Relationship between early-flowering mutation and LET-Gy combination of ion beam irradiation in einkorn wheat

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Bread wheat (*Triticum aestivum*) is a hexaploid species with the genome constitution AABBDD that were derived from three wild diploid ancestral species: the A genome from *T. urartu*, the B genome from *Aegilops speltoides* or another species classified in the Sitopsis section, and the D genome from *Ae. tauschii*. Therefore, the hexaploid wheat genome contains triplicated homoeologous genes, and unfortunately, this characteristic increases the difficulty of screening for mutants in bread wheat. To avoid this problem, we have chosen to use cultivated diploid einkorn wheat (*T. monococcum*) with A^m genome, similar to the A genome in bread wheat, for developing a large-scale mutant panel[1)]. To avoid the rainy season for harvesting, early maturing is one of the more important properties of bread wheat in East Asia, including Japan. Therefore, we focused on identifying early-flowering mutations in the screening of the mutant panel.

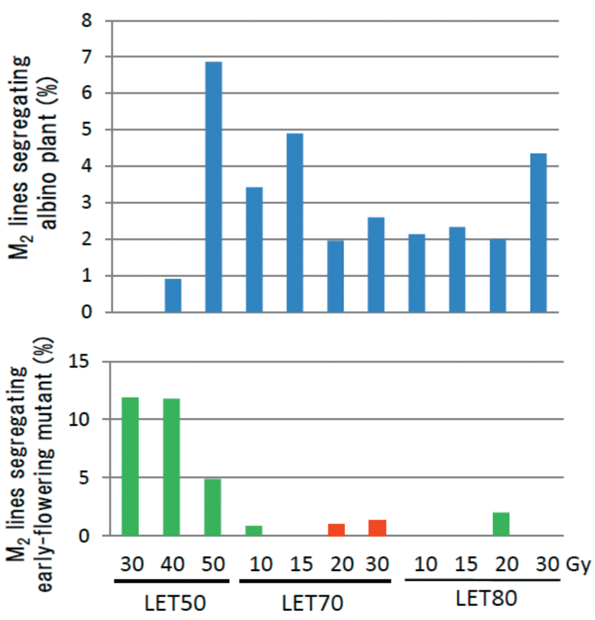

Fig. 1. Appearance of albino plants and early-flowering mutant plants in M_2 generation. Percentages (%) of M_2 lines segregating mutant plants are shown in each LET-Gy combination of ion beam irradiation. Green and red bars indicate early-flowering mutants and extra early-flowering mutants, respectively.

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In a previous study, we identified four extra early-flowering mutants, named *extra early-flowering1* (*exe1*), *exe2*, *exe3*, and *exe4*, which were headed 30–45 days earlier than the wild-type strain KU104-1[2)]. Here we report our recent study on the relationship between the early-flowering mutation and the LET-Dose (Gy) combination of ion beam irradiation.

Dry seeds of the diploid einkorn wheat (*T. monococcum*) strain KU104-1 were irradiated with 10, 15, 20, 30, 40, or 50 Gy of $^{12}C^{6+}$ ions with 50, 70, or 80 keV μm^{-1} LET to determine the optimal conditions for mutant generations, using the E5 beam line of Ring Cyclotron (RRC) in the RIKEN RI-beam factory. The germination rate was examined using the irradiated seeds (called M_1 seeds) that were sown in wet-paper-containing petri-dishes. 150 seeds (50 seeds with three replications) were tested for each LET-Gy combination. The germination rate was not affected by ion beam irradiation. The M_1 seedlings were planted in the field in October 2013, and the survival ratio was observed in May 2014 at the heading stage. The survival ratio was reduced to less than 80 % when LET of ion beam was 70 or 80 keV μm^{-1}. The harvested seeds from each individual M_1 plant were used to produce the next generation (M_2) lines. 23 - 134 M_2 lines (997 lines in total) for each LET-Gy combination were sown in October 2014 in the fields; ten seeds of each M_2 line were sown. The frequency of lines with albino plant(s) among the ten plants was determined to assess the comparative mutation ratio of the different irradiation conditions. The frequency of albino plants in the M_2 generation was different for different LET-Gy combinations (Fig. 1). The highest ratio was observed for the LET50-50Gy treatment condition. The data of survival ratio suggests that LET50-50Gy treatment was the optimal condition for einkorn wheat.

In the mutant screening in 2015, we observed early-flowering mutation within one week and extra early-flowering mutation before one week, compared with the wild-type. Fig. 1 shows the percentages of the M_2 lines segregating (extra) early-flowering and mutant plant(s). The early-flowering mutants were obtained under relatively moderate treatment conditions with LET50, while extra early-flowering mutants were obtained under harsher treatment conditions with LET70. The genes for early-flowering phenotypes would be good candidates in genome editing for fine-tuning of heading time in wheat.

References

1) K. Murai et al.: Nuclear Instruments and Methods in Physics Research B 314. **2013**, 59.
2) A. Nishiura et al.: Breeding Science 64. **2014**, 213.