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TRACKING THE DWARF27-LIKE (D27-LIKE) GENES IN TOMATO

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Initially identified both as the most relevant germination stimulus for parasitic weeds, and as one of the prominent signals responsible for the establishment of symbiotic interactions with arbuscular mycorrhizal fungi (AMF), strigolactones (SLs) were only later recognized also as plant hormones. These carotenoid-derived molecules were subsequently found to be involved in plant response to stresses caused by the lack of nutrients (i.e., nitrogen and phosphorus), resulting in a modification of the architecture of the shoots and roots. Their biosynthesis in tomato is catalysed by four enzymes – *D27*, *CCD7*, *CCD8* and *MAX1* – which, starting from the all-*trans*-β-carotene, leads to the biosynthesis of carlactonic acid (CLA), which is further converted into the different SLs by the activity of several P450 cytochromes.

The first enzyme in the pathway, *D27* (*DWARF27*), is an isomerase that catalyses the reversible conversion of the all-*trans*-β-carotene to 9-*cis*-β-carotene. The CRISPR/Cas9 *d27* knock-out tomato lines show a milder phenotype than the other knock out mutants of the downstream genes. This result might be explained by the effects of the spontaneous isomerization of all-*trans*-β-carotene into 9-*cis*-β-carotene and/or suggests the presence of additional isomerases. A phylogenetic analysis extended to *Solanaceae*, *Brassicaceae* and *Poaceae* was performed. A similarity-based search revealed the presence of two *D27-LIKE* genes (*D27-LIKE 1* and *D27-LIKE 2*) in tomato, whose expression profiles were retrieved from public databases. Two

CRISPR/Cas9 constructs were assembled to produce the two *d27-like* tomato knock-out mutants and the activity of the sgRNAs tested by hairy root assay. The production of such mutants will help elucidate the role of additional isomerases in SL biosynthesis and clarify their function in other biological processes in tomato.