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Ultrastructural Changes of Cell Organelles in *Arabidopsis* Stems after Gamma Irradation

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We examined ultrastructural changes of the cell organelles of *Arabidopsis* stems in response to gamma irradiation. Seedlings treated with 0 to 5 Gy developed normally, while height growth in plants exposed to 50 Gy was significantly inhibited. Based on TEM observations, the chloroplasts were extremely sensitive to such irradiation. In particular, the thylakoids were heavily swollen, some portions of the mitochondria and endoplasmic reticulum were structurally altered, and the plasmalemma had pulled away from the cell wall in places. However, no ultrastructural changes in cell organelles occurred at doses of 0 to 5 Gy.

Keywords: chloroplast, gamma irradiation, thylakoid, transmission electron microscopy, ultrastructure

Gamma ray is a form of ionizing radiation that interacts with atoms and molecules to produce free radicals. These processes can damage or excite various important compounds. Low- or high-doses of ionizing radiation were used to stimulate or inhibit seed germination, plant growth, and productivity in various plants respectively. For example, low-doses radiation can enhance cell division, growth, and development in various plant and animal organisms (Luckey, 1980; Planel et al., 1987; Sagan, 1987; Kim et al., 1997, 2000; Korystov and Narimanovo, 1997). In contrast, seedling growth is inhibited after high dose gamma irradiation (Bagi et al., 1988). However, the mechanisms for these responses are not well known and are limited. By determining the morphological changes to cellular organelles at the ultrastucural level, we can gain insight into the mode for ionizing radiation. Therefore, our objective was to study the effect of prolonged, low- and high-doses of gamma irradiation on cells in the stems of *Arabidopsis*.

MATERIALS AND METHODS

Plant Material and Gamma Irradiation

Seeds of *Arabidopsis thaliana* L. ecotype Columbia were surface-sterilized with 0.01% (v/v) Triton X-100 (in 70% ethanol) for 10 min. After successive washing with absolute ethanol, they were plated in effendorf tubes containing 0.1% (w/v) agarose solution and kept in the dark at 4°C for 3 d. The seedlings were then maintained for 5 weeks in a growth chamber set at 22/20°C (day/night), under a 16-h photoperiod. Irradiation treatments include exposure to 1, 2, 5, or 50 Gy, as generated by a gamma irradiator (^{60}Co, ca. 150 TBq capacity, AECL) at the Korea Atomic Energy Research Institute, Korea. Those doses were confirmed with thermoluminescence dosimeters. Following these treatments, heights of 20 seedlings were measured at 2-d interval.

Transmission Electron Microscopy (TEM)

Arabidopsis stems were cut into 1-mm^2 segments and placed immediately in a freshly prepared mixture of 2% (w/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 50 mM sodium cacodylate buffer (pH 7.4). The segments were then de-gassed and fixed under vacuum for 4 h at room temperature. After washing in the same buffer, they were post-fixed in 1% (w/v) osmium tetroxide (in the same buffer) and washed again. Dehydration was done through a graded acetone series. The specimens were then infiltrated and embedded in a low-viscosity epoxy resin (Spurr, 1969). Ultrathin sections (80 nm) were mounted on uncoated nickel grids (300 mesh) and sequentially stained with uranyl acetate and lead citrate before being

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examined at 80 kV under a transmission electron microscope (J-1010, Jeol, Japan).

RESULTS AND DISCUSSION

Seedling Growth

We used seedling height to determine how morphological changes are related to growth rates and doses of gamma irradiation. No significant alterations in phenotype were observed with plants treated at 0 to 5 Gy, but stem lengths were remarkably different between those exposed to 50 Gy versus the control plants (Fig. 1), particularly at 2 weeks after gamma irradiation (Fig. 2). For all experiments, seedling heights progressively increased throughout the measuring period; exposure to relatively low doses (i.e., 1 to 2 Gy) appeared to slightly, but not significantly, enhance growth compared with the performance of the controls. This stimulating effect, however, was not appreciable under treatment conditions at 5 Gy. In contrast, the rate of height growth was significantly decreased when seedlings were exposed to a relatively high dose of 50 Gy

