Effect of heavy-ion irradiation on survival rate of *Torenia fournieri*

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A heavy-ion beam is a powerful mutagen that has high linear energy transfer (LET). We previously demonstrated that the value of LET affects the size and type of the induced mutations in a model plant *Arabidopsis thaliana*; a C-ion beam with an LET of 30.0 keV/μm predominantly induce small mutations that affect almost single genes, while an Ar-ion beam with an LET of 290 keV/μm can induce chromosomal rearrangements including translocation and large inversions or deletions.[1] We focus on the induction of chromosomal rearrangements because they can affect multiple genes resulting in the induction of new phenotypic traits that have not been ever observed by single gene mutations.

To investigate such a broad spectrum of phenotypes, we aim at observing the floral phenotypes of *Torenia fournieri* after the irradiation of heavy-ion beams with high LET, because its simple floral structure with colorful petals allows us to observe the change in the phenotypes with high visibility.[2] Moreover, *T. fournieri* is a useful plant for genetic analysis and molecular biology because of its small genome size ($2n = 18$, 171 Mb), and because of the transformation techniques established.[3,4] Once an interesting mutant is isolated, mutations including rearrangements responsible for its phenotype can be determined by whole-genome resequencing. Dry seed irradiation and screening of seed propagated mutants needs to be conducted using an inbred strain, to efficiently perform the investigation of the phenotypic mutation spectrum followed by whole-genome resequencing. In this study, prior to performing mutant screening, dose dependence on survival was examined and the effect of LETs on survival was compared.

The inbred line 'Zairai murasaki' was kindly provided by Dr. Nishijima of the National Institute of Floricultural Science. Dry seeds of 'Zairai murasaki' were irradiated with Ar ions with LETs of 184 keV/μm and 290 keV/μm, respectively, at a dose range of 25–200 Gy in the RIKEN RI-beam factory. C-ion beam irradiation with an LET of 50 keV/μm was carried out by the Wakasa Wan Multi-purpose Accelerator with Synchrotron and Tandem at the Wakasa Wan Energy Research Center at a dose range of 100–500 Gy. Irradiated M_1 seeds were surface-sterilized and incubated on 0.7% agar containing 1/2 MS medium supplemented with 3% sucrose at 25°C under long-day conditions (16 h light, 8 h dark). About 30–50 seeds were sown for each irradiation treatment. After 1.5 months, the survival rate (the number of plants having true leaves per total number of germinated plants) was determined.

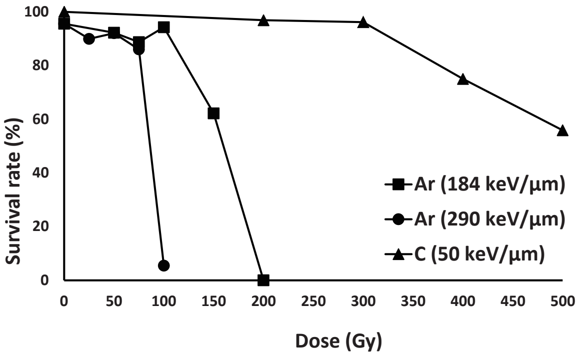

Fig. 1. LET-dependent effect on survival rate in *T. fournieri*.

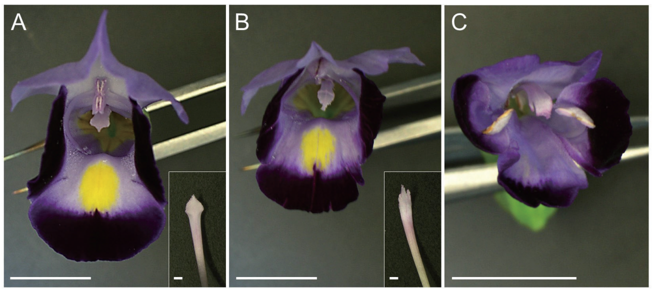

Fig. 2. Photographs of wild-type flower (A); a mutant flower with a rough-edged pistil and petals (B), and a mutant flower without the yellow spot (C). Bars: 1 cm for whole photos, 1 mm for the photos of pistils.

In any LET, survival rates were decreased as the irradiation dose increased (Fig. 1). The effect on survival rate differed among LET values; it increased as LET values increased. The Ar ion beam with the LET of 290 keV/μm was the most effective for the reduction of the survival rates, although the effect of higher values of LET will need to be tested. Based on the rule of thumb that a dose showing around 90% survival is the most effective in mutation induction, the most effective doses on mutation induction were estimated from the current results; 300 Gy, 75 Gy, and 50 Gy for the C ion (50 keV/μm), Ar ion (184 keV/μm), and Ar ion (290 keV/μm), respectively.

Mutant screening is carried out in the M_2 generation because a phenotype caused by recessive mutation is expressed in the M_2 generation. However, we identified two mutants from 30 and 16 M_1 plants after irradiation with LETs of 290 keV/μm and 184 keV/μm, respectively. The former has jagged pistil and petals (Fig. 2B), and the latter has no yellow spot (Fig. 2C). These phenotypes were observed in all flowers produced on the same branch. The detection of mutations responsible for these phenotypes is in progress.

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