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► To cite this version:

Nacera Talbi, Nouredine Lazar, Carl H. Mesarich, Simine Pakzad, Yohann Petit-Houdenot, et al.. Characterization of epistatic interactions within a family of structurally conserved fungal effectors and of their broad-spectrum recognition by a plant resistance protein. integrated control of oilseed crops meeting, May 2022, Virtual conference, France. hal-04433092

HAL Id: hal-04433092

<https://hal.science/hal-04433092>

Submitted on 1 Feb 2024

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Characterization of epistatic interactions within a family of structurally conserved fungal effectors and of their broad-spectrum recognition by a plant resistance protein

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Recognition of a pathogen avirulence (AVR) effector protein by a cognate plant resistance (R) protein triggers a set of immune responses that render the plant resistant. Pathogens can escape this resistance by different mechanisms including the acquisition of new effectors that suppress AVR recognition. *Leptosphaeria maculans*, causal agent of oilseed rape stem canker, develops a high diversity of mechanisms to efficiently escape plant resistance including suppression of R protein-mediated recognition by an AVR effector. Indeed, AvrLm4-7 suppresses the recognition of AvrLm3 and AvrLm5-9 by the R proteins Rlm3 and Rlm9, respectively [1,2]. To decipher the epistatic interaction between these *L. maculans* AVR effectors, we determined the crystal structure of AvrLm5-9 and obtained a 3D model of AvrLm3, based on the crystal structure of Ecp11-1, a homologous AVR effector from *Fulvia fulva*. Despite a lack of sequence similarity, AvrLm5-9 and AvrLm3 are structural analogues of AvrLm4-7 (structure previously characterized [3]). Structure-informed sequence database searches identified a larger number of putative structural analogues among *L. maculans* effector candidates, including AvrLmS-Lep2, as well as among effector candidates from other phytopathogenic fungi [4]. Remarkably, transformants of *L. maculans* expressing one of these structural analogues, Ecp11-1, triggered Rlm3-mediated immunity. Furthermore, this recognition was suppressed by AvrLm4-7. On these bases, we continued to decipher the interactions between Ecp11-1 / AvrLm3 (and their closely related homologues) and Rlm3 by: (i) performing *L. maculans* complementation experiments with different homologues; (ii) determining whether AvrLm4-7 could suppress that recognition; (iii) finely characterizing the amino-acids / regions of Ecp11-1 and AvrLm3 which induced recognition by Rlm3. This analysis is a first step towards the understanding of broad-spectrum resistances that may allow multi-pathogen disease management.

Keywords: *Leptosphaeria maculans*, oilseed rape, avirulence, 3-D structure, evolution, specific resistance, broad-spectrum resistance

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