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LIBRO DE RESÚMENES

P4-05 The loss of function of the tomato homolog of *ROTUNDIFOLIA3* impairs leaf expansion and plant growth

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We characterized the tomato *2489etmm* recessive mutant, which belong to our T-DNA insertional mutant collection (1). Homozygous mutant plants showed slower growth rate at greenhouse conditions, as well as wrinkled leaves and enlarged stem. Despite these developmental defects, *2489etmm* mutant plants were able to develop flowers and yield seed-bearing fruits after almost ten months growing under optimal conditions, in contrast to the three months required by the wild-type plants. By combining mapping-by-sequencing and CRISPR/ Cas9 genome editing methods, we proved that a 2-bp deletion in the tomato homolog of the Arabidopsis *ROT3* gene (*SlROT3*) was responsible for the phenotype observed in the *2489etmm* mutant. We engineered knockout mutations at the *SlROT3* locus by using the CRISPR/Cas9 system with a single guide RNA. Independent first-generation CRISPR lines homozygous or biallelic for edited mutant alleles showed slower growth, wrinkled leaves, and enlarged stem, a phenotype resembling that observed in the *2489etmm* mutant. The *ROTUNDIFOLIA3 (ROT3)* gene encodes a cytochrome P450 family protein involved in regulating leaf length of the model species *Arabidopsis thaliana*. Specifically, *ROT3* is required for the conversion of typhasterol to castasterone in the early C6-oxidation pathway of brassinosteroid biosynthesis (2). Brassinosteroids are a group of plant steroid hormones and playing a key function during plant growth and development. Together, our results suggest that *SlROT3* may act regulating tomato leaf expansion in a similar manner as occurs in Arabidopsis, by probably participating in brassinosteroid biosynthesis pathway.

1. Pérez-Martín et al. (2017). Plant Biotechnology Journal, 15: 1439-1452.
2. Ohnishi et al. (2006). The Plant Cell 18, 3275–3288.

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