Analysis of DNA damage response in *Cyrtanthus* pollen after Ar-ion beam irradiation

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Heavy-ion-beam mutagenesis has been applied for various plant materials, and many mutants have successfully been obtained through the screening process. As one of the plant materials for the irradiation, pollen was used for mutant induction.[1,2] This implies that the DNA damage induced by a heavy-ion beam would be repaired during the double-fertilization process, and the genomic information including mutations would be transmitted to the next generation. DNA damage response (DDR) in male gametes of *Cyrtanthus mackenii* after C-ion beam (22.5 keV/μm) irradiation has been reported, and the male gametes repaired the DNA lesions during the pollen tube growth.[3] In the present study, we irradiated mature pollen of *C. mackenii* with an Ar-ion beam, which has a higher linear energy transfer (LET) than the C-ion beam, and analyzed the DDR in the male gametes. Moreover, we compared the DDR after irradiation with Ar-ion and C-ion beams.

Anthers of *C. mackenii* in 0.2-mL tubes were irradiated with Ar ions (280 keV/ μm) at a dose of 2.5–40 Gy and then stored at −20°C. Pollen grains from the anthers were cultured in 2 mL of liquid pollen culture medium at 25°C in the dark.[4] Sperm cell formation in the pollen tube was observed after 4′,6-diamidino-2-phenylindole staining of the pollen tube. Immunocytochemical analysis for the evaluation of DDR in male gametes was performed according to a protocol described previously.[3]

When the germination rate of the pollen grains and pollen tube length were measured after 24 h of *in vitro* culture, the germination rate and pollen tube length in irradiated pollen showed no decrease compared to non-irradiated pollen. These results were similar to those for C-ion irradiation.[3] Since *C. mackenii* forms bicellular pollen, sperm cells are divided from a generative cell in the pollen tube. Although the sperm cell formation rate was not decreased up to 10-Gy irradiation, sperm-cell formation was inhibited by high-dose irradiation of 20 and 40 Gy (Fig. 1). In the C-ion-beam irradiation, the sperm-cell formation rates were decreased to approximately 70% at 40 Gy and 30% at 80 Gy.[3] Thus, it is interpreted that the Ar-ion beam has a greater effect of inhibition of sperm-cell formation compared to the C-ion beam.

To investigate the cause of inhibition of sperm-cell formation, male gametes were isolated from pollen tubes and immunostained by using anti-α-tubulin antibody for cell-cycle confirmation and by using anti-

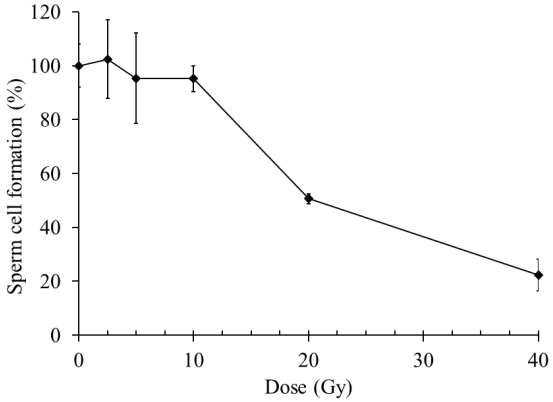

Fig. 1. Sperm cell formation after Ar-ion beam irradiation. Values ± SD are expressed relative to the unirradiated control (value set at 100%).

phosphorylated histone H2AX (γH2AX) for the detection of DNA double-strand breaks (DSBs) in the chromosome. After 40-Gy irradiation, the cell cycle in a part of the generative cells was arrested at the metaphase in pollen mitosis II (PMII) at 24 h of culture. In the male gametes, proportions of metaphase cells were 0% at 0 Gy, 1% at 10 Gy, and 35% at 40 Gy, suggesting that cell-cycle arrest was induced by the high-dose irradiation. The proportions of metaphase cells at 24 h of culture after the C-ion-beam irradiation were approximately 8% at 10 Gy and 11% at 40 Gy.[3] These results indicated that one of the causes of the inhibition of sperm-cell formation is the cell cycle arrest. Approximately half of the metaphase cells with 40-Gy Ar-ion irradiation showed γH2AX foci in the chromosomes, indicating that unrepaired DSBs remained in the genome. Therefore, a spindle assembly checkpoint in the metaphase would be activated by chromosomal lesions including the DSBs and arrested in the cell cycle progression.

In the C-ion-beam irradiation, chromosomal bridges were formed between the sperm cells, and generative-cell-like sperm cells, which are defined as cells with generative-cell-like nuclei and sperm-cell-like microtubule arrays that completed PMII but failed in chromosome separation, were also formed.[3] We also analyzed the male gametes that completed PMII in the Ar-ion-beam irradiation, and those data will be useful for understanding the effects of heavy-ion-beam irradiation on male gametes.

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References

1) K. Naito *et al.*, Genet. **169**, 881 (2005).
2) Y. Kazama *et al.*, Sci. Rep. **6**, 18917 (2016).
3) T. Hirano *et al.*, AoB Plants **5**, plt004 (2011).
4) T. Hirano, Y. Hoshino, J. Plant Res. **122**, 225 (2009).