

ZUCCHINI *TINY4* GENE REGULATES PLANT DEVELOPMENT THROUGH BRASSINOSTEROID SIGNALLING PATHWAY

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Brassinosteroids (BRs) are steroid plant hormones necessary for the regulation of physiological processes essential for plant growth and survival. The analysis of mutants altered in these responses has provided insight into the genes involved in the BRs signalling pathway, identifying the molecular components essential for perception and transmission of this hormonal signal. The characterization of a collection of EMS mutants of *Cucurbita pepo* morphotype Zucchini allowed us to select five mutants that show alterations in vegetative development, which we have called *tiny* (*tin1/5*) due to their small plant size. In this work, by combining whole-genome sequencing and mapping of molecular markers of codominant inheritance, it has been possible to identify the first known mutant allele of the zucchini *TINY4* gene, homologous to the *SERK* (*SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE*) gene of *Arabidopsis thaliana*. *SERK* encodes a protein kinase with leucine-rich repeats (LRR-RLK) located in the plasma membrane, which together with two other LRR-RLK proteins, namely BRASSINOSTEROID-INSENSITIVE1 (*BRI1*) and *BRI1*-ASSOCIATED KINASE1 (*BAK1*), form a complex for perception and signalling of brassinosteroid. The *bri1* mutant is characterized by short petioles and inward-curved leaves. The *serk* mutation enhances these phenotypic traits, and thus the *bri1 serk* double mutant show reduced petiole length, small rosette size and excessive leaf curl. The zucchini *tin4* mutant exhibits severe compaction of the vegetative organs of the plant, caused by reduced petiole size of leaves and stems, a phenotype that resemble the *Arabidopsis bri1 serk* double mutant. To confirm that the *tin4* mutation is responsible for the mutant phenotype, new loss-of-function alleles of this gene are being generated using CRISPR-Cas9 gene editing technology. These results will contribute to the functional genomics of this species and provide further insight into the functionality of the *TIN4* gene in the brassinosteroid perception pathway and thus in vegetative morphogenesis of zucchini.

Keywords: chemical mutagenesis; whole-genome sequencing; plant development; dwarf.

Acknowledgments. This work has been funded by the Ministerio de Ciencia e Innovación (project CUCURVAR RTC2019-007247-2).