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Agrobacterium-mediated in planta transformation of cut coleoptile: a new, simplified, and tissue culture-independent method to deliver the CRISPR/Cas9 system in rice

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Abstract

Author keywords

Indexed keywords

SciVal Topics

Chemicals and CAS Registry Numbers

Metrics

Funding details

Abstract

Background: Agrobacterium-mediated transformation and particle bombardment are the two common approaches for genome editing in plant species using CRISPR/Cas9 system. Both methods require careful manipulations of undifferentiated cells and tissue culture to regenerate the potentially edited plants. However, tissue culture techniques are laborious and time-consuming. Methods and results: In this study, we have developed a simplified, tissue culture-independent protocol to deliver the CRISPR/Cas9 system through in planta transformation in Malaysian rice (Oryza sativa L. subsp. indica cv. MR219). Sprouting seeds with cut coleoptile were used as the target for the infiltration by Agrobacterium tumefaciens and we achieved 9% transformation efficiency. In brief, the dehusked seeds were surface-sterilised and imbibed, and the coleoptile was cut to expose the apical meristem. Subsequently, the cut coleoptile was inoculated with A. tumefaciens strain EHA105 harbouring CRISPR/Cas9 expression vector. The co-cultivation was conducted for five to six days in a dark room (25 ± 2°C) followed by rooting, acclimatisation, and growing phases. Two-month-old plant leaves were then subjected to a hygromycin selection, and hygromycin-resistant plants were identified as putative transformants. Further validation through the polymerase chain reaction verified the integration of the Cas9 gene in four putative T_0 lines. During the fruiting stage, it was confirmed that the Cas9 gene was still present in three randomly selected tillers from two 4-month-old transformed plants. Conclusion: This protocol provides a rapid method for editing the rice genome, bypassing the need for tissue culture. This article is the first to report the delivery of the CRISPR/Cas9 system for in planta transformation in rice. © 2023, The Author(s), under exclusive licence to Springer Nature B.V.

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Agrobacterium-mediated; Genome editing; In planta transformation; Malaysian rice; Tissue culture-independent

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