Genetic characterization of large flower mutant *ohbana1* induced by heavy-ion beam irradiation in *Arabidopsis thaliana*†

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Flower size is an important trait that influences the economic value of ornamental plants, and most cultivars have relatively larger floral organs than their corresponding wild species. Thus, the genetic control of floral organ size is a principal target for ornamental plant breeding. The development of floral organs is based on two distinct processes: cell proliferation and cell expansion, which increase the cell number and cell size, respectively. Positive and negative regulatory genes corresponding to each developmental process have been identified in *Arabidopsis thaliana*. In this study, an Arabidopsis large flower mutant, *ohbana1* (*ohb1*), was isolated from a mutant library induced by heavy-ion beam irradiation.[1–3] We characterized the flower phenotypes of *ohb1* and revealed the gene responsible for the mutant phenotype by the resequencing of the mutant genome to gain more insight on the regulatory network for floral organ size.

Flowers at stage 14, which had fully expanded petals but had not been pollinated, were fixed with a fixative solution containing 86% ethanol and 14% acetic acid. After fixation, the flowers were dissected using a stereoscopic microscope, and abaxial epidermal cells in the distal region of the petals were observed and photographed. The genomic DNA for whole-genome resequencing was isolated from the leaves of 40 M_3 generation mutants. Library construction was performed according to the method reported by Hirano *et al.*[4] The obtained reads were input into the automated mutation analysis pipeline.[5] The mutations in the mutant induced by heavy-ion beam irradiation were selected by removing pre-existent polymorphisms in the wild type (WT) and false-positive mutations.

The sepal and petal sizes in the mutant were compared to those in the WT to evaluate the phenotypic characteristics of the *ohb1* mutant; the *ohb1* mutant formed large petals and sepals. The petal area in the *ohb1* mutant was approximately 1.4 times larger than that in the WT (Fig. 1A). Furthermore, cell sizes in the petal of the *ohb1* mutant were also 1.4 times larger than those of WT (Fig. 1B). We concluded that the large petal formation in *ohb1* is mainly caused by cell-size increment because the increasing rates in whole petals and its organizing cells are almost the same in *ohb1* petals.

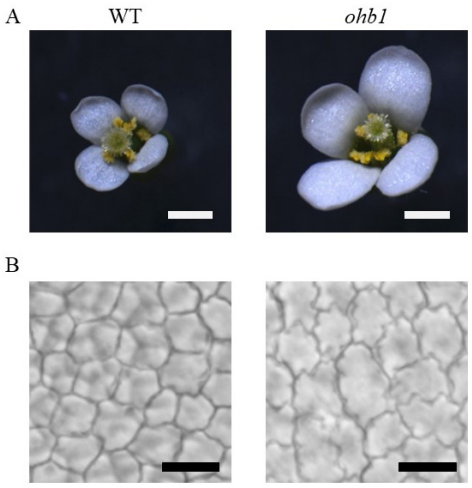

Fig. 1. Flower phenotypes of the *ohb1* mutant. Whole flower (A) and epidermal cells in the petal (B). Bars = 1 mm (A) and 20 μm (B).

In the M_3 generation, five homogenous mutations in three loci, which had been predicted to affect gene function, were detected in the mutant genome. The results of the linkage analyses and complementation test indicated that the mutated gene in AT4G04920 is responsible for the large flower phenotype. The gene in AT4G04920 encodes one mediator subunit, *MEDIATOR* (*MED*) *16*, and it has been identified as a multifunctional regulator. MED16 restricted post-mitotic cell expansion to control the final flower size in Arabidopsis, and these results agree with a previous study.[6]

Interestingly, MED16 also contributed to seed-size regulation. In double mutants crossed between *ohb1* and the representative large flower mutants, some of them formed larger and heavier seeds than each parental single mutant. Therefore, size-regulation pathways are believed to be partly different between floral organs and seeds. The *ohb* mutants derived from heavy-ion irradiation are valuable genetic resources for investigating the size-regulation pathways, and they can lead to further understanding of plant size control.

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