PB-006

Genome Editing Using CRISPR/Cas9 for Knock-out Flowering Suppressor Gene PCKP

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[Introduction]

Every year, South Korea suffers serious damage to rice growth and productivity due to typhoons. In particular, there is a high possibility that may become dry, discolored and infertile in panicle, if occured typhoon during the flowering period of rice. Therefore, by knock-out the flowering suppressor gene *PCKP* using the CRISPR/Cas9 system, we tried to develop an early maturing cultivar and heavy panicle type that can obtain a stable yield even in the event of a typhoon.

[Materials and Methods]

To knock-out *PCKP* of Ilmi, a guide RNA was designed around the *PCKP* domain region, and the PAM sequence used 5'-NGG-3'. After inserting the designed gRNA into pRGEB32, a vector for *agrobacterium*-mediated transformation was constructed. It was confirmed whether the *PCKP* gene sequence was edited using the regeneration plant.

[Results and Discussion]

The 200 callus aged 20 days of Ilmi were inoculated into using the *agrobacterium*-mediated transformation method, and 15 (7.5%) of regeneration plants were obtained. To check whether *PCKP* was edited, genomic DNA was extracted, a *PCKP* domain region was amplified using PCR, and checked the *PCKP* sequence. In 3 of 15 (20%) regeneration plants, indels occurred in front of the PAM sequence. When the relative expression level of the *PCKP* was confirmed using qPCR, the relative expression level was decreased in regeneration plants 6, 7, and 14 than Ilmi. In the future, we tried to develop early maturing cultivar and heavy panicle type by selecting and generation advancement, plant whose flowering time has been accelerated due to suppression of the expression of the *PCKP*.

[Acknowledgement]

This work was supported by a grant from the New breeding technologies development Program (Project No. PJ01479 3012020), Rural Development Administration, Republic of Korea

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