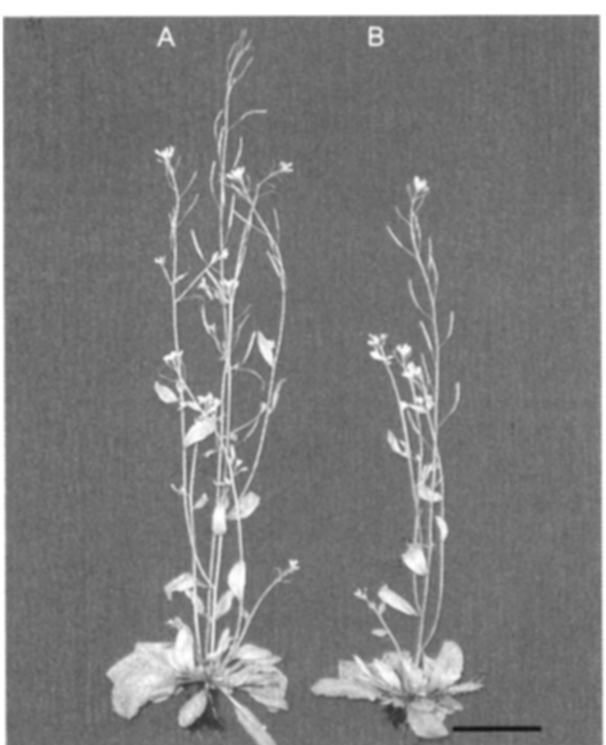

Figure 1. Phenotypes of control plant (**A**) and seedling irradiated with 50 Gy (**B**), at 6 d after treatment. Bar = 5 cm.

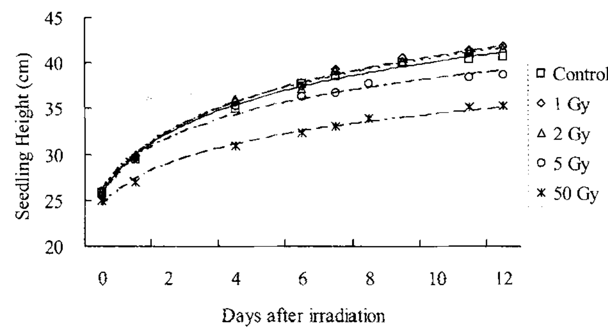

Figure 2. Seedling height growth over 12 d of cultivation for 5-week-old seedling treated with gamma irradiation.

(Fig. 2). These trends have been reported for other plant species as well (Kim et al., 1997, 2000; Kim et al., 2004). We believed that differences in seedling height are the result of physiological and biochemical changes that are induced by relatively low or high gamma irradiation.

Ultrastructural Changes in Cell Organelles

Arabidopsis stem sections were studied by TEM, focusing on the ultrastructural changes in cell organelles of stem exposed to 1, 5, or 50 Gy. Their structures were generally similar between low- and high-dose treatments. Cortical cells were highly vacuolated with the cytoplasm forming a thin layer between the cell wall and vacuole (Fig. 3). The electron density was high in the cytoplasm, which contained various organelles, e.g., chloroplasts, endoplasmic reticulum (ER), mitochondria, and ribosomes (Fig. 3A-D). For both the control and the relatively low-dose treatments (1 and 5 Gy), the chloroplasts showed a typical structure, having an ellipsoidal shape and well-displayed thylakoid membranes in the grana and stroma (Fig. 3A-C). In contrast, the chloroplast structure in cortical cells of stem after 50 Gy irradiation was obviously altered, with thylakoids being considerably swollen and destructed (Fig. 3D). However, chloroplast sizes were not significantly different between the low (1 to 5 Gy) and the high (50 Gy) doses. For both the control and the lower doses (1 to 5 Gy), the mitochondria possessed well-organized cristae (Fig. 4A-C). However, in stems exposed to 50 Gy, the mitochondria distorted and ER membranes were distended (Fig. 4D). After low dose (1 to 5 Gy) treatment, those mitochondria were slightly enlarged, but were still similar in size to the control at the higher dose of 50 Gy (Fig. 4).

