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Identification of Gene Editing in Wheat Protoplast Populations with HRM

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[Introduction]

CRISPR/Cas9 system is an accurate tool for wheat genome editing. Verification of genome editing through Sanger sequencing is widely adopted. Identification of gene edits by Sanger sequencing is more time-consuming and expensive than by HRM. This study aims to build an HRM gene editing detection system to quickly and accurately identify CRISPR/Cas9-induced mutations without Sanger sequence analysis.

[Materials and Methods]

Protoplasts were isolated using the first leaf of ‘Bobwhite’ and ‘Chinese spring’. Protoplasts were transformed with a CRISPR/Cas9 vector designed by targeting gene related to pre-harvest sprouting resistance by PEG + Lipofectamine method and transferred to regeneration buffer. After 16 hours of culture, gDNA was extracted from the transformed protoplasts. And HRM was performed in each DNA sample.

[Result and Discussion]

Using DNA extracted from protoplasts, we performed HRM with primers covering guide sequence targeting region. Several DNA samples exhibited different melting curve patterns compared to that of the wild type. Then we performed Sanger sequence analysis to confirm mutations induced by CRISPR/Cas9. However, mutation on target DNA was not detected from Sanger sequence analysis. The protoplasts used for DNA extraction consisted of cells without mutation or included cells with mutations with a low ratio. We will use more sensitive methods like NGS sequencing to detect mutations on the target gene. And we will prove that protoplast gene editing can be easily confirmed by HRM.

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