Identification of chromosomal region responsible for the phenotype of the novel torenia mutant *frilly petal undulation 1*

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Torenia fournieri is widely used as a horticultural plant in parklands and belongs to the Linderniaceae family. Its conspicuous flowers facilitate mutant screening by allowing observation of floral phenotypes after heavy-ion-beam irradiation. The relatively small genome size of *T. fournieri*, approximately 171 Mbp,[1] makes it easy to identify genes responsible for specific phenotypes. Leveraging this property, we have generated beneficial mutants of T. fournieri using heavy-ion-beam irradiation. One of these is the novel mutant, *frilly petal undulation 1* (*fpu1*), produced through irradiation of carbon-ion beams accelerated by the synchrotron equipped in the Wakasa Wan Energy Research Center. In this mutant, the petal edges are serrated, and the entire petal exhibits strong undulation (Fig. 1). To identify the gene responsible for this mutant phenotype, we performed long-read sequencing to assemble the genome of *T. fournieri*, subsequently identifying the chromosomal regions associated with this phenotype.

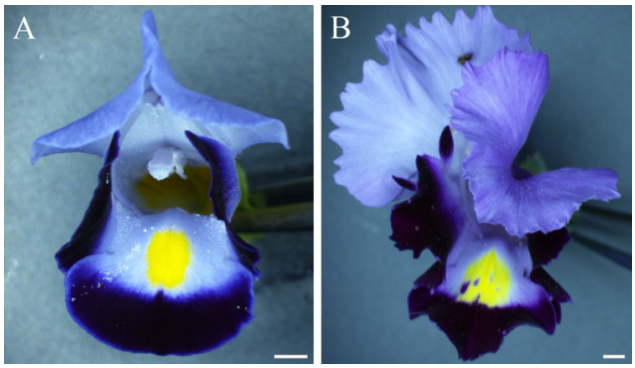

Fig. 1. Flower phenotypes of wild type (A) and *fpu1* (B).

The genomic DNA was extracted from leaves of the inbred line 'Zairai murasaki.' Extracted DNA was subjected to whole genome sequencing. The obtained reads were assembled by GeneBay Inc. Then, genomic DNA were extracted from the bulk of 36 BC2 individuals that showed the same phenotype as that of fpu1, and sequenced. The resulting reads were analyzed by using the mutation detection pipeline: AMAP[2] to detect the chromosomal regions responsible for *fpu1* mutant phenotypes.

The resulting genome assembly comprised of 150 contigs. Its features are as follows: a maximum contig length of 11.7 Mb, N50 length of 6.11 Mb, no gaps in the sequence, and total yield of 144 Mb.

The AMAP analysis identified three homozygous mutations in two distinct regions of contig 11, which is 8.2 Mbp in length (Fig. 2). One is a 4-bp deletion located in the first intron of the *PLENA* (*PLE*) gene, known to play a role in the development of pistils.[3] The other two mutations are a 14-bp deletion accompanied by a base substitution from C to A in two areas, and a single base substitution from G to T. Both were found in the promoter region of the *XYLEM NAC DOMAIN 1* (*XND1*) gene, which is involved in the development of lignocellulose in the xylem.[4] Any of these mutations could potentially be responsible for the *fpu1* mutant phenotype. Identification of the responsible gene is in progress.

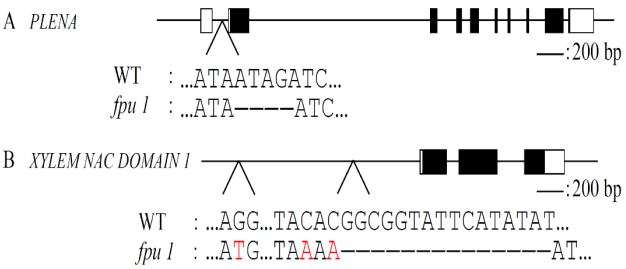

Fig. 2. Candidates of mutations responsible for the *fpu1* phenotype. (A) Gene structures of *PLE*. (B) Gene structures of *XND1*. White boxes, black boxes, red letters, and dashes indicate non-coding regions, coding regions, base substitutions, and deletions, respectively.

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References

1) S. Kikuchi *et al.*, Chr. Res. **14**, 665 (2006).
2) K. Ishii *et al.*, Genes Genet. Syst. **91**, 229 (2016).
3) K. Sasaki *et al.*, Planta **251**, 101 (2020).
4) C. Zhao *et al.*, Plant J. **53**, 425 (2008).