

**Immunocytolocalization of Peroxidase in Pumpkin (*Cucurbita ficifolia* Bouché) Seedlings  
Exposed to High Dose Gamma Ray**

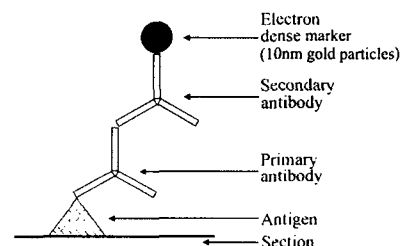
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**Objectives**

In this study, we applied immunogold labeling to establish the location of induced POD and to examine the differences of distribution patterns for this enzyme spatially after gamma irradiation. For the visualization of the localization of POD, we used a polyclonal antibody against horseradish POD in combination with transmission electron microscopy (TEM).

**Materials and Methods**

- Plant material
  - pumpkin (*Cucurbita ficifolia* Bouché)
  
- Methods
  - Gamma irradiation
    - The 9-day-old seedlings were exposed to 1 kGy.
  - Methods
    - Immuno-cytochemistry of Peroxiase
    - Conventional indirect immuno-gold labeling was employed with ultrathin sections for localization of POD (Kim et al., 2002).



**Results and Discussion**

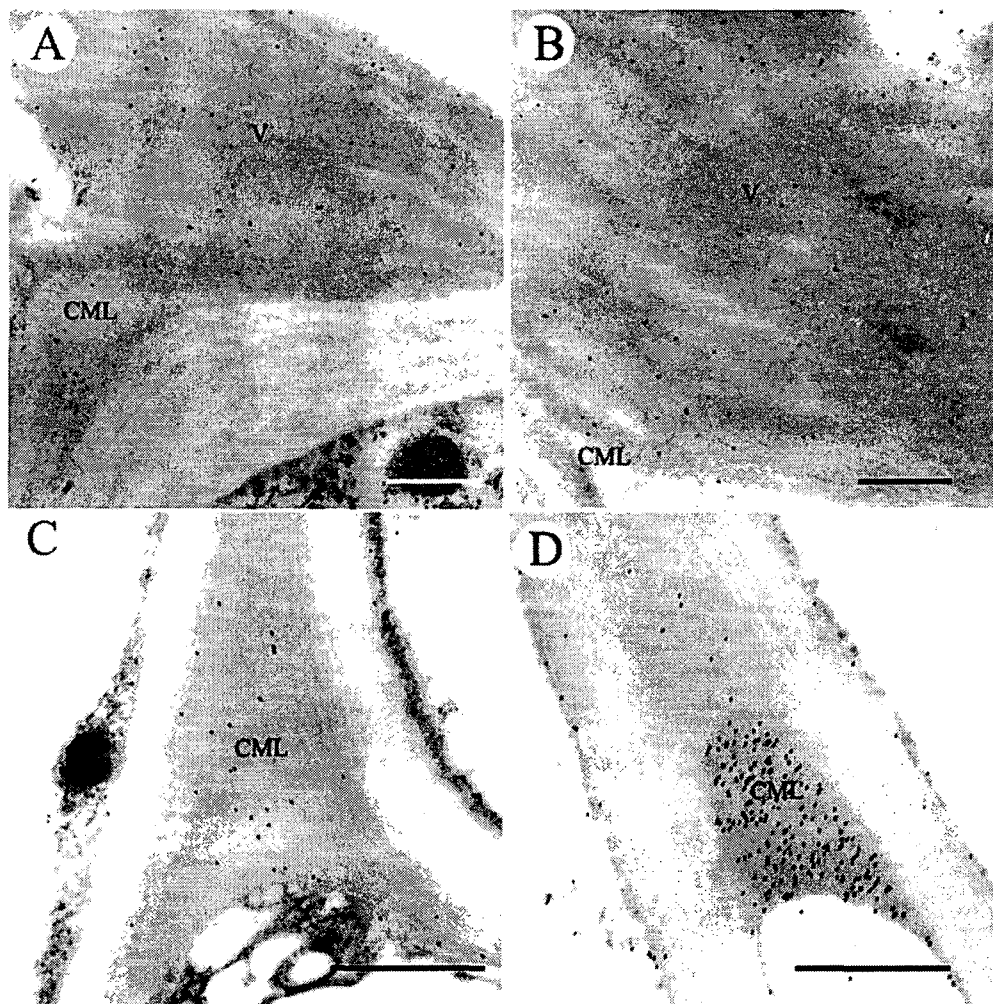
- The localization pattern of POD varied depending on the different cell and tissue types of pumpkin.
- POD was more inductive in parenchyma cell than in vessel to gamma ray.
- The density of gold particles was mainly increased on cell corner middle lamella by gamma ray, especially in petiole and hypocotyl.

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**Table 1.** Quantitative evaluation of the distribution of the gold particles as a marker of peroxidase (gold particles per  $\mu\text{m}^2$ ). Data are expressed as the means of 3-5 fields.

|           | Vessel    |          |                |          | Parenchyma cell |          |                |            |
|-----------|-----------|----------|----------------|----------|-----------------|----------|----------------|------------|
|           | Cell wall |          | Middle lamella |          | Cell wall       |          | Middle lamella |            |
|           | control   | 1 kGy    | control        | 1 kGy    | control         | 1 kGy    | control        | 1 kGy      |
| Leaf      | 9.4±1.3   | 9.1±1.8  | 19.6±4.2       | 39.1±3.9 | 2.1±0.5         | 6.1±0.7  | 0.2±0.1        | 8.2± 2.3   |
| Petiole   | 11.9±1.4  | 12.7±0.9 | 14.4±3.9       | 12.1±0.7 | 2.8±0.8         | 8.8±0.9  | 21.4±3.6       | 233.3±19.2 |
| Cotyledon | 10.9±2.1  | 15.2±2.3 | 38.3±4.7       | 36.8±3.9 | 4.3±1.1         | 19.0±2.9 | 6.1±1.9        | 31.7± 4.6  |
| Hypocotyl | 16.2±1.2  | 16.6±1.8 | 31.1±2.9       | 27.9±3.9 | 8.6±1.3         | 9.5±1.7  | 5.4±0.9        | 77.5± 4.9  |



**Fig. 1.** Immuno-localization of peroxidase in vessel (A and B) and parenchyma cell (C and D) of petiole from control (A and C) and a irradiated plant with 1 kGy (B and D). CML, cell corner middle lamellae; V, vessel wall. Bar=0.5  $\mu\text{m}$ .