Unusual evolutionary mechanisms to escape Effector-Triggered-Immunity in the fungal phytopathogen Leptosphaeria maculans

Clémence Plissonneau, Bénédicte B. Ollivier, Isabelle Fudal-Grolier Fudal, Thierry T. Rouxel, Marie-Helene Balesdent

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PLISSONNEAU Clémence (1), OLLIVIER Bénédicte (1), FUDAL Isabelle (1), ROUXEL Thierry (1), ***BALESDENT Marie-Hélène*** *(1)*
(1) -BIOGER INRA, AgroParisTech, Thiverval-Grignon, France

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Leptosphaeria maculans is responsible for the stem canker (phoma) disease of oilseed rape (*Brassica napus*). AvrLm3 and AvrLm4-7, two avirulence effector genes of *L. maculans*, are involved in an unusual relationship: the presence of AvrLm4-7 induces the Rlm7-mediated resistance but suppresses the Rlm3-mediated recognition (Plissonneau et al., 2015; doi: 10.1111/nph.13736). To investigate this relationship, we assessed the sequence diversity of AvrLm3 in *L. maculans* populations. The analysis of more than 200 isolates revealed a high level of allelic polymorphism for this gene, but no deletion event nor inactivating mutations were found. This observation contrasts with the presence/absence or RIP-(Repeat-induced-point-mutation) inactivation polymorphisms usually found for other *L. maculans* avirulence genes and questioned the role of AvrLm3 in fungal fitness. Two distinct mechanisms responsible for the «double virulent» phenotype (i.e. virulent toward both Rlm3 and Rlm7, «a3a7») were identified. In isolates displaying an inactivation of AvrLm4-7, amino acid changes in AvrLm3 were responsible for the virulent phenotype towards Rlm3. However, 56% of the a3a7 isolates displayed an avirulent allele of AvrLm3, combined with point mutations in AvrLm4-7. Such situations allow the fungus to escape Rlm7-mediated resistance while maintaining the suppression of the AvrLm3 phenotype effective. They also allow the fungus to escape to the recognition by two resistance genes while keeping the secretion of the two corresponding effector proteins. The complex evolutionary mechanisms displayed by *L. maculans* to escape Rlm3-mediated resistance while preserving AvrLm3 sequence integrity, along with the reduced virulence in isolates silenced for AvrLm3 confirmed the importance of this effector in pathogenicity towards *B. napus*.

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