

in five clusteral phylogroups. Virulence assays carried out on rice near isogenic lines carrying defined resistant genes demonstrated a significant difference in genotype by strain interaction.

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Variabilität Protein-kodierender Genombereiche des *Cherry leaf roll virus*

Variability of protein-coding genome regions of Cherry leaf roll virus

Das *Cherry leaf roll virus* (CLRV) der Gattung *Nepovirus* (Comovirinae, Secoviridae) ist weltweit in einer Vielzahl von verschiedenen Wirtspflanzenarten aus 26 Pflanzengattungen, vornehmlich in Gehölzen, verbreitet. Die beiden genomischen einzelsträngigen RNA-Moleküle des CLRV kodieren für Polyproteine, die durch die virale Protease in die funktionellen Proteine gespalten werden. Die Genomvariabilität wurde anhand der RNA1-kodierten Proteine VPg, Protease, RdRP und des RNA2-kodierten Hüllproteins von CLRV-Isolaten aus verschiedenen Wirtspflanzen bestimmt. Auf der Basis von Nukleotid- und Aminosäuresequenzidentitäten differieren die Variabilitätswerte der untersuchten Proteine nur geringfügig bei maximal 22,7 % bzw. 15,1 %. Dagegen zeigte das Verhältnis von synonymen zu nicht-synonymen Nukleotidsubstitutionen, dass insgesamt auf alle untersuchten Protein-kodierenden Genombereiche ein hoher ($dS/dN > 1$), auf die Protease aber der signifikant höchste negative Selektionsdruck wirkt. Dieses lässt vermuten, dass beim CLRV die genetische Evolution der Protease stark eingeschränkt ist und in anderen Protein-kodierenden Genombereichen beispielsweise funktionelle Interaktionen mit wirtsartspezifischen Faktoren eine höhere Variabilität bedingen können.

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Heterologe Expression der viralen Proteinase des *Cherry leaf roll virus* (CLRV)

Heterologous expression of the viral proteinase of Cherry leaf roll virus (CLRV)

Cherry leaf roll virus (CLRV), ein *Nepovirus* der Subgruppe C, gehört zur 2009 eingeführten Familie der Secoviridae (Sanfacon et al., 2009). Das bipartite Genom besteht aus einzelsträngiger RNA, die zwei Polyproteine (P1 und P2) kodiert. P1 beinhaltet charakteristische Domänen für einen Proteinase-Cofaktor (PCo), eine Helikase (Hel), ein genome-linked Protein (VPg), eine Proteinase (Pro) und eine RNA-abhängige Polymerase (Pol). P2 beinhaltet neben einer Region am 5'-Ende, der noch keine Funktion zugeordnet werden konnte, das movement Protein (MP), sowie das coat Protein (CP) (von Bargaen et al., 2012). Die Polyproteine werden posttranslational durch die virale Proteinase zu funktionellen Einheiten prozessiert. Die Analyse der Vollängensequenz zeigt diverse putative Prozessierungsstellen, die analog zu experimentell bestätigten Schnittstellen verwandter Proteinasen aus den Nepoviren *Tomato ringspot virus* (ToRSV, Wang et al., 1999, Wang und Sanfacon, 2000) und *Arabidopsis mosaic virus* (ArMV, Wetzel et al., 2008) liegen. Zur funktionalen Charakterisierung der Proteinase von CLRV wird diese, sowie Bereiche des P2-Polyproteins, die putative Erkennungsstellen kodieren, heterolog in *E. coli* exprimiert. Anschließend erfolgt die native Aufreinigung der Proteine unter Verwendung eines N-terminalen His-Tags über NTA-Agarose. Die proteolytische Aktivität der Proteinase, sowie die putativen Prozessierungsstellen des P2 werden *in vitro* experimentell verifiziert.

