

Studies on an effector NLP1 expressed during the late phase of plant infection by *Colletotrichum orbiculare*

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Chapter I. Inappropriate expression of an effector NLP1 in *Colletotrichum orbiculare* impairs infection on Cucurbitaceae cultivars via plant recognition of the C-terminal region

A hemibiotrophic pathogen *Colletotrichum orbiculare* preferentially expresses a necrosis and ethylene-inducing peptide 1 (Nep1)-like protein named NLP1 during the switch to necrotrophy. I here revealed that the constitutive expression of NLP1 in *C. orbiculare* blocks pathogen infection in multiple Cucurbitaceae cultivars via their enhanced defense responses such as ROS generation and callose deposition. NLP1 has a cytotoxic activity that induces cell death in *Nicotiana benthamiana*. However, *C. orbiculare* transgenic lines constitutively expressing a mutant NLP1 lacking the cytotoxic activity still failed to infect cucumber, indicating no clear relationship between cytotoxic activity and the NLP1-dependent enhanced defense in cucurbits. NLP1 also possesses the microbe-associated molecular pattern (MAMP) sequence called nlp24 recognized by *Arabidopsis thaliana* at its central region similar to other pathogens' NLPs. Surprisingly, inappropriate expression of a mutant NLP1 lacking the MAMP signature is also effective for blocking pathogen infection, uncoupling the infection block from the corresponding MAMP. Notably, the deletion analyses of NLP1 suggested that the C-terminal region of NLP1 is critical to enhance defense in cucumber. The expression of mCherry fused with the C-terminal 32 amino acids of NLP1 was enough to trigger the defense of cucurbits, revealing that the C-terminal region of the NLP1 protein is recognized by cucurbits, and then terminates *C. orbiculare* infection.

Chapter II. Functional studies of *NLP1* and *NLP1* homologs in *Colletotrichum orbiculare*

The NLP family is broadly conserved among microorganisms including bacteria, fungi and oomycetes. In Chapter I, I revealed that the 22 amino acid residues at C-terminal of *C. orbiculare* NLP1 is recognized by Cucurbitaceae cultivars, whereas the central region of NLP1 (nlp24) is recognized as MAMP by *Arabidopsis*. In order to gain further understanding on two MAMP sequences, I performed the transient expression of NLP1 lacking 22 amino acids of C-terminal region (NLP1 Δ 22) or NLP1 lacking nlp24 sequence (NLP1Mut) in *N. benthamiana*. NLP1Mut largely reduced the cytotoxic activity in *N. benthamiana* whereas NLP1 Δ 22 slightly reduced the activity, indicating the importance of the two MAMP sequence for the cytotoxic activity is distinct. To gain insights on the role of NLP1 in fungal virulence, I generated the targeted deletion mutants for *NLP1*. I show that the *nlp1* null mutants exhibit virulence on host plants the same as the wild-type strain. I further studied the two *NLP* homologs of *C. orbiculare* expressed at the early phase of infection in contrast to *NLP1* expressed at the necrotrophic phase. I revealed that the overexpression of the two homologs in *C. orbiculare* did not give negative effects on virulence of the pathogen on cucumber, suggesting that these NLPs expressed at the early infection phase are unlikely recognized by cucurbits. In addition, I found that the two homologs have no strong activity to induce cell death in *N. benthamiana*. These findings suggest that these two NLP1 homologs might have unidentified roles for the early infection phase, that is not related to cytotoxicity.