22-triggered expression of *Acre31* and *WRKY22*, marker genes of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), but this did not require the E3 activity of RHA1B. Moreover, transgenic potato overexpressing the *RHA1B* transgene exhibited enhanced susceptibility to *G. pallida*. Thus, our data suggest RHA1B facilitates nematode parasitism not only by triggering degradation of NB-LRR immune receptors to block ETI signaling but also by suppressing PTI signaling via a yet unknown E3-independent mechanism.

Discovery of the first pectin methylesterase gene in a plant-parasitic nematode

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The root lesion nematode (RLN) *Pratylenchus penetrans* is considered one of the most damaging plant-parasitic nematode (PPN) species worldwide affecting important agronomic and horticultural crops (e.g. potato, corn). The successful invasion of roots by RLNs is related to their ability to overcome the barrier imposed by the plant cell wall. Like other PPNs, RLNs are equipped with a protrusible stylet that mechanically disrupts the cell wall and through which cell wall-degrading enzymes are secreted to facilitate penetration and migration of the nematode through host roots. Herein, we identified and characterized a pectin methylesterase gene for *P. penetrans*. Sequence analysis confirm the eukaryotic gene structure of *Pp-pme*. Expression of the *Pp-pme* gene was localized in the esophageal glands of *P. penetrans* as determined by *in situ* hybridization. The possible function and activity of the gene were assessed by transient expression of *Pp-pme* in plants of *Nicotiana benthamiana* plants via a Potato Virus X-based vector. PME's have been so far described for plants, fungus, bacteria, and to a restrict number of insects. To our knowledge, this is the first report a PME within the phylum Nematoda.

Investigating early aphid-induced calcium signals and their role in determining aphid-host compatibility

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As hemipteran pests and vectors of many significant plant viruses, aphids pose a substantial and increasing threat to plant health. Despite this, little remains known about the mechanisms by which plants can detect and defend themselves against aphid attack. Cytosolic calcium, a fundamental plant second messenger, has recently been implicated in the interactions between *Arabidopsis thaliana* and the generalist aphid, *Myzus persicae*. Elevations in cytosolic calcium concentrations occur as rapid and local signals within the epidermal and mesophyll tissues during probing by the aphid before it locates its stylet within the phloem for feeding. By using live *in vivo* imaging of early calcium signaling responses to aphid species of different host compatibilities, we are now gaining a unique insight into the key features of these signals and their functions within aphid-plant interactions. These findings will prove fundamental in understanding the colonization ability of different aphid species and the mechanisms underlying aphid-host compatibility.

Aphid elicitors induce plant immunity in a cysteine protease-dependent manner

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PAMP-triggered immunity (PTI) is the first layer of plant defence response to pathogens and pests, and involves the recognition of a conserved molecule (PAMP) of the pathogen by a plant cell surface Pattern Recognition Receptor (PRR). In investigations of aphid-plant interactions, we previously demonstrated that the PRR co-receptor BAK1 is required for the plant defence response induction during aphid feeding and upon application of aphid extract. Here, we present an immunoactivity-led purification strategy to identify aphid PAMPs that trigger PTI in plants using an *Arabidopsis* luciferase reporter. Interestingly, we show that low molecular weight components in aphid-derived fractions can rapidly induce defence pathways in a cysteine protease-dependent manner. The immuno-activity of these fractions elutes as two major peaks from a reverse-phase C₁₈ HPLC column. We have identified several candidate putative PAMPs within these peaks via LC-MS/MS which appear to be previously uncharacterised as elicitors of plant immunity. This study aims to underpin future research on the identification of sources of genetic resistance in crops to aphids and potentially other piercing-sucking insect pests.

Understanding the role of strigolactones in cyst nematode-plant interaction

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Cyst nematodes are obligate, biotrophic parasites of many important agricultural crops that have the fascinating ability to reprogram plant cells and form a unique feeding structure called syncytium. The syncytium functions as a metabolic sink from which feeding nematodes acquire their nutrients for several weeks. Establishment of a successful cyst nematode-plant interaction strongly depends on plant hormone homeostasis. We have recently discovered that strigolactones play distinct roles in the interaction of beet cyst nematode (*Heterodera schachtii*) with *Arabidopsis*. Strigolactone signaling mutant *max2* negatively affects nematode attraction to the roots, whereas both *max2* and the strigolactone biosynthesis mutant *max4* enhance syncytium development. Moreover, the exogenous application of the synthetic strigolactone GR24 expands the zone above the root tip where *H. schachtii* invades the host. We want to dissect the role of strigolactone pathway in the overall susceptibility of *Arabidopsis* to *H. schachtii*. Furthermore, we aim at understanding the molecular mechanisms underlying the effect of strigolactone signaling and responses in nematode attraction, host invasion, and syncytium development.

Effectors of specific C13 peptidases induce host pathogenic programmed cell death - A molecular pathogenic mechanism of *Bursaphelenchus xylophilus*

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The molecular mechanism of the pinewood nematode (*Bursaphelenchus xylophilus*) killing pine trees is unclear. Early histopathological studies observed parenchyma cell vacuolization and vacuoles breakdown resulting in a rapid cell death in pine host at the initial stage of pathogenesis, which similar to VPE-mediated programmed cell death (PCD) in plants. By comparative genome analysis, we found cysteine peptidases C13 family (legumain/VPE/AEP) are significantly abundant in PWN genome. We introduced *BxVPEs* into the *Arabidopsis* *γpe*-null mutant and detected cell death triggered by fungal toxin FB1. The result showed that specific *BxVPEs* could best complement the function of the *Arabidopsis* *γVPE*. Effector properties of *BxVPEs* were determined by hybridization *in situ* and yeast secretion assay. Comparative transcriptome analysis of *Arabidopsis* *γpe*-null mutant, wild-type and *BxVPE* transformed plants identified 59 differentially expressed genes (DEGs), with 26 upregulated and 33 downregulated in WT and *BxVPE* transformed plants, compared to *γpe*-null mutant. Functional annotation showed that these genes are mainly encoding transcription factors, E3 ubiquitin ligases and related proteins, F-box proteins, protein kinases, etc., which involved in