PB-8

Application of Tissue Culture Bypass Gene Editing of Korean Wheat Variety 'Keumgang' through *in planta* Bombardment Technology

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[Introduction]

The genome editing technology using CRISPR/Cas9 has been applied to create gene-edited plants in various major crops, such as wheat (*Triticum aestivum* L.), because of the convenience of inducing editing of desired DNA target sites in a simple way. However, to apply gene editing technology to plants, tissue culture is required in advance, and it is very difficult to apply such gene editing technology to plants with poor tissue culture efficiency. The *in planta* bombardment (iPB) technique recently developed by Japanese researchers succeeded in developing transgenic plants that bypassed tissue culture by directly genetically altering the shoot apical meristem (SAM) region of mature seeds. This technology made gene editing technology, which was only applicable to varieties with high tissue culture efficiency, possible in various wheat varieties.

[Materials and Methods]

The Korean wheat variety 'Keumgang' was grown in a greenhouse, fully matured seeds were harvested, and iPB was applied for gene editing. The iPB process was performed with slight modifications by referring to the process previously reported by Hamada et al. (2018). After bombardment, embryos were germinated in half-MS medium for 1~2 weeks, transferred to soil, and grown in a greenhouse. Genome editing was confirmed by the high-resolution melting (HRM) analysis using gDNA extracted from the leaves after the 5th leaf stage of each plant and the flag leaf of each spikes.

[Results and Discussion]

For the mature embryos extracted from 100 seeds, most of the plants grew with only roots after applying bombardment or did not grow normally and withered after leaves appeared, and only five embryos (5%) germinated as regular plants. This result may be due to damage to the apical meristem during the process of exposing the SAM. As a result of HRM analysis performed in the 5th leaf stage on five regularly plants, the morphology of chimera showing different patterns for each repeated analysis was found in one plant. Finally, as a result of HRM analysis performed on the flag leaf of each spike after heading of one plant with a chimeric pattern, a curve with a different pattern from the wild-type cv. Keumgang was detected in all repeated experiments. In the future, we plan to verify the InDel of the gene through sequencing and to test for transgene integration using PCR. This study can be useful information as a tissue culture bypass technique for applying gene editing technology to the Korean wheat varieties.

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