

Effects of carbon-ion irradiation to male gametes on double fertilization in *Cyrtanthus mackenii*

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Heavy-ion-beam mutagenesis has been applied for various plant materials, and many mutants have been successfully obtained through the screening process. As one of the plant materials for the irradiation, pollen was used for mutant induction.^{1,2)} When pollen grains of *Cyrtanthus mackenii* (Amaryllidaceae) were irradiated with a carbon-ion beam, inhibitory effects on pollen tube growth were not observed.³⁾ It is reported that generative cells in the irradiated pollen grains can recognize and manage genomic lesions using DNA damage response pathways during pollen tube growth. However, with high-dose irradiation (40 Gy), sperm cell formation is prevented and the generative cells are not divided into two sperm cells; however, the cell cycle progresses. The male gametes are called generative-cell-like sperm cells (GC-like SCs). In this study, we analyzed the reproduction process involved in the irradiated pollen grains to reveal the behavior of male gametes during double fertilization.

Anthers of *C. mackenii* in 0.2-mL tubes were irradiated with C ions (22.5 keV/ μm) at a dose of 10 or 40 Gy and then stored at -20°C . The pollen grains with or without carbon ion beam irradiation were crossed with unirradiated female organs. The ovules were fixed with an FAA solution (formaldehyde, acetic acid, and ethanol) for 3 days after the crossing (DAC), and we observed the developmental states of the ovules by using the paraffin section method.

In the embryo sacs at 3 DAC with the unirradiated pollen grains, a degenerated synergid cell, an undivided egg, and central cells were observed. In the sexual reproduction process of higher plants, one of the synergid cells, which succeed in interacting with the pollen tube, is degenerated. Therefore, it is indicated that the pollen tubes reached one of the synergid cells until 3DAC, and the embryo sacs were considered immediately before or after fertilization. In addition to the embryo sacs at the fertilization phase, we observed abnormal embryo sacs without the egg apparatus and central cell (Fig. 1). The proportions of the embryo sacs in the fertilization phase, abnormal development, and unfertilization were 79%, 9%, and 12%, respectively.

In the pollen grains irradiated at 10 Gy, pollen tubes also arrived at the embryo sacs until 3 DAC. The proportions of the embryo sacs in the fertilization phase, abnormal development, and unfertilization were 79%,

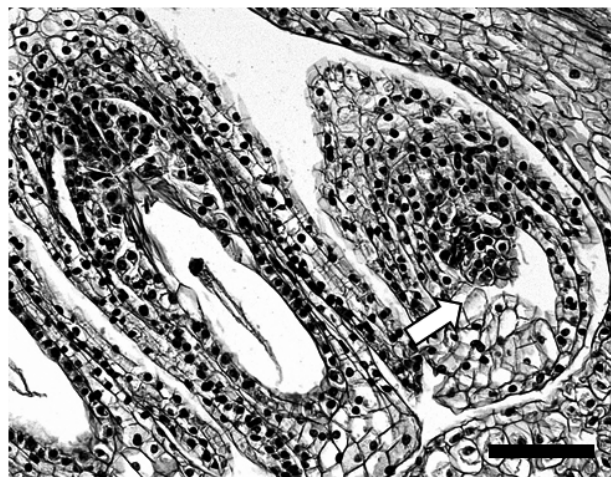


Fig. 1. Abnormal embryo sac formed 3 days after the crossing with unirradiated pollen grains. The arrow indicates the abnormal embryo sac. The embryo sac on the left side has a central cell. Bar = 50 μm .

6%, and 15%, respectively, and were the same as that in the case of unirradiated pollen grains. After 40 Gy irradiation, the proportion of embryo sacs in the fertilization phase was 58% and that in the abnormal development was 28%. These results indicated that pollen tubes from the irradiated pollen grains also arrived at the embryo sacs until 3 DAC.

Since the abnormal embryo sacs were commonly observed with or without carbon-ion irradiation, the abnormal development is interpreted to be affected by environmental factors or the plant status. Based on the interpretation, the proportion of embryo sacs at the fertilization phase is thought not to be drastically decreased in the pollen grains irradiated at 40 Gy. It is also suggested that inhibitory effects on pollen tube growth would not be observed *in vivo*. However, there is a possibility that under high-dose irradiation, pollen tubes or male gametes promote abnormal embryo sac formation.

We are now observing embryo sacs after 14 DAC. Those data are expected to reveal double fertilization and the embryogenesis process involved in the irradiated male gametes and will be useful for understanding abnormal embryo sac formation.

References

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