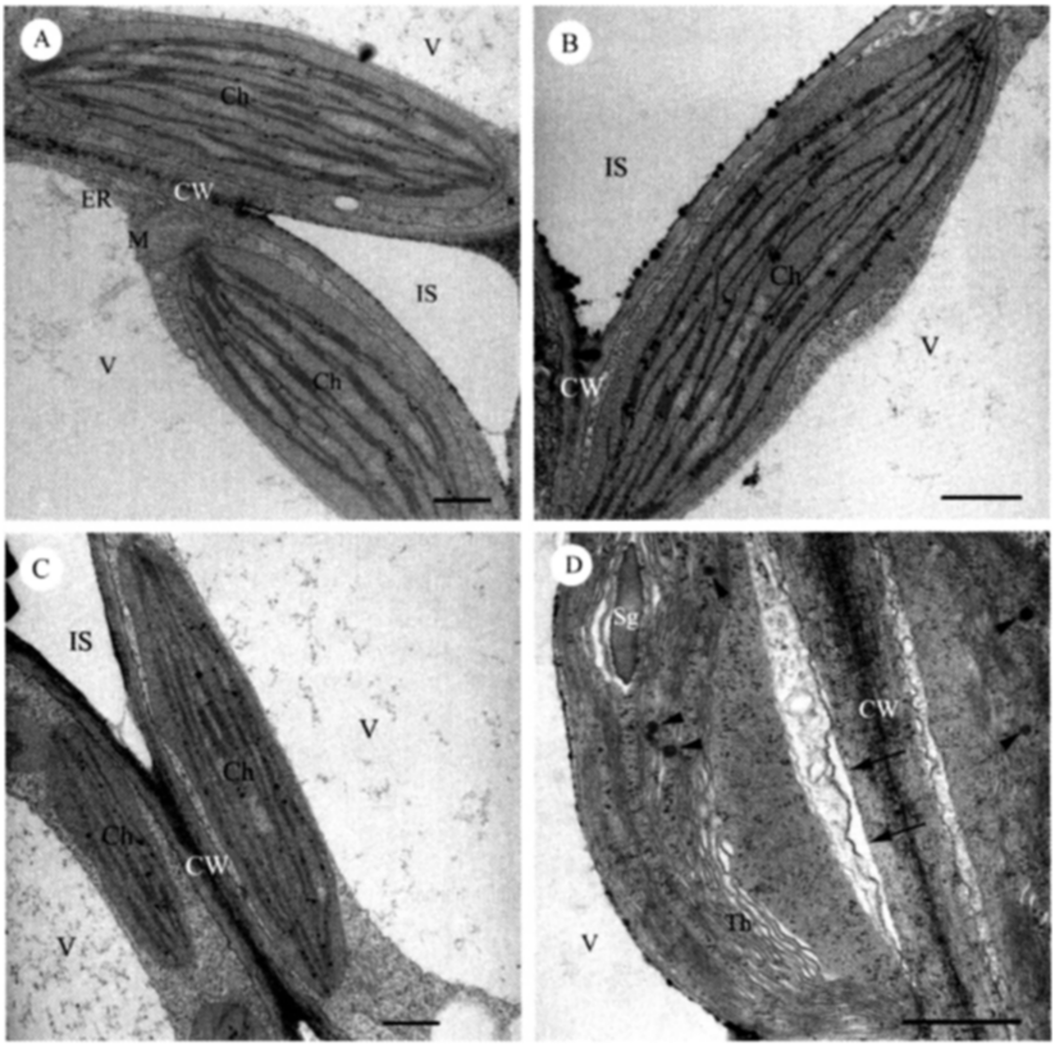

Figure 3. Chloroplasts in cortical cells from stems of control seedling (**A**) and plants irradiated at 1 (**B**), 5 (**C**), or 50 (**D**) Gy. Ch, chloroplast; CW, cell wall; ER, endoplasmic reticulum; IS, intercellular space; Sg, starch grain; V, vacuole; Bar = 500 nm. Note the numerous plastoglobuli (arrow heads) and plasmolysis (arrows).

The cytoplasm was well preserved in the cells, and the nucleus contained fairly disperse chromatin and a prominent nucleolus. In addition, the nuclear envelope showed no evidence of damage in any of our experiments (Fig. 5A-D). However, the plasmalemma was separated (Fig. 3D, 4D, and 5D) in plant treated at the highest dose (50 Gy). The ultrastructures of the cell organelles under relatively low doses were similar to those recorded for control plant, thereby demonstrating that the chloroplast is very sensitive to higher doses of gamma rays (i.e., 50 Gy).

These responses are similar to those reported for plant tested by environmental stresses, such as UV radiation, toxic metals, acidic rain, and high light intensities (Stoyanova and Tchakalova, 1997; Molas, 2002; Gabara et al., 2003; Quaggiotti et al., 2004). Gamma radiation is known to induce the production of free radicals, which can become harmful in plant cells (Kovács and Keresztes, 2002). These compounds damage the membrane lipids and proteins as well as nucleic acids (Bolwell and Wojtaszek, 1997), and decrease membrane integrity (Lee et al., 1998). This results in diminished plant growth and development (Ogawa and Iwabuchi, 2001). Thus, the results of our TEM observation suggest that an increase in free radicals by high-dose gamma irradiation may have been involved in changing those membrane structures.

