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Poster Communication Abstract – PH.43

DMR6 KNOCK-OUT: A STRATEGY TO CONFER RESISTANCE AGAINST PATHOGENS IN TOMATO

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Susceptible genes (S-genes) encode for proteins required by the pathogen to infect the plant at some step of the infection process. Disabling these genes represent an intriguing strategy to confer resistance against pathogens in plants. Noteworthy, the resistance mediated by S genes mutation can be pathogen-specific or broad-spectrum and long lasting. So far different S-genes have been characterized in many plant species and, amongst them, we focused on Downy Mildew Resistance 6 (DMR6). DMR6 is involved in the conversion of salicylic acid (SA) to 2,3-dihydroxybenzoic acid (2,3-DHBA) and negatively regulate the defence genes expression. DMR6 has been linked to susceptibility against Phytophthora capsici and P. infestans in Arabidopsis and potato. Due to its role in pathogen defence we decided to analyze the role of this gene in susceptibility against late blight (P. infestans), powdery mildew (Oidium neolycopersici) and Botrytis cinerea in tomato. Two DMR6 homologues were identified in tomato (DMR6-1 and DMR6-2). The transcript levels of the two DMR6 orthologs genes have been preliminary evaluated through qPCR in tomato leaves infected by B. cinerea. The expression of DMR6-1 increased significantly 72 hours post infection when the first signs of damage appeared on the leaf, while DMR6-2 did not show a clear activation in response to pathogen infection. A CRISPR/Cas9 approach was used to induce knock-out mutation on DMR6-1 by using a multiplexing strategy based on three gRNAs targeting the first three gene exons. Five mutated regenerated plants showed biallelic and chimeric genomic status, with a high editing efficiency on three targeted exons. Regenerated plants and their offspring will undergo pathogen assay to verify an increased resistance. A new CRISPR/Cas9 transformation has been recently set up in order to target simultaneously the gene knock-out on DMR6-1 and DMR6-2 and these plants are now under regeneration process.