Growth inhibition of marine red alga *Agardhiella subulata* through C-ion beam irradiation

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In the Japanese way of life, seaweeds such as laver, kelp, wakame, and hijiki have a long history of use and are consumed in large quantities. Recently, seaweeds have gained attention as materials for the production of carrageenan, alginic acid, and agar,[1] and their production has been increasing in Asia as an aquaculture marine resource rather than for food. Furthermore, seaweeds contain fucoidan, which has proven to be effective in hair growth promotion,[2] immunostimulatory activities,[3] and anti-aging.[4] This information has led to increasing attention to seaweeds as raw materials for functional materials.

Agardhiella subulata, commonly known as edible red algae, has been discovered as a new material that produces fucoidan,[5] and there is much interest in increasing the production of its active ingredients. Breeding new cultivars with properties such as high yield, high environmental adaptability, and high concentrations of constituents with human health benefits is desirable for enhanced value. In this report, to determine the effective conditions of the C-ion beam for red algae mutagenesis, we calculated the growth in the area of irradiated regenerating buds.

Small fragments of *A. subulata* were cultured with NORI SEED (Daiichi Seimo Co., Ltd.), a highly enriched culture solution. A culture medium was prepared using 160 μL of NORI SEED diluted with 300 mL of autoclaved sea water. Small fragments developed into regenerating buds for three weeks in a stirred 300-mL marine flask containing the culture medium. The flask was placed at 25°C for a 12 hours photoperiod with a light intensity of 50 μmol photons $\cdot$ m^{-2}s^{-1}, and the culture medium in the flask was replaced every week. The regenerating buds were replaced into 5-mL tubes at each irradiation condition.

Heavy-ion-beam irradiation was performed using C (LET = 30 keV/μm) at 8 irradiation doses of 5, 10, 20, 40, 70, 100, 200, and 400 Gy generated at the RIKEN RIBF.[6] Four hours after irradiation, eight regenerating buds were inoculated into each 36-mm dish containing 3 mL of fresh culture medium for each irradiation condition. The dishes were placed at 25°C for a 12 hours photoperiod with a light intensity of 50 μmol photons $\cdot$ m^{-2}s^{-1}, and 150 μL of the 20-fold concentrated culture medium was added every 2–3 d. The regenerating buds were photographed every few days and the sizes of 8 reproducing individuals ($n = 8$) were calculated using Image J software version 1.5.1[7] (Fig. 1).

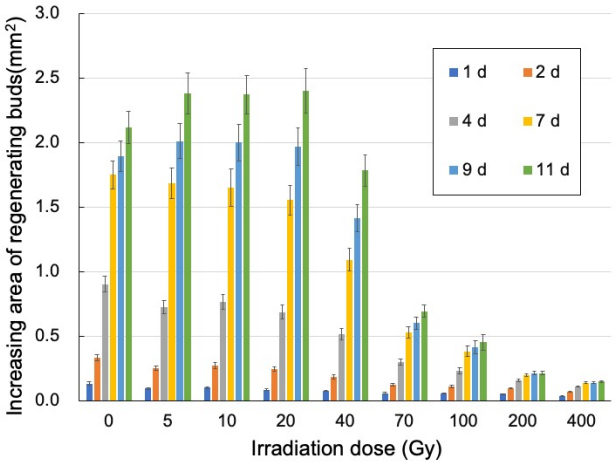

Fig. 1. Correlation between increasing area of regenerating buds and C-irradiation dose. A correlation chart using the average values of increasing area was constructed for the unirradiated control and irradiated regenerating buds. The vertical vars represent the standard errors.

An increase in the area representing the regenerating buds irradiated up to 20 Gy in the correlation chart indicates that they proliferate in the same way as unirradiated controls (Fig. 1). In contrast, the growth inhibition of the regenerating buds is observed in a dose-dependent manner effected by 40–400 Gy C-ion beams (Fig. 1). In future studies, we will carry out large-scale screening under each irradiation condition, and the optimum irradiation conditions will be determined based on the frequency of the appearances of the mutant lines.

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References

1) Y. C. Liao *et al.*, Bioengineered **12**, 3787 (2021).
2) I. S. Moon *et al.*, Hear. Res. **282**, 236 (2011).
3) R. Li *et al.*, Mar. Drugs **21**, 18 (2023).
4) R. Zhang *et al.*, Carbohydr. Polym. **15**, 228 (2020).
5) C. Faggio *et al.*, Nat. Prod. Res. **30**, 2016 (2015).
6) T. Abe *et al.*, in *Plant Mutation Breeding and Biotechnology*, edited by Q. Y. Shu *et al.* (CABI, Oxfordshire, 2012), p. 99.
7) C. Schneider *et al.*, Nat. Methods **9**, 671 (2021).