

## Isolation of morphological mutants in a bryophyte model *Marchantia polymorpha* using heavy-ion mutagenesis

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Since land plants emerged approximately 470 million years ago, their body plans have changed significantly during evolution through a transition from gametophyte-dominant to sporophyte-dominant life histories and the development of many specialized tissues and organs, such as stomata, vascular tissues, roots, leaves, seeds, and flowers. Bryophytes occupy a basal position in land-plant evolution, and the haploid ( $n$ ) gametophyte is dominant over the diploid ( $2n$ ) sporophyte in their life histories, while angiosperms (flowering plants), the last diverged group of land plants, have diploid ( $2n$ ) sporophyte-dominant life histories. For better understanding of the evolution of body plan in land plants, the accumulation of knowledge about the molecular mechanism for the gametophytic organ development in bryophytes is needed. Forward-genetic approaches focusing on the gametophyte development in bryophytes are especially important.

The bryophyte *Marchantia polymorpha* is a classical but recently emerging model plant. Recently, powerful experimental tools for molecular genetics have been established in *M. polymorpha*, including high-frequency transformation techniques and genome-editing techniques using the CRISPR/Cas9 system,<sup>1</sup> in addition to the whole genome sequence information.<sup>2</sup> As it exhibits low genetic redundancy in most regulatory pathways,<sup>2</sup> *M. polymorpha* should be advantageous for forward-genetic approaches. Similar to other bryophytes, the spores are haploid; therefore, the effect of the induced mutations can be immediately observed in the first gametophyte generation developed from the spores.

In this study, we adopted a heavy-ion beam as the mutagen for screening mutants with altered gametophyte morphology in *M. polymorpha*. Spores, which are single

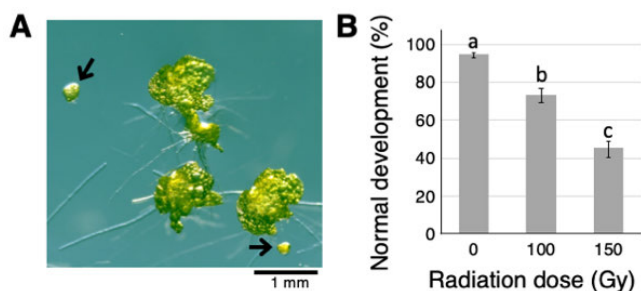


Fig. 1. Assessment of the mutagenicity of carbon-ion beams on the spores of *M. polymorpha*. Plants grown from the spores irradiated with carbon-ion beams were observed (A), and the ratio of normal thallus development was evaluated (B) ( $N = 4$ ,  $n = 69$ – $466$ , bar = SD). Values accompanied by different letters are significantly different (Tukey's test,  $p < 0.05$ ). Arrow, growth-arrested plants.

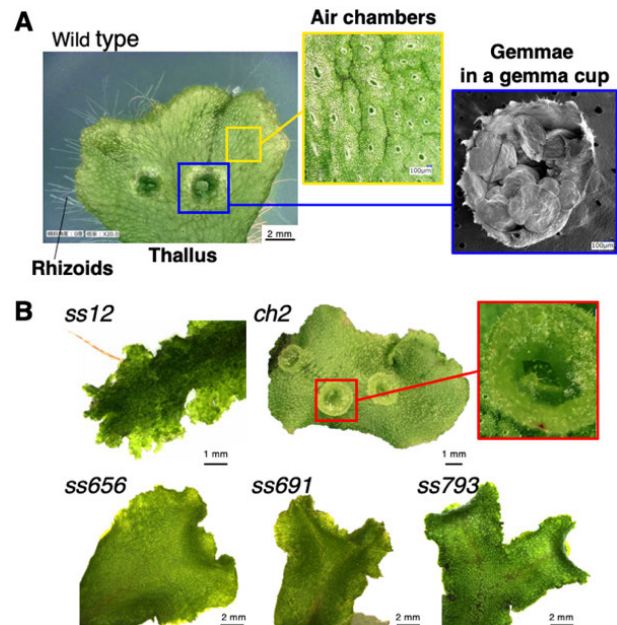


Fig. 2. Wild-type thallus (A) and isolated morphological mutants (B) of *M. polymorpha*.

cells and the start point of the development of the thallus (plant body) in *M. polymorpha*, were irradiated with a carbon-ion beam (LET:  $30 \text{ keV}/\mu\text{m}$ ) at a dose of 100 Gy or 150 Gy. Carbon-ion irradiation decreased the ratio of the normal thallus development from the spores in a radiation-dose-dependent manner (Fig. 1A and B).

Subsequently, the plants developed from spores irradiated with carbon-ion beams were further cultured for several weeks to screen for mutants. To date, 19 mutants showing abnormal thallus development were successfully isolated among  $\sim 9,000$  individuals. As shown in Fig. 2A, on the thallus surface of the wild-type plant, air chambers (yellow square) and gemma cups with clonal progenies, gemmae (blue square), are developed. Two of the 19 isolated mutants develop defective air chambers, 10 have abnormal gemmae and gemma cups, and 7 showed defects in both air-chamber and gemma-cup development. For example, a mutant *ss12* shows a marked decrease in the frequency of air-chamber formation and *ch2* has gemma cups with no gemma (red square), while *ss656*, *ss691*, and *ss793* are deficient in the development of gemma cups (Fig. 2B). We are currently conducting the detailed characterization of morphological phenotypes in these mutants, in parallel with the identification of the causal genes using next-generation sequencing (NGS). These mutants seem promising to reveal important factors functioning in the gametophytic organ development in the model basal land plant *M. polymorpha*.

### References

- 1) K. Ishizaki *et al.*, *Plant Cell Physiol.* **57**, 262–270 (2016).
- 2) J. L. Bowman *et al.*, *Cell.* **171**, 287–305 (2017).

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