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Cytochemical Localization of Hydrogen Peroxide at Leaf Sheath of Rice (*Oryza sativa* L. cv. Pokkali) under Salt Stress

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Objectives

Salinity is one of environmental stress, affects plant growth and crop productivity in many parts of the agriculture. Although considerable effort has been done in the selection and development of rice cultivars resistant (Pokkali) or sensitive (IR-29) to salinity stress, progress seems slow due to inadequate understanding of the mechanism of salt stress. To understand better the biochemical and physiological mechanisms of ROS by salt stress, it is necessary to study the spatial distribution of ROS in tissue. In this study, we applied cerium chloride reagent to in order to examine the sites of H_2O_2 accumulation induced by salinity stress.

Materials and Methods

o The uniformly germinated seeds were selected and transferred to MS medium with 100mM NaCl or without the NaCl supplement. Then rices were grown in growth chamber at 28℃ for 7 days.

o H_2O_2 was detected by the cerium chloride ($CeCl_3$) method, as described by Bestwick et al. (1997). This cytochemical method is based on the reaction of H_2O_2 with $CeCl_3$ to produce electron dense insoluble precipitates of cerium perhydroxydes, $Ce(OH)_2OOH$ and $Ce(OH)_3OOH$.

o Ultrathin sections (70-90 nm in thickness) were mounted on the uncoated nickel grids (300 mesh) and examined with a TEM in conjunction with EDXA at 80kV without post-staining.

Results and Discussion

o In the NaCl-untreated sample, precipitates of electron dense cerium perhydroxides, were mainly located in cell corner region. There was only little presence of cerium perhydroxide participates in plasmalemma and cytoplasm.

o The sections illustrated Fig. 1 shown electron dense deposits of cerium perhydroxide formed by reaction of H_2O_2 with $CeCl_3$ were localized on the tonoplast of bundle sheath. However, the staining with $CeCl_3$ in presence of H_2O_2 in the cell wall and at plasmalemma-cell interface was not as apparent. Cerium participates on the tonoplast were also confirmed using TEM-EDXA (Fig. 2).

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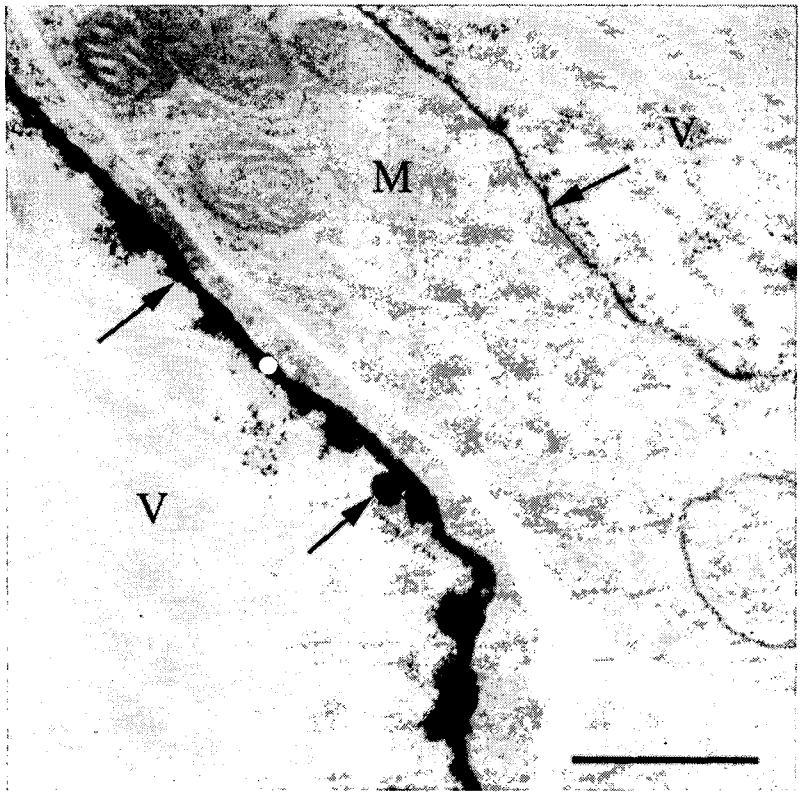

Fig. 1. Visualization of H_2O_2 by cerium deposits in the leaf sheath of rice under salt stress. Cerium participates are present in the tonoplast (arrows) surrounding vacuole. M, mitochondria; V, vacuole; White point, region of EDXA. Bar= 500nm.

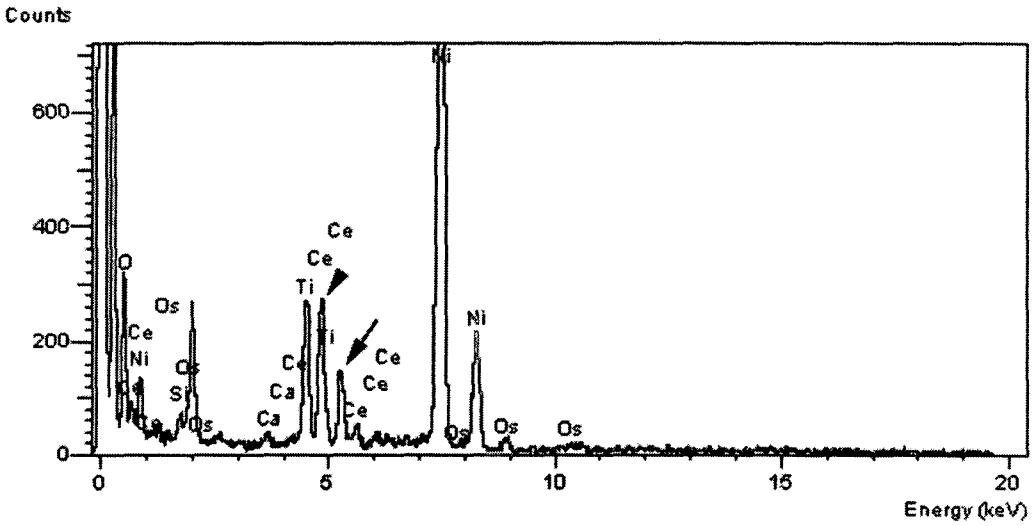

Fig. 2. Spectrum of EDXA obtained from white point of Fig. 1A section. Localization of cerium deposits are confirmed in the tonoplast using EDXA. Nickel, osmium, and titanium derive from grid, postfixative, and EDXA detector, respectively. O, oxygen; Ni, nickel, Ce, cerium; Os, osmium; Ti, titanium.