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Alteration of allergen potential by *Cherry Leaf Roll Virus* (CLRV) in infected birch pollen

Our group has a major focus on the *Cherry Leaf Roll Virus* – CLRV a virus in trees which was correlated to a birch decline observed in Finland. The plant virus *Cherry leaf roll virus* infects many woody and herbaceous species and is widespread in temperate regions. The medical importance of the plant virus CLRV was never investigated. A negative impact on human health has to be seen in an allergen reaction to the virus modified pollen. Up to 80 %

of all allergenic pollen is originated from birch. Exposure to as few as 10 grains/m³ can result in seasonal rhinitis and asthma in birch sensitized individuals. The major birch pollen allergen, Bet v 1 belongs to the group of Pathogenesis-related class 10 proteins, which are classified by sequence homology and induced expression in response to pathogen infection. The mRNAs of PR-10 genes are detected in birch pollen amongst other tissues. The promoter of the Bet v 1 gene is strong pollen specific. The infection of pollen by *CLRV* virus may determine the allergen potential of the infected pollen or influence the allergic reaction. Any research step in this field needs interdisciplinary and bilateral collaboration. Presented is preliminary work on *CLRV* analysis in pollen and a first characterization of birch pollen allergens in extracts of *CLRV* infected birch pollen. We give an outlook on the current and future projects.

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Changes in the composition of volatile organic compounds (VOCs) of maize cobs infected with mycotoxin producing *Fusarium* spp.

Volatile organic compounds (VOCs) are hydrocarbons with low molecular weight. They belong to several chemical classes and can travel large distances in heterogeneous environments. In a plant-pathogen system they are known to be either plant derived (*de novo* synthesis upon biotic or abiotic stress) or pathogen derived.

To investigate the changes in volatile profiles of healthy and fungal-infected maize, we inoculated cobs of commercial hybrid maize at flowering stage (BBCH 65) with spore suspensions of *Fusarium graminearum*, *F. verticillioides* and *F. subglutinans* as well as mixed spore suspensions of *F. graminearum* and *F. verticillioides*.

A destructive static headspace sampling (solid phase microextraction, SPME) as well as a non-destructive dynamic headspace sampling (open-loop stripping using activated charcoal cartridges as volatile traps) were carried out to collect volatiles from maize cob material at different time points (4 dpi - 24 dpi). Fungal biomass was determined using quantitative real-time PCR and mycotoxin production was checked with HPLC-MS. Collected volatile samples were analyzed by gas chromatography coupled with mass spectrometer for identification and flame ionization detector for quantitative purposes.

We observed a considerable change in composition and quantity of VOCs between infected and healthy maize cobs as well as between cobs infected with *F. graminearum* and *F. verticillioides*. Among the specific set of volatile biomarkers of *Fusarium* infection in maize are plant-derived signals, such as green leaf volatiles, known fungal volatiles as well as terpenoid compounds released by the plant and the fungus. These markers are detectable within 4 - 8 dpi. At this early time point no infection symptoms are visible to the human eye. The set of volatile biomarkers can be used as a tool for early prediction of fungal infection.

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The role of plant defense proteins during early phases of plant-microbe interactions in the legume *Medicago truncatula*

Legumes are among the most economically important crop families playing a vital role in human and animal diet as excellent sources of protein, vitamins, minerals and other nutrients. Grain legumes including chickpea, pigeon pea, soybean, dry beans, etc, form an extremely essential protein source for millions of people in semi-arid and tropical regions of many Asian and African countries. Legumes are unique in establishing *rhizobia* bacteria association which allows nitrogen fixation and hence able to grow in nitrogen starved soils. Legumes are also capable of establishing symbiotic association with arbuscular *mycorrhizal* fungi. However, also pathogenic interactions with oomycete root rot pathogens like *Aphanomyces euteiches* often lead to major yield losses. The infection physiology involves protein-protein interactions between the pathogen and the host plant, where the latter generates symbiotic and pathogenic specific cellular responses. Of focus were the two initial response mechanisms using the model legume *Medicago truncatula* Jemalong 17 after inoculations with *rhizobia* bacteria (*Sinorhizobium meliloti*) *Arbuscular mycorrhizal* fungi (*Glomus intraradices*) and *A. euteiches*. *Medicago truncatula* Gaertn. (barrel medic) is established as a model legume mainly because of its small diploid genome size and ability to enter into both symbiotic and pathogenic associations with microorganisms.