DOI 10.18699/SBB-2022-371

Does *DEEPER ROOTING 1* (*DRO1*) gene of cucumber (*Cucumis sativus* L.) regulate a lateral root angle?

Kiryushkin A.S.*, Ilina E.L., Guseva E.D., Demchenko K.N.

Laboratory of Cellular and Molecular Mechanisms of Plant Development, Komarov Botanical Institute, RAS, St. Petersburg, Russia

** akiryushkin@binran.ru*

Key words: CRISPR/Cas, root system architecture, genome editing, *DRO1*, lateral root, cucumber

Introduction: The orientation or gravitropic set point angle (GSA) of underground plant organs are regulated by different gene families. The IGT (*LAZY1*/*TAC1*/*DRO1*) gene family is one of them. The representative of the IGT family, the *DRO1* gene, was initially described as a regulator of rice rooting depth. *DRO1* homologues were subsequently identified in other plant species, both monocots and dicots. The aim of this study was to elucidate whether the *DRO1* gene of cucumber (*Cucumis sativus* L., Cucurbitaceae) regulates a change in lateral root angle.

Methods: The results were obtained using phylogenetic analysis of cucumber IGT protein family, quantitative real time PCR (qPCR), confocal laser scanning microscopy (CLSM), and CRISPR/Cas9-mediated editing of cucumber *DRO1* genes.

Results: Three putative orthologues of rice and Arabidopsis DRO1 proteins encoding by *CsDRO1a*, *CsDRO1b*, and *CsDRO1c* genes were identified in cucumber. The phytohormone auxin plays a central role in formation of root GSA, so using qPCR the effect of exogenous auxin (1-Naphtaleneacetic acid) on *CsDRO1a*, *CsDRO1b*, and *CsDRO1c* expression was examined. The expression of *CsDRO1a* gene was significantly increased, while transcript levels of *CsDRO1b* and *CsDRO1c* were not changed. Study of the expression of pCsDRO1a, pCsDRO1b, and pCsDRO1c promoter-reporter fusions in the cucumber root tips was conducted using Agrobacterium-mediated hairy root transformation following by analysis of root sections using CLSM. Expression of *CsDRO1a*, *CsDRO1b*, and *CsDRO1c* was observed in central cylinder, pericycle, endodermis, and cortex up to an average distance of 700 μm from the initial cells. Then, expression maxima of *CsDRO1a* was retained only in endodermis cells involved in lateral root development, whereas *CsDRO1b* and *CsDRO1c* continued to express in cells layers of central cylinder, in pericycle, and in endodermis. The role of the single *CsDRO1* gene (*CsDRO1a*, *CsDRO1b* or *CsDRO1c*) in regulation of lateral root angle were clarified using CRISPR/Cas9-mediated knockout. The efficiency of *CsDRO1a* editing was 94 %, for *CsDRO1b* – 75 %, and for *CsDRO1c* – 50 %. Although editing events resulted in complete knockout of the single *CsDRO1* gene in individual transgenic roots, no statistically significant changes in lateral root angle were detected.

Conclusions: The results suggest that *CsDRO1a*, *CsDRO1b*, and *CsDRO1c* may have degenerate function to regulate lateral root angle in cucumber parental root.

Acknowledgements: The Ministry of Science and Higher Education of the Russian Federation supported this study in the framework of Bio-resource collections (Grant No. 075-15-2021-1056).