The Pattern of Distribution of Peroxidase and H_2O_2 in Hypocotyls of Pumpkin irradiated with Gamma Ray

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1. Introduction

Gamma radiation, one of ionizing radiations, has been reported to affect the morphological, anatomical, biochemical and physiological changes of plants at different dose levels. These effects at high level include inhibition in plant growth [1]. Peroxidases (PODs) are mainly participations the process of lignification on the cell wall [2-3] and protect the cell organelles in cytosol against the oxidative stresses by ROS (reactive oxygen species) [4]. Hydorgen peroxide (ROS) is normal metabolite in aerobic cells and the physiological steady concentrations (between 10^{-7} and 10^{-9}) are not particularly cytotoxic [5]. When these concentrations are increased by ionizing radiation, they lead to cell lethality. Thus radiation-induced H_2O_2 may appear as an important agent causing cell damage.

In this study, a polyclonal antibody against peroxidase and cerium chloride as a trapping agent for H_2O_2 were used to obtain for better information on the occurrence and distribution of POD and H_2O_2 in the cytoplasm and walls of vascular bundle in hypocotyls of pumpkin.

2. Materials and Methods

2.1 Plant Material and Gamma-irradiation

Pumpkin (*Cucurbita ficifolia* Bouche) plants were used in these experiments. Seedlings were irradiated with 1 kGy at 7 days after sowing. The gamma radiation was generated by a gamma irradiator (^{60}Co, *ca*. 150 TBq of capacity, AECL) in Korea Atomic Energy Research Institute. Plants were grown in a growth chamber at 28/20 ^{o}C (D/N) with a 14 h photoperiod.

2.2 localizaton of POD and H_2O_2

Peroxidase was localized by immuno-gold labeling methods of Kim *et al*. (2002) using a polyclonal antibody against horseradish peroxidase and H_2O_2 was detected by the cerium chloride ($CeCl_3$) method, as described by Bestwick *et al*. (1997).

3. Results and Discussion

3.1 Vessel

Electron microscopic analyses of sections incubated with the peroxidase antibodies revealed that a few particles in vessel wall and plasma membrane of control sections were appeared (Fig. 1A). The density of gold particles in vessel wall of pumpkin irradiated with 1 kGy was significantly higher than the control (Fig. 1B).

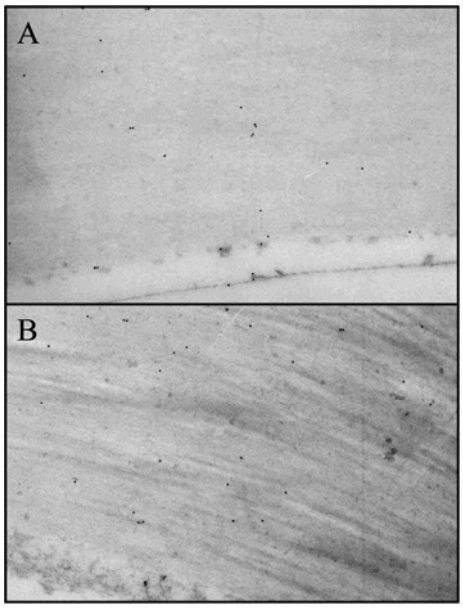

Figure 1. Immunogold labeling of peroxidase in vessel wall of pumpkin. A; control, B; 1 kGy.

In control sample, the precipitates of electron-dense cerium perhydroxide, indicating the presence H_2O_2, were commonly detected in plasma membrane, associated with xylem vessel. After the irradiation of gamma ray, H_2O_2 was clearly detected at junction between vessel and parenchyma, using $CeCl_3$ to generated electron-dense deposits of cerium perhydroxides. In most of the cases, the reaction was associated with plasma membrane and middle lamellae. Deposits in vessel wall were not detected.

3.2 Cortical cells

There is no gold labeling in the cortical cell wall in both control and 1 kGy irradiated samples. The density of gold particles in cortical cell wall of pumpkin irradiated with 1 kGy were not significantly different from those of the non-irradiated control, but, plasma membrane and cytoplasm in these cells were distinctly labeled in control

and 1 kGy irradiated sample, respectively. Hydrogen peroxide was detected as cerium perhydroxide electron-dense spots at cell walls in both control and irradiated samples (Fig. 2). However, the intensity of the reaction from cortical cells of pumpkin after gamma irradiation was stronger than that from control samples.

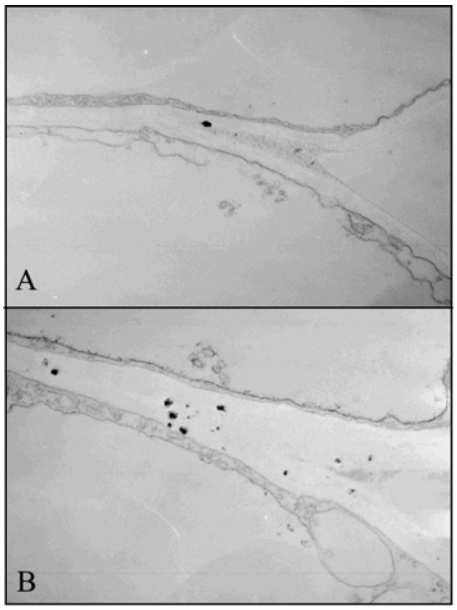

Figure 2. Localization of H_2O_2 accumulation with in the cortical cell. A; control, B; 1 kGy.

3.3 Cytoplasm

The cytoplasm of irradiated sections showed increased labeling of peroxidase, compared to the control sample and also a distinct labeling of chloroplast in there (Fig. 3). In addition, labeling was commonly seen over golgi body (data not shown). However the intensity of H_2O_2 deposits was not different among the control and irradiated samples.

3. Conclusion

The cytoplasm and cell wall of vascular bundles after gamma irradiation were more densely labeled than those of control samples. The localization of peroxidase in the vessel wall and cytoplasm of pumpkin provides an indication that the activities of wall-bounded and cytosolic peroxidases would be increased by the gamma radiation.

H_2O_2 in control samples was typically present around vessel wall and within the cell walls of cortical cells. However, after gamma irradiation highly localized accumulation of H_2O_2 was found around the vessel wall, and within the cortical cell wall. The intensity of cerium perhydroxide was increased in cell walls of all tissues by gamma irradiation. These results suggest that excess H_2O_2 is generated by gamma irradiation.

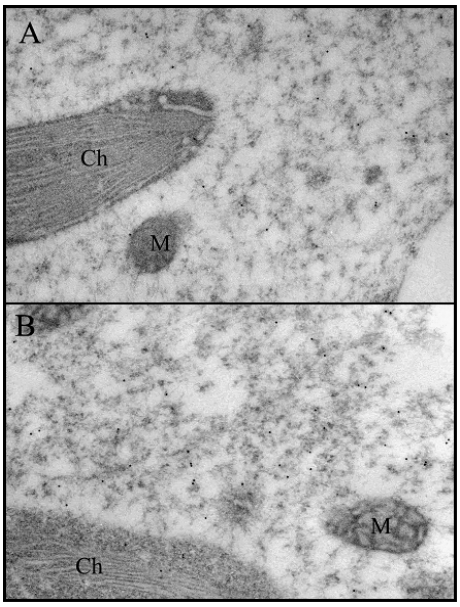

Figure 3. Immunogold labeling of peroxidase in cytoplasm. A; control, B; 1 kGy. Ch; chloroplast, M; mitochondria.

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