Sphingolipid metabolism and programmed cell death in tomato

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On the cover: A germinating spore of *Alternaria alternata* f.sp. *lycopersici* with a hypha penetrating an epidermal leaf cell of tomato. Pictures are kindly provided by Margriet Ferwerda. The front and the back sides present different levels.

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Promotor: Prof. Dr. J.Hille

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Prof. Dr. O.P. Kuipers
Prof. Dr. H.J.J. Nijkamp

aan Dilyana

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Stefka

Contents:

Scope of the thesis

Programmed cell death is an extensively studied process in animal systems. The interest is due to the importance of the process for development, immune response and cell proliferation. In plants, the interest in programmed cell death increased in the last five years. The process is associated with plant development and plant-pathogen interactions. While in animals significant knowledge has been gained regarding the initial signalling, second messengers and execution phase of programmed cell death, addressing these issues has only just begun in plants. The work in this thesis is focused on possible signals in plant programmed cell death.

The role of sphingolipid signalling in programmed cell death is well established in animal systems. In plants, there is little known about the role of sphingolipids as cellular signals, though their chemical structure has been elucidated and their structural role in the tonoplast and plasma membrane has been established. ***Chapter 1*** summarises the contemporary knowledge of the structure of plant sphingolipids, enzymes involved in the plant sphingolipid metabolism and points to the first evidence in plants that sphingolipids and sphingoid metabolites play a role in cellular signalling and in particular in programmed cell death.

Asc-1 in tomato is a gene conferring resistance to the *Alternaria* stem canker disease and insensitivity to AAL-toxin. AAL-toxin, produced by the fungal pathogen *Alternaria alternata* f.sp. *lycopersici*, is a structural homologue of sphingoid long-chain bases and a competitive inhibitor of (dihydro)ceramide synthase. Cloning of the *Asc-1* gene revealed that *Asc-1* belongs to a gene family with homologues in all eukaryotic kingdoms. ***Chapter 2*** deals with the expression of the tomato *Asc-1* gene in the yeast *lag1Δlac1Δ* double mutant. *LAG1* and *LAC1* are the two yeast homologues of tomato *Asc-1,* deletion of which results in a lethal or slow growth phenotype. The slow growth phenotype yeast strain, which was chosen for the *Asc-1* complementation study is characterised by severe cell wall defects and delayed glycosylphosphatidylinositol-anchored protein transport. The functioning of Asc-1p in yeast sphingolipid biosynthesis is discussed. ***Chapter 3*** analyses the function of Asc-1p in tomato. Here, the effect of AAL-toxin on sphingolipid biosynthesis in the presence or in the absence of the *Asc-1* gene is presented. The possible programmed cell death signals generated from sphingolipid metabolism are discussed in the chapter. Plant sphingolipids can be divided in two different groups: inositolphosphorylceramides and glucosylceramides. In ***Chapter 4*** these two groups of sphingolipids are analysed with respect to the Asc-1p-AAL-toxin interaction and cell death signalling.

In ***Chapter 5*** an alternative approach to study programmed cell death in plants is used. The chapter describes engineering a lesion mimic mutant in tomato via virus-induced gene silencing of the lethal leaf spot-1 homologue. Characteristic for lesion mimic mutants is that they exhibit a hypersensitive response-like

phenotype in the absence of a pathogen. What could be the possible signal triggering programmed cell death in the lethal leaf spot-1 mutant is discussed.