Screening for high-growth mutants in sporophytes of *Undaria pinnatifida* using heavy-ion beam irradiation†

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Undaria pinnatifida (Wakame) is primarily cultivated as a food resource on Japan's Sanriku coast, but production yield is declining due to environmental changes and a decrease in the number of producers. To meet domestic market demand, excellent strains that improve productivity per producer must be developed. Until now, there have been no results of mutation breeding using heavy ion beams in brown macroalgae. Our previous study developed a technique for producing mutants in the gametophyte from zoospores obtained by irradiance of heavy-ion beams on the sporophylls.[1)] However, this method limits the time for obtaining mature sporophylls. Therefore, in this study, gametophytes and young sporophytes of this alga were irradiated with a heavy-ion beam, and the subsequent culture was conducted using a tank system (CFCS)[2)] to select elite cultivars without aquaculture on the sea.

The mother plant for the irradiation was collected from Hirota Bay, Iwate Prefecture. According to the method described in previous studies,[1,2)] the zoospores were induced and cultured to gametophytes and sporophytes. Gametophytes, and sporophytes were irradiated with C ions (135 MeV/nucleon, LET, 30.0 keV/μm) at a dose of 0.5–25 Gy or Ar ions (95 MeV/nucleon LET, 284 keV/μm) at a dose of 0.2–10 Gy. The sporophytes obtained from these ions irradiated gametophytes, and the irradiated sporophytes were cultivated in a 7 L plastic tank for about two weeks and then transferred to a CFCS for cultivation. UV-sterilized seawater was used throughout the cultivation. The seawater temperature and light intensity were set at 10°C and 100 μmol photons m^{-2} s^{-1}, respectively. On the 100th day after the start of cultivation, mutant candidates (M_1) were selected based on a total length of 100 cm or more and an individual weight of 40 g or more. Cultivation was continued until sporophylls were formed and zoospores could be collected. The sporophytes obtained by crossing male and female gametophytes derived from the individual zoospore were cultured for 20 days, and the total length was measured. Subsequently, the culture of M_2 was continued, and those with a weight twice that of the non-irradiated group, at 100 days after germination, were selected as excellent mutant candidates (M_2). M_2 was then cultured until sporophylls were formed, released zoospores, and obtained gametophytes.

The survival rate of irradiated individuals varied depending on the irradiated generation, ions, and dose. The decreasing rate was more pronounced in the sporophyte than in the gametophyte at higher doses. Although the survival rates of female gametophytes irradiated with heavy-ion beams with C and Ar ions decreased with increasing dose, those of male gametophytes did not decrease after both irradiations. These differences in sensitivity to ions may be influenced by differences in haploid and diploid stages or differences in tissue structure.

In the M_1 generation, 18 individuals were selected from 210 sporophytes obtained from irradiated gametophytes, and 30 were selected from 297 irradiated sporophytes. As a result of cultivating the sporophyte obtained by self-crossing of these 48 individuals and confirming their growth, a total of four lines was obtained for particularly large individuals compared to others; 3 lines [1302G-R68 (C ions, 5 Gy), 1302G-B60 (Ar ions, 0.2 Gy), 1302G-W40 (Ar ions, 2.5 Gy)] from M_1 candidate of irradiated gametophytes, and one line [1302S-R40 (C ions, 2 Gy)] from the M_1 candidate of the irradiated sporophyte (Fig. 1). Among these four lines, the weight of the largest individuals on the 100th day of cultivation was two to three times that of the non-irradiated group, so they were selected as M_2 individuals. In addition, a sensory test was conducted after cutting off a part of the blade and boiling it; the taste and texture of M_2 candidates did not differ from those of the non-irradiated individuals. As a result of cultivating the next-generation sporophytes from the gametophytes obtained from M_2, all four lines showed earlier growth in size than non-irradiated individuals, similar to the M_2 generation. These lines are stored as M_3, confirming the fixation of the mutation. In the future, these high-growth mutants can be used for food production and biomass utilization, including feed and fertilizer.

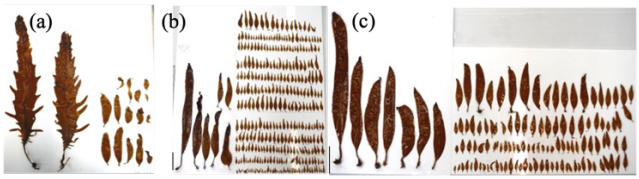

Fig. 1. Sporophytes in selected M_2 lines (3 lines out of total 4 lines) containing high-growth mutants 100 days after germination. (a): 1302G-B60 line, (b): 1302G-W40 line, (c) 1302S-R40 line. Bars = 10 cm.

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References

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