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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 over-expressed 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.