

INNOVATIVE GENETIC APPROACHES TO EXPLORE RESISTANCE AGAINST THE PARASITIC WEED *Phelipanche ramosa* IN TOMATO

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Broomrapes are root holoparasites belonging to the genus *Orobanche* spp. and *Phelipanche* spp. Their growth and survival are strictly dependent on the presence of a host plant from which they obtain the nutrients. This results in a significant reduction in yield and quality in various crops around the world. Conventional management practices are often inadequate to control broomrape infestation, mainly due to the underground mechanism of parasitism and seed banks difficult to eradicate.

Strigolactones (SLs), a class of phytohormones derived from carotenoids, are known to trigger the germination of *Orobanche* seeds when exuding into the soil from the host roots. *Phelipanche ramosa* (branched broomrape) represents a serious threat to the cultivation of tomato (*Solanum lycopersicum* L.), one of the most important and traditional vegetable in

Italy. In order to reduce tomato susceptibility to *P. ramosa* infestation, we have adopted several approaches. In particular, altering the release of SLs in the rhizosphere could represent a valid resistance strategy. Through the use of CRISPR/Cas9-mediated mutagenesis, tomato knock-out mutants were produced for the four main genes (*SLD27*, *SlCCD7*, *SlCCD8*, *SlMAX1*) involved in the biosynthesis of SLs. Phenotyping of *Slccd7* and *Slccd8* mutant progenies highlighted the expected phenotype of SL- plants and a significant reduction in *P. ramosa* infection in soilless experiments. In parallel, further CRISPR/Cas9 editing experiments were performed on other classes of genes involved, or potentially involved, in the transport/release of SLs in root exudates (*SlPDR1*, *SlPDR2* and *SlPDR3*), thus leaving the SL biosynthetic pathway unaltered. The mutants for both SL biosynthesis and transport will provide novel genetic material that: 1) will generate CRISPR alleles that can potentially be introduced into commercial tomato varieties to confer resistance to *P. ramosa*; 2) will help to clarify some biological aspects of the biosynthesis and transport of SLs in Solanaceae.

In previous studies, the wild species *S. pennellii* was poorly colonized by the aforementioned parasitic weed. Consequently, we are also evaluating the resistance/susceptibility towards *P. ramosa* of the main 48 introgression lines (ILs) of *S. pennellii* in tomato. Using the protein sequences of the genes abovementioned as a query, a BLAST analysis to identify orthologous genes in *S. pennellii* is currently in progress. These screenings could potentially provide us valuable genetic material useful for elucidating the complex host-parasite interactions and a source of new genes that control *P. ramosa* resistance in tomato.