Distinctive development of embryo and endosperm caused by male gametes irradiated with carbon-ion beam†

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Heavy-ion beam irradiation has been applied to pollen for mutant induction and genetic analyses.[1,2] However, the details of the fertilization process of irradiated male gametes and early development of the embryo and endosperm have not received much attention and remain largely unclear. In *Cyrtanthus mackenii*, DNA damage responses in male gametes of heavy-ion beam-irradiated pollen were investigated. *C. mackenii* forms bicellular pollen, and the generative cell divides into two sperm cells during pollen tube growth. Irradiated generative cells with DNA double-strand breaks (DSBs) are arrested at metaphase, and the DSBs are repaired.[3,4] Sperm cells passing through metaphase show abnormalities in chromosomal separation, and chromosomal bridges are formed frequently. To understand the mechanisms that maintain genome stability and influence mutation selection during the double fertilization process, it is necessary to investigate the fertilization of gametes with DNA damage or mutations and the processes of embryo and endosperm development. Therefore, in this study, male gametes irradiated with a carbon-ion beam were fertilized, and the embryo and endosperm development were analyzed.

Anthers of *C. mackenii* were irradiated with a carbon-ion beam (22.5 keV/μm) at absorbed doses of 10 and 40 Gy and then stored at -20°C until further analysis. Two days after emasculation, unirradiated and irradiated pollen grains were pollinated. The pistils were collected 14 days after pollination (DAP) and fixed in 4% paraformaldehyde. Enlarged seeds were removed from the ovaries under a stereomicroscope and dissected using tweezers and glass needles. The isolated embryo sacs were stained with 1 μg/mL of Hoechst 33258 and 0.1% (v/v) polyoxyethylene (20) sorbitan monolaurate in PBS for 30 min and mounted on slide glass in an antifade reagent. Images were obtained at 1.0 μm steps along the Z-axis using a confocal laser scanning microscope.

To investigate the effects of fertilization with carbon-ion beam-irradiated male gametes on embryo and endosperm formation, the embryo sacs in immature seeds at 14 DAP were observed. When non-irradiated pollen grains were pollinated, embryo sacs containing an embryo and an endosperm with a syncytium structure were observed (Fig. 1a). In contrast, after pollination with irradiated pollen grains, embryo sacs with an egg cell or an undivided zygote and a syncytium endosperm were observed in addition to normal embryo sacs (Fig. 1b). The embryo sacs were categorized based on embryo and endosperm formation. In fertilization of irradiated male gametes, 2% and 14% of the embryo sacs showed no embryo development at 10 and 40 Gy, respectively, and the number of embryo sacs without embryo development increased in a dose-dependent manner. No embryo sacs without endosperm development were observed.

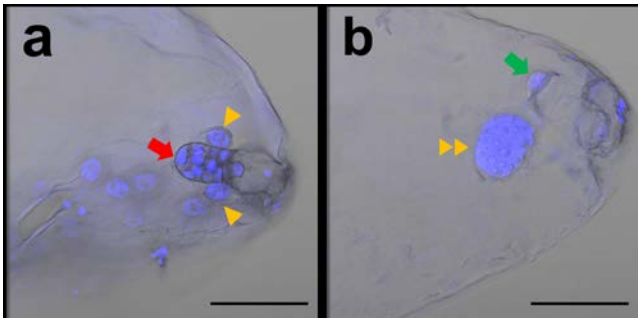

Fig. 1. Embryo sacs isolated from immature seeds. Embryo (red arrow) and endosperm nuclei (yellow arrowhead) derived from pollination of 0 Gy-irradiated pollen grain (a). An egg cell or an undivided zygote (green arrow) and enlarged endosperm nucleus (yellow arrowheads) derived from pollination with 40 Gy-irradiated pollen grain (b). Bars = 100 μm.

Abnormalities in chromosome segregation and enlarged nuclei were observed only in the endosperm nuclei, irrespective of the presence or absence of embryogenesis. Therefore, it is considered that the male gamete nuclei fuse with the polar nuclei, and that DNA damage and mutations in the male gametes are inherited by the endosperm nuclei and caused by endoreduplication through repeated cell cycles with incomplete nuclear division. In contrast, no chromosomal aberrations were observed in the embryo nuclei. This suggests that cell cycle checkpoints in the zygote are stricter than those in the endosperm and that mitosis does not proceed in the presence of DNA damage or abnormalities in chromosome segregation. Since carbon-ion irradiation causes chromosomal rearrangements even at low doses,[3,4] it is expected to induce relatively few genome-wide mutations. The effects of chromosomal rearrangements on double fertilization could be analyzed in more detail using pollen irradiation with a carbon-ion beam.

References

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