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Functional analysis of AvrLm10a and AvrLm10b, two neighbor effector genes from L. maculans displaying a 'two genes for one gene' interaction with the resistance gene Rlm10 from Brassica nigra

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Leptosphaeria maculans is a hemibiotrophic ascomycete which causes stem canker of oilseed rape (Brassica napus). The most economically and environment friendly method to control stem canker is the genetic control by using host resistance. Specific resistances are rare in oilseed rape, and efforts are made to find resistance genes in other Brassica species. We investigated the AvrLm10 avirulence that induces a resistance response when recognized by the Brassica nigra resistance gene Rlm10. Using map-based cloning, we identified two AvrLm10 candidates (AvrLm10a and AvrLm10b) as two head-to-head genes located in a sub-telomeric repeat-rich region of the genome. Both genes harbor typical features of fungal effectors: they encode small secreted proteins, are co-regulated and overexpressed 7 days post-infection of oilseed rape cotyledons. Complementation and silencing assays indicated that both genes are necessary to trigger Rlm10 resistance. Interaction of two avirulence genes against one resistance gene suggests that AvrLm10a and AvrLm10b could directly interact and / or that they could target the same plant protein. Yeast two-hybrid, Bimolecular Fluorescence Complementation and FRET-FLIM assays showed that the two AvrLm10 proteins physically interact in vitro and in planta. Interestingly, recognizable orthologues exist for both genes in other phytopathogenic species, with a similar genome organization, which may point to an important conserved effector function. Indeed, silencing by RNA interference of AvrLm10a induces an increase of lesion size on oilseed rape cotyledons and both AvrLm10a and AvrLm10b are able to suppress cell death after transient expression on tobacco leaves.