Localization of Hydrogen Peroxide in Pumpkin (Cucurbita ficifolia Bouché) Seedlings Exposed to High-Dose Gamma Ray

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Hydrogen peroxide (H₂O₂) was detected cytchemically, via transmission electron microscopy (TEM), in pumpkin tissues exposed to high-dose gamma ray. Its reaction with cerium chloride produced electron-dense precipitates of cerium perhydroxides. Their patterns of deposition in the tissues of both control plants and those irradiated with gamma ray (PIG) were typically found in the plasma membranes and cell walls. However, gamma irradiation remarkably increased the intensities of cerium perhydroxide deposits (CPDs) in the plasma membranes and cell walls for all tissue types, but especially the leaves. The only exception was for vessels in the cotyledons. After gamma irradiation, the H₂O₂ content in all tissues was higher than in the control samples, except for the cotyledons of PIG, where the H₂O₂ content was lower than for all others. The increased appearance of CPDs may have been due to the enhancement of H₂O₂ accumulation by gamma radiation. This accumulation also varied according to the cell or tissue type examined.

Keywords: cerium chloride, gamma irradiation, hydrogen peroxide, pumpkin, transmission electron microscopy

Stresses such as UV exposure, herbicides, drought, temperature stress and intensive light induce the production of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), hydroxyl radicals (OH) and singlet oxygen, in plant tissues (Noctor and Foyer, 1998; Desikan et al., 2003). These oxidative stresses can directly damage cells by modifying target molecules, including proteins, lipids and DNA (Fridovich, 1986; Wolff et al., 1986; Imlay and Linn, 1988; Bolwell and Wojtaszek, 1997) and by decreasing membrane integrity (Lee et al., 1998).

H₂O₂, a key player in oxidative stress (Cho and Sohn, 2004), is required for a variety of physiological processes associated with cell wall biosynthesis (Olson and Varner, 1993; Wi et al., 2005b). It can be produced by a number of enzymatic systems, and is commonly synthesized in response to various environmental stimuli (Sutherland, 1991). In addition, although H₂O₂ is a normal metabolite and not particularly cytotoxic at optimal concentrations, when these concentrations are increased by environmental stresses and ionizing radiation, they lead to cell lethality (Halliwell, 1974). Thus, H₂O₂ is one of the most important agents in terms of cell damage.

H₂O₂ contents can be remarkably increased by water radiolysis derived from gamma rays (Crout et al., 1982). High doses also inhibit plant growth, promoting H₂O₂ production that is harmful to cell organelles, while also inducing the formation of leaf trichomes and the alteration of morphologies (Nagata et al., 1999; Wi et al., 2005a). Although these techniques have described this relationship between H₂O₂ and gamma rays, none has yet demonstrated the distribution of H₂O₂ after such irradiation.

Histochemical localization of H₂O₂ production has relied on starch/KI reagents (Olson and Varner, 1993; Schopfer, 1994) or staining for peroxidase activity (Angelini and Federico, 1989). However, these techniques are indirect methods and are limited to detecting H₂O₂ produced only at the cut surfaces of tissue sections (Ros Barceló, 1998). Precise histochemical detection of H₂O₂ on an ultrastructural level is based on its reaction with cerium chloride (CeCl₃), which forms electron-dense insoluble precipitates of cerium perhydroxide, Ce(OH)₂OOH + Ce³⁺ + 2H₂O₂ → Ce(OH)₃OOH + H⁺. Although the Ce³⁺ that originates from CeCl₃ only slowly penetrates into the tissues, this second method is now widely used for cytchemical detection (Bestwick et al., 1997; Wi et al., 2005b).

In this study, we applied the cerium chloride

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method to tissues of pumpkin seedlings to examine the effects of high-dose gamma rays on H$_2$O$_2$ production, and to obtain more detailed information on the pattern of H$_2$O$_2$ deposition after irradiation.

**MATERIALS AND METHODS**

**Plant Materials and Gamma Irradiation**

Seedlings of pumpkin (*Cucurbita ficifolia* Bouché)

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**Figure 1.** Microphotographs of leaf (A and B), petiole (C and D), hypocotyl (E and F), and cotyledon (G and H) of control (A, C, E, and G) and plant irradiated at 1 kGy (B, D, F, and H). Bar = 100 μm.