主論文の要約

Investigation on the molecular mechanisms of sesquiterpenoid phytoalexin metabolism in Solanum species

（Solanum 属植物におけるセスキテルペノイドファイトアレキシンの生成および代謝に関する研究）

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植物病理学研究分野

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Plants are constantly confronted with a large number of pathogens, such as fungi, oomycetes, bacteria and viruses. In order to cope with these threats, plants have evolved various defense strategies. One such strategy is the hypersensitive response, where plant cells deliberately commit cell-death, in order to deprive invading pathogens of nutrients. While this strategy is effective against biotrophic pathogens, which require living plant cells to feed on, this measure is ineffective against hemibiotrophic and necrotrophic pathogens, which can feed on dead plant tissue. In such cases, the rapid production of phytoalexins, a generic term for a wide range of toxic compounds, can represent an effective defense measure. These phytoalexins accumulate near the site of infection where they ideally kill, or at least slow down an invading pathogen. These toxic compounds vary between species and fall into different chemical classes, such as flavonoids, indoles, terpenoids and others.

Solanaceae, the plant family that encompasses the important nutritional plants potato, tomato, eggplant and pepper, produces a substantial number of phytoalexins that belong

to the class of sesquiterpenes, such as capsidiol, rishitin, lubimin, oxylubimin, solavetivone, debneyol and others. Not all of these phytoalexins have been scrutinized extensively, but at least some of these compounds were found to damage the plant's own cells as well. The use of these phytoalexins is therefore a double-edged sword, and in the case of potatoes, the main phytoalexin rishitin is therefore eventually detoxified to a less harmful compound once the pathogen threat has subsided.

Although many solanaceous phytoalexins have already been discovered decades ago, our understanding of how plants produce, regulate and detoxify these compounds lacks far behind. With exception to capsidiol and solavetivone, the genes required for the biosynthesis of phytoalexins have not yet been discovered. Discovery of these genes could be valuable, since this knowledge could eventually be used for efficient breeding of more resilient cultivars of important crops such as potato and tomato. To this end, this thesis aimed to contribute to advancing our knowledge of the Solanaceae phytoalexin pathway.

In order to do so, various potato tuber genes were cloned and tested for their involvement in the metabolic pathway of potatoes' main phytoalexin rishitin, which is thought to derive via the production of solavetivone, lubimin and oxylubimin. Production of solavetivone in *E. coli*, yeast and *N. benthamiana* was attempted, via expression of potato homologs of HPS and HPO. This was made in order to use these expression systems as basis for further screening of solavetivone derived phytoalexins. It was found that *E. coli* and yeast are not suitable for large scale testing of candidate genes and expression in

INF1 elicited *N. benthamiana* was found to be the best system for this purpose. Candidate genes, were co-expressed with solavetivone producing genes in the leaves of *N. benthamiana*. Using this approach, a cytochrome 450 gene was identified which was thereafter named sesquiterpenoid phytoalexin dihydroxylase (SPH). SPH was found to encode for a protein, which detoxifies rishitin via hydroxylation to 13-hydroxrishitin, also referred to as rishitin-M1. Interestingly, this protein was found to metabolize a wide range of other phytoalexins as well, including solavetivone, lubimin, oxylubimin and even capsidiol-type phytoalexins that do not naturally occur in potato. This wide-range of phytoalexin substrates, was determined to occur via a conserved isopropenyl tail, present in many sesquiterpenoid phytoalexins. This flexibility in substrate choices and the conservation of closely related homologs in other Solanaceae indicates that SPH presumably plays an important role in phytoalexin detoxification, even in species that do not produce rishitin.

While the genes responsible for the synthesis of solavetivone derived phytoalexins were ultimately not discovered, this work has laid the groundwork which will improve further testing of phytoalexin synthesis genes. This includes not only methodological improvements, but also a set of novel candidate genes, which should further help to narrow down possible actors in this pathway. These candidates were the results of a large scale RNAseq analysis of elicited potato tubers, which were analyzed using weighted gene co-expression network analysis (WGCNA). Three co-expression modules were found which demonstrated a strong positive correlation to phytoalexin accumulation in

potato tubers. Although strong up-regulation during pathogen attack is expected for phytoalexin synthesis genes, it is possible that enzymes which catalyze the formation of solavetivone-derived phytoalexins, may not be rate-limiting, and therefore do not require a strong up-regulation, which could explain why these genes have been overlooked in past attempts. The candidates discovered via WGCNA are therefore valuable, since they also include genes, which correlate strongly with phytoalexin accumulation, but are only moderately up-regulated.