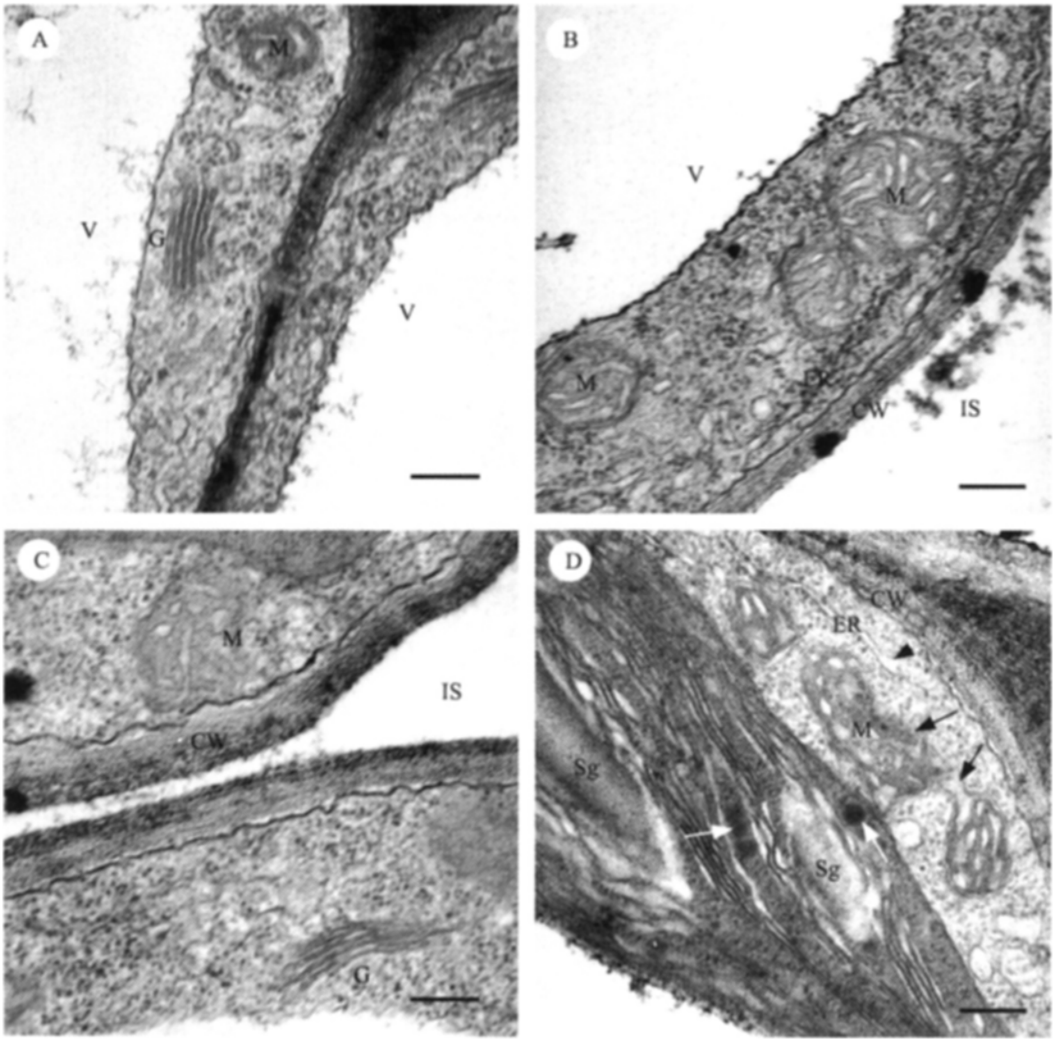

Figure 4. Mitochondria in stems of control seedling (**A**) and plants irradiated at 1 (**B**), 5 (**C**), or 50 (**D**) Gy. Ch, chloroplast; CW, cell wall; ER, endoplasmic reticulum; G, Golgi apparatus; M, mitochondria; IS, intercellular space; Sg, starch grain; Th, thylakoid; V, vacuole. Bar = 200 nm. Note the plastoglobuli (white arrows), membrane of ER (arrow head) and mitochondria (black arrows).

Numerous plastoglobuli appeared on the chloroplasts of stem cells exposed to high-dose irradiation; their sizes were significantly increased in the stroma. In addition, starch grains accumulated in their chloroplasts (Fig. 3D, 4D). However, at relatively low rates (1 and 5 Gy) no starch accumulation was observed (Fig. 3A-C). This irradiation influence was accompanied by damage to and disorientation of grana and thylakoids, indicating that carbohydrate transport was inhibited (Carmi and Shomer, 1979; Bondada and Oosterhuis, 2003).

