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Identification of Cochlioquinone 9 through *FNSII* Knock-out and Breeding of Functional Rice

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[Introduction]

Rice has long been used as a staple food all over the world. Rice improvement has been started since the 1970s, and the focus was on increasing the yield. However, after increasing the quantity to some extent, people have been trying to breed rice varieties that taste good and add functionality. Anthocyanins act as antioxidants and are substances that can prevent various diseases. Therefore, in this study, *FNSII* was knocked-out by using genetic scissors, and the presence of genome editing and the phenotype of regenerated plants were confirmed.

[Materials and Methods]

In this research, *FNSII* was knocked out in Ilmi. The guide RNA was designed through CRISPR direct (https://crispr.dbcls.jp/). The guide RNA was designed as 20mer excluding the PAM sequence, and the GC content was 40-60%, the out-of-frame score was 68.0 or higher, and the mismatches were 0bp, 1bp, and 2bp values of 1-0-0. The designed guide RNA was ligated to pRGEB32 vector, and transformed into agrobacterium using a vector into which the guide RNA was correctly inserted through colony PCR and sequencing. Constructed *agrobacterium* was inoculated into Ilmi's callus which cultured for 3 weeks, and transfered selection medium and regeneration medium for plant regeneration.

[Results and Discussion]

A total of 3 guide RNAs were designed to knock-out *FNSII*. These were located at 23, 147, 161 in the gene sequence of *FNSII*, respectively, and all mismatches were 1-0-0. Guide RNA was synthesized using PCR, treated with a *Bsa*I restriction enzyme to insert into pRGEB32, a CRISPR/Cas9 vector, and then ligation with guide RNA. It was confirmed that the guide RNA was completely inserted through transformation in *E. coli*. When each guide RNA::pRGEB32 colony was picked and confirmed by three, it was completely inserted in all colonies, and colony PCR was performed to transformation into *agrobacterium* using the clear band. *Agrobacterium* was inoculated with Ilmi's callus.

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