Effects of heavy-ion beam and X-ray irradiation on flowering rate in *Arabidopsis thaliana*

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Heavy-ion-beam irradiation is widely used in plant mutation breeding, as it induces a wide variety of mutations with high efficiency. The effects of different types of radiation on plant development vary depending on the linear energy transfer (LET).[?,?] To induce mutations effectively, appropriate LET values (called LETmax) should be selected in each plant species. In *Arabidopsis thaliana*, LETmax was determined as 30 keV/μm.[?] Appropriate doses also need to be determined for each LET value by observing the flowering rate in M_1 generation. Generally, the dose range showing around "shoulder" of the survival rate was used as the appropriate choice. The "shoulder" refers to the dose range in which the survival rate of irradiated plants remains relatively high before experiencing a sharp decline at higher doses. In *A. thaliana*, the flowering rate was used to indicate the survival rate, and the "shoulder" doses in 23 keV/μm, 30 keV/μm, 62 keV/μm, and 290 keV/μm were found to be 450 Gy, >500 Gy, 250 Gy, and 50 Gy, respectively.[?] The optimal doses for mutation induction at 23 keV/μm, 30 keV/μm, 62 keV/μm, and 290 keV/μm were 250 Gy, 400 Gy, 150 Gy, and 50 Gy, respectively. The optimal dose for mutagenesis is estimated to be 56–100% of the "shoulder" dose. Given the limited availability of irradiation opportunity for heavy-ion beams, we hypothesized that X-ray irradiation can be used to estimate the survival rate shoulder for ion beam irradiation. Here, we compared the flowering rates between heavy-ion irradiation and X-ray irradiation.

Dry seeds of A. thaliana (Col-0) were irradiated with X-rays using Hitachi MBR1520R-4 (150 kV, 1-mm alminium filter, 3.6 Gy/min) or with carbon-ion beams at the RIBF. The LET values for the carbon-ion beams were 30 keV/μm. The absorbed doses ranged from 400 Gy to 700 Gy, as 400-Gy irradiation at 30 keV/μm have previously shown approximately 90% flowering rate.[?] After irradiation, the seeds were sown after sterilization on the 1/2MS plates with 0.7% agar supplemented with 3% sucrose under long day condition (16 hours light/8 hours dark). Then, germinated plants were transplanted to soil in flowerpots and grown in the same condition. Thirty-days after transplanting, the flowering rate was calculated as the number of individuals that reached flowering divided by the number of transplanted individuals. The 110 individuals were transplanted per test plot for each irradiation condition, and each experiment was repeated at least three times.

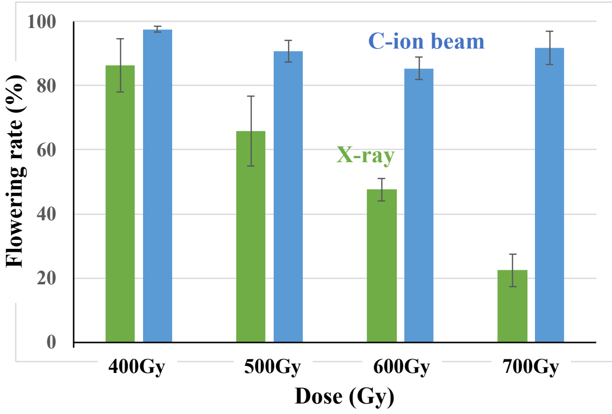

Fig. 1. The effects of irradiation on flowering rates. Each value is expressed as the mean ±SE.

The flowering rates were different between X-ray and carbon-ion-beam irradiations (Fig. 1). The shoulder dose for X-ray irradiation was approximately 400 Gy, while determining the shoulder dose for 30 keV/μm was challenging. Although the shoulder dose estimated from the optimal dose for mutation induction with 30-keV/μm carbon-ion irradiation was above 400 Gy,[?,?] the flowering rate did not decrease at 700 Gy. These results suggest that carbon-ion beams induce varying biological effects from X-rays, particularly in terms of the flowering rate.

The differences in DNA damage or mutation induced by each type of irradiation in the transplanted plantlets are interesting. This outcome highlights the importance of precise LET and dose selection for plant mutation studies. Further research is necessary to refine dose-response relationships and optimize irradiation conditions of carbon-ion beam irradiation for mutation breeding using X-ray irradiation.

References

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