In conclusion, our TEM results demonstrate that the chloroplast structures are obviously altered under relatively high-dose treatment (50 Gy), such that the organelle membranes become swollen and disrupted. This indicates that those structures are remarkably sensitive to ionizing radiation. Therefore, we can assume that structural changes are affected by an increase in free radical formation under gamma radiation. Further studies are needed to examine whether those free radicals produced by directly affect the membrane structure of cell organelles.

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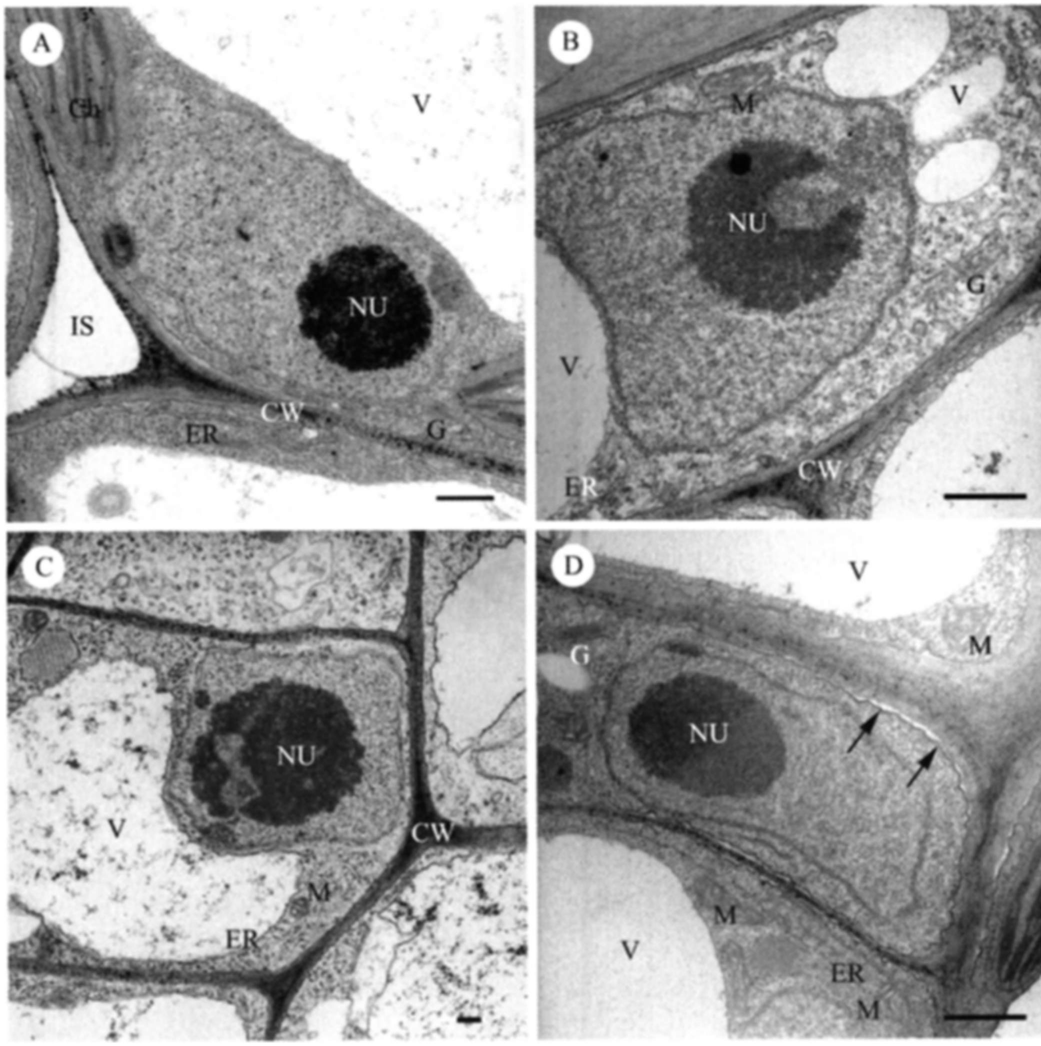

Figure 5. Nuclei in stems of control seedling (**A**) and plants irradiated at 1 (**B**), 5 (**C**), and 50 (**D**) Gy. CW, cell wall; ER, endoplasmic reticulum; Nu, nucleolus; M, mitochondria; G, Golgi apparatus. Plasmolysis was detected in xylem cell (arrows). Bar = 500 nm.

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