Effects of heavy-ion beam irradiation on sporophyte survival and growth in *Undaria pinnatifida*

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Undaria pinnatifida, called wakame in Japanese, is one of the most popular seaweed in Japan, Korea, and China. It is the most important cultivated seaweed in Iwate and Miyagi Prefectures in Japan. In recent years, the wakame yield has been decreasing because of changing environment, unimproved productivity, and damage due to Tsunami. For the purpose of yield restoration and sustainable growth market of *U. pinnatifida*, the development of new cultivars with properties such as high yield or high environmental adaptability is necessary. We used heavy-ion-beam irradiation to induce mutagenesis in *U. pinnatifida* for the production of new cultivars.[1)] However, since not enough studies have been conducted in the mutagenesis of marine algae, it is important to produce efficient mutation breeding in *U. pinnatifida* to assess the relationship between survival rate after irradiation and mutation frequency. In this study, we irradiated *U. pinnatifida* sporophytes with C-ion and Ar-ion beam and analyzed the effects on sporophyte survival and growth in the M_1 generation toward successful mutant screening in M_2 generation.

Sporophytes of *U. pinnatifida* were obtained after fertilizing the male and female gametes. The sporophytes of 1-mm length were transferred into 15-ml plastic tubes for C-ion irradiation and hybribags for Ar-ion irradiation, both containing sterilized seawater, and were irradiated at dose ranges of 0–25 Gy (C ions) or 0–10 Gy (Ar ions). Each tube or hybribag contained 50 sporophytes. After the irradiation, the sporophytes were cultivated in 500-ml Erlenmeyer flasks containing 1/4 PESI medium, [2)] with aeration, at 15°C, photoperiod of 12 h/12 h (light/dark), and a light intensity of 90 µmol photons m^{-2} s^{-1}. The survival rate was measured after 3 weeks of culture. The plants surviving and reaching 20-mm with in the culture period were further cultivated in the Rotating and Flowing Land Tank System (PAT.P) at 10°C, photoperiod of 12 h/12 h (light / dark), and a light intensity of 180 µmol photons m^{-2} s^{-1} for 8 weeks. The total length and fresh weights of all the sporophytes were measured after blotting them dry.

In the flask cultivation after irradiation, dead sporophytes were observed. Almost all the dead sporophytes had formed within 1 week of cultivation and had wrinkled leaves (data not shown). The survival rates after 3 weeks of cultivation were decreased with increasing irradiation dose (Fig. 1). The survival rates in sporophytes irradiated using C-ion beam in sporophytes irradiated using C-ion beam were almost similar to those in female gametophytes derived from sporophylls irradiated using C-ion beam.[1)]

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The surviving sporophytes of 20-mm length were transferred to the land tanks. The total length and individual weight differed between the doses and ion species. The sporophytes irradiated with 2 Gy of C-ion beam were the largest: the maximum length and weight were 75 cm and 45.6 g, respectively. These values were approximately two times higher than those for the largest sporophytes in the control. The sporophytes irradiated with 25 Gy of C-ion and 2.5–10 Gy of Ar-ion remained withered for up to 2 weeks after culturing in the tanks.

The sporophytes irradiated with 2-12.5 Gy of C-ion and 1 Gy of Ar-ion formed sporophylls after cultivation for 2 months. Currently, we have cultured and performed mutant screening of the sporophytes in the M_2 generation. We will analyze the next generation of the large sporophytes obtained after C-ion irradiation at 2 Gy to confirm whether the phenotype is inheritable.

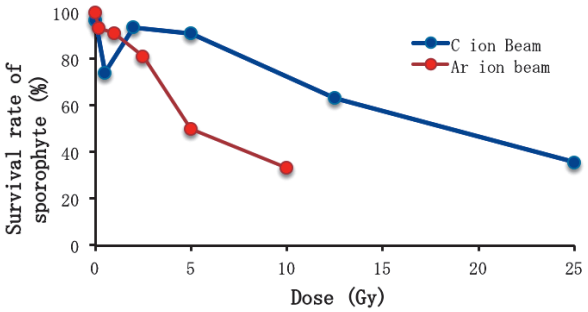

Fig. 1 Survival rates of young sporophytes irradiated with C-ion and Ar-ion beams.

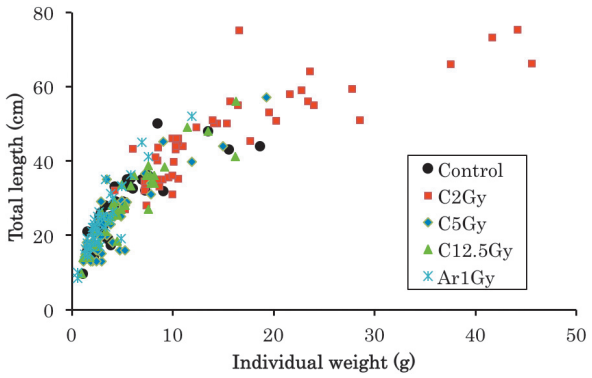

Fig. 2 Total length and individual weight of *Undaria pinnatifida* sporophytes measured 8 weeks after the irradiation with C-ion and Ar-ion beams.

References

1) Y. Sato et al.: RIKEN Accel. Prog. Rep. 46, 267 (2012)
2) M. Tatewaki: Phycologia 6, 62 (1966)