Rapid evaluation of mutational effects resulting from heavy-ion irradiation of *Undaria pinnatifida*

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Undaria pinnatifida, called wakame in Japan, is a major commercial seaweed. Recently, there has been a demand for new cultivars with enhanced properties, such as high yield, high environmental adaptability, or high concentration of available contents for human health, to expand the market for wakame. Therefore, we performed the mutation breeding of *U. pinnatifida* through heavy-ion-beam irradiation.[1)] Optimization of the dose and linear energy fransfer (LET) in heavy-ion mutagenesis is essential for efficient mutant induction.[2)] However, a method for evaluating mutation frequency has never established in *U. pinnatifida*, and the optimization is still difficult. Moreover, in macroalgae, the analysis of mutation frequency in M_2 generation consumes much time and requires large space. In the present study, we irradiated zoospores with heavy-ion beams. Because the female and male gametophytes developed from the zoospores are in the haploid stage of its life cycle, mutant screening can be performed in M_1 generation. Through the mutant screening and investigation of the mutant phenotypes, we tried to develop a method for the effectiveness of heavy-ion mutagenesis.

Samples (3 cm × 3 cm) of sporophylls of *U. pinnatifida* were irradiated with C ions (LET: 30.0 keV/μm) at a dose of 0–25 Gy or Ar ions (LET: 280 keV/μm) at a dose of 0–10 Gy. The irradiated pieces were placed in beakers filled with sterilized seawater, and zoospores were induced. The zoospore suspension were diluted with Provasoli's enriched seawater with Iodine[3)] and poured into plastic dishes. The dishes were incubated at 20 ºC with 12 h photoperiods and a light intensity of 5 μmol m^{-2} s^{-1}. The gametophyte size was measured after 3 weeks of culture. The gametophyte over 100 μm in size of the longest cell filament was defined as a developed gametophyte, and the formation rate of developed gametophyte was calculated. Mutant screening was performed after 5 weeks of culture.

When we investigated the growth of the gametophytes developed from the irradiated zoospores, the number of developed gametophytes decreased with increasing irradiated dose (Fig. 1). A comparison between C-ion and A-ion irradiation revealed that the Ar-ion irradiation has high biological effect to the cell division or cell growth. After 5 weeks of culture, some mutants in cell shape, cell size, and intracellular structure were observed. One of the mutants showed a reduction in cell elongation (Fig. 2C, D). In the untreated control, the female gametophyte cells were larger than the male gametophyte cells (Fig. 2A, B). There were at least two types of cell elongation mutants: small-cell type (Fig. 2C) and large-cell type (Fig. 2D). One possible interpretation is that the small-cell type and the large-cell type cells were induced in male and female gametophytes, respectively. The mutation frequency for the cell elongation mutant tended to increase in a dose-dependent manner (data not shown). Therefore, the mutants can be used as an indicator for investigating the effectiveness of heavy-ion mutagenesis. We will evaluate LET-dependent effects for mutation induction in *U. pinnatifida* using this method.

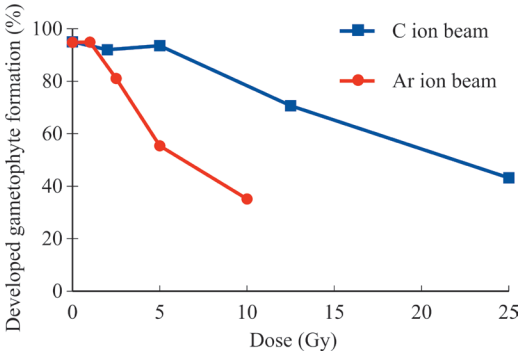

Fig. 1 Effect of heavy-ion irradiation on gametophyte development. The formation rates after 3 weeks of culture are expressed as the mean of two individual experiments.

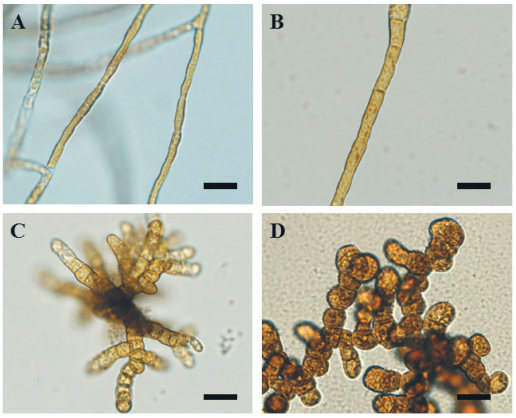

Fig. 2 Morphological mutants induced by C-ion irradiation. The male (A) and female gametophytes (B) from unirradiated zoospores were cultured for 5 weeks. The cell elongation mutants after 5 weeks of culture derived from the C-ion irradiation at 5 Gy (C) and 12.5 Gy (D). Bars indicate 20 μm.

References

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