AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION OF PODOSPAERA FUSCA

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The powdery mildews are obligate biotrophic pathogens that depend on living plant cells to complete its life cycle. These pathogens develop a specialized structure called haustorium that serves for nutrient uptake and exchange of factors with the plant. Thus, great interest has been focused on understanding how they are assembled and how they mediate fungus-plant communication. A bottleneck in these studies is the difficulty to manipulate genetically this group of fungi, and indeed transformation of powdery mildew fungi remains elusive to researchers. In this work, we show the genetic manipulation of the cucurbit powdery mildew pathogen Podosphaera fusca using Agrobacterium-mediated transformation. Using this method, we obtained stable transformants for a variety of constructs: i) a plasmid expressing the egfp gene under the control of a constitutive promoter from Aspergillus nidulans, ii) plasmids expressing the hygromycin resistance cassette or a β-tubulin allele that confers resistance to carbendazim, a MBC fungicide. Moreover, we performed fluorescence microscopy studies with a translational fusion of CSEP5191, a candidate effector of P. fusca, fused to GFP, and found how this candidate effector is localized into large vesicles of the haustorium. These results support the feasibility of this transformation method to manipulate genetically P. fusca and open the possibility to be used with other powdery mildews.

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