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Study of functional features of plant root systems using CRISPR/Cas-mediated genome editing



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CRISPR/Cas-mediated genome editing is a powerful tool of plant functional genomics. Hairy root transformation is a rapid and convenient approach for obtaining transgenic roots. When combined, these techniques represent a fast and effective means of studying gene function [1, 2].

A common construct for efficient genome editing and selection of hairy roots is comprised of three components, i.e., a cassette carrying the gene encoding the Cas nuclease, a cassette expressing the guide RNA (gRNA), and a cassette encoding a screenable or selectable marker [2]. After design and construction, the resulting vector is used to transform plant using appropriate *Rhizobium rhizogenes* strain.

Over 26 plant species have been used in experiments combining genome editing and hairy root transformation to date [2]. Possible applications of CRISPR/Cas9 genome editing using hairy root transformation include different directions like test the efficiency of the CRISPR/Cas9 genome editing; obtaining whole genome-edited plants regenerated from individual edited hairy roots; investigation of root development or root function, root nodule symbiosis, resistance to biotic or abiotic stresses, or metabolic engineering [2].

The basic principles of plant CRISPR/Cas genome editing like the different components of CRISPR/Cas vectors, the types of Cas nuclease, design principles of gRNAs, as well as the possible applications of CRISPR/Cas genome editing in hairy roots will discuss. The application of this method for multigene editing strategy will also be demonstrated on *DEEPER ROOTING1* genes of cucumber.

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Keywords: CRISPR-Cas9; hairy roots; cucumber.

REFERENCES

1. Gogolev YV, Ahmar S, Akpinar BA, et al. OMICs, epigenetics, and genome editing techniques for food and nutritional security. *Plants*. 2021;10(7);1423. DOI: 10.3390/plants10071423

2. Kiryushkin AS, Ilina EL, Guseva ED, et al. Hairy CRISPR: Genome editing in plants using hairy root transformation. *Plants.* 2022;11(1);1–39. DOI: 10.3390/plants11010051

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