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Over-expression of BdCHI Improves Anthocyanin Content in Brachypodium Seed

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[Introduction]

Brachypodium distachyon is a member of Poaceae and has been widely used as a model plant for important cereal crops and biofuel feedstocks such as wheat, barley, and switchgrass. *Brachypodium* has a small genome and the reference genome has been released. Gamma radiation has been a major source of creating genetic variability. Seed color is an important economic trait and affects customer preference. Anthocyanin accumulation in *Brachypodium* seed coat is responsible for *Brachypodium* seed coloration.

[Materials and Methods]

Brachypodium seedlings were subjected to gamma radiation at doses of 50, 100, 150, 200, and 250 Gy. A mutant line showed a seed coat color change from brown to yellow through several generations (M_3 to M_5). The mutant line was used for whole genome re-sequencing and a *chalcone isomerase* gene was selected as a candidate gene for the seed coat color change. The full-length CDS of *BdCHI* was inserted into an over-expression vector and the vector was subsequently transformed to *Agrobacterium*. Immature embryos collected from the mutant line and the wild-type were used for *Brachypodium* tissue culture. Embryogenic callus were used for *Agrobacterium*-mediated transformation. PCR reaction targeting the *npt* Π gene in the over-expression vector and *npt* Π ELISA assay were performed to detect transgenic plants.

[Results and Discussion]

Over-expression lines were obtained for the mutant line and the wild-type. T_1 seeds were harvested from PCR-positive and ELISA-positive plants. T_1 plants were used for *npt* \square ELISA assay to identify copy number of transgene. More than 50% of T_0 transgenic lines showed a 1:3 segregation ratio in the T_1 generation. Seed coat color recovery was observed in *CHI* over-expressing mutant lines. The yellow seed of the mutant line changed to brown caused by *CHI* over-expression. A reduction of anthocyanin in seeds of the mutant line was recovered in *CHI* over-expressing mutant lines.

[Acknowledgement]

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1A4A1030348).

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