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Localization of acid phosphatase in root cap of rice plant

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Abstract. Localization of acid phosphatase in root cap of rice was studied by Gomori's reagent with electron microscopy. Ultrastructure studies showed that the site of lead deposits varied with cell localities of root cap. In the cap column, acid phosphatase was mainly detected in the nuclei, the cell wall and intercellular spaces and minorly in amyloplasts, mitochondria, Golgi, and the endoplasmic reticulum. In the peripheral regions of dying and peel off cells, lead deposits were found in almost all organelles and, especially, accumulated in large quantity in the vacuoles.

Key words: Acid phosphatase; Localization; Rice; Root cap.

Introduction

The functions of acid phosphatase in plant tissue are related to the following: 1) autolysis of cytoplasmic organelles (Evert, 1977); 2) secondary wall formation (Charvat and Esau, 1975; Cronshaw and Bentwood, 1977); 3) cell secretion (Schulz and Jensen, 1981); 4) abscission layer formation (Hall and Sexton, 1974); and 5) sugar transport (Leigh and Walker, 1980). Changes in their isozyme patterns are also affected by hormones (Gabard and Jones, 1986; Hooley, 1984) and environmental stress (Gabbrielli *et al.*, 1989). Cytochemical studies with lead deposits showed that acid phosphatase occurred in various organelles, such as the cell wall (Hall and Sexton, 1974), plasmalemma (Nishizawa and Mori, 1980), mitochondria (Mizata and Suda, 1980), plastids (Schulz and Jensen, 1981), dictyosomes (Catesson and Rao, 1987), the endoplasmic reticulum (Chen *et al.*, 1990), and the nucleus (Berggrer, 1987). However, the sites of lead deposits in organelles varied with the developmental stage of cells in rice tissues (Huang and Chen, 1989) and with xylogenesis in *Zinnia elegans* (Lin *et al.*, 1991). In the present study, acid phosphatase occurring in root cap of rice are cytochemically examined.

Materials and Methods

Plant Material

Seeds of *Oryza sativa* were sterilized in 2% sodium hypochoride for 20 min. After being thoroughly washed, seeds were soaked at 37°C for one day, and then transferred to hydroponic culture containing half strength of Kimura medium (Kao, 1980) with a 14 h light and 10 h dark period at 30°C day and 25°C night temperature. Ten day old seedlings were collected for further studies.

Electron Microscopy

Root tips of rice seedlings were cut with a razor blade into small cubes in fixation buffer containing 2.5% glutaraldehyde. These were transferred to fesh fixation buffer for 2 h, washed three times, postfixed in 1% osmium tetraoxide for 4 h, dehydrated through an ethanol series, and then infiltrated and embedded in Spurr's resin. Sections in golden color were collected, doubly stained with uranyl acetate and lead citrate, and observed with a Hitachi H-600 electron microscope at 75 KV.

For cytochemical localization of acid phosphatase, cubes were incubated in Gomori's reagent (Huang and Chen, 1991), containing p-nitrophenyl phosphate as the

substrate. Incubation medium without substrate and with NaF were set as the controls.

Enzyme Preparation and Assay

Enzyme extraction following the methods of Minocha and Halperin (1976) and Fukuda and Komamine (1982) were used for the fractionation of acid phosphatase. Rice root tips were homogenized with cold 50 mM tris-maleate buffer (pH 7.0). Crude extracts were first filtered through four layers of cheesecloth. This was centrifuged at 500 xg for 5 min at 4°C, and supernatant again at 18,000 xg for 20 min. The supernatent collected represented the soluble fraction of acid phosphatase. After thoroughly washed with buffer, the pellet was treated with 0.2 M $CaCl_2$ for 2 h and then centrifuged at 18,000 xg for 20 min. The supernatent collected, represented the ionically bound fraction. The pellet was washed in 0.1 M acetate buffer (pH 5.0) containing 0.2 M $CaCl_2$ and digested for 16-20 h in 0.1 M acetate buffer containing 1% cellulase and 1% macerozyme. This was centrifuged at 18,000 xg for 20 min. the supernatent represented the covalent bound fraction of acid phosphatase.

For the enzyme assay of acid phosphatase, the method of Hooley (1984) was adopted. The reaction mixture contained 0.1N sodium acetate (pH 5.0) and 0.05 M disodium p-nitrophenyl phosphate. The reaction was carried out at 37°C for 20 min and terminated by adding 0.6N Na_2CO_3. The absorbance at 400 nm was measured by spectrophotometry. One unit activity was the amount of enzyme that liberated 1 μM p-nitrophenol per min. Protein content was determined by Braford's method (1976).

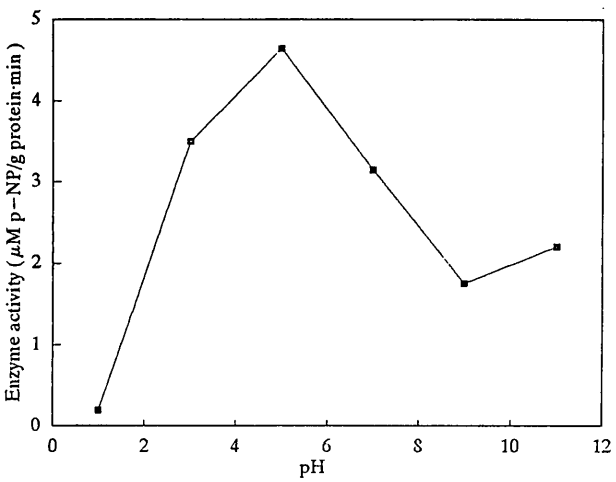


Fig. 1. The optimal pH of acid phosphatase in root.

Six phosphoric esters (all in 0.05 M) were examined to judge the substrate specificity of three fractions of acid phosphatase (Pan *et al.*, 1987). Eleven different ions (all in 0.5 mM) affected on the activity of different acid phosphatase fractions were also tested.

Results

As shown in Figure 1, the optimal pH of acid phosphatase in crude extract of root tip is around 5. In rice root tips, there are three fractions of acid phosphatase, soluble, ionically bound and covalently bound fractions.

Table 1. *Substrate specificity of three fractions of acid phosphatase*

Substrate (0.05 M)	Relative activity (%)		
	Soluble	IB*	CB*
p-Nitrophenyl phosphate	100.0±5.7+	100.0±5.0	100.0±3.9
β-Glycerophosphate	4.7±0.2	11.0±0.5	3.9±0.1
Glucose-1-phosphate	15.2±1.0	20.0±0.3	16.9±0.3
Glucose-6-phosphate	6.6±0.5	9.1±0.3	2.8±0.1
Fructose-6-phosphate	17.9±0.9	16.5±0.2	12.3±0.5
Fructose-1, 6-diphosphate	128.1±4.6	55.1±4.6	42.1±2.3

*IB, Ionically bound fraction; CB, Covalently bound fraction.
+ Mean ± standard deviation (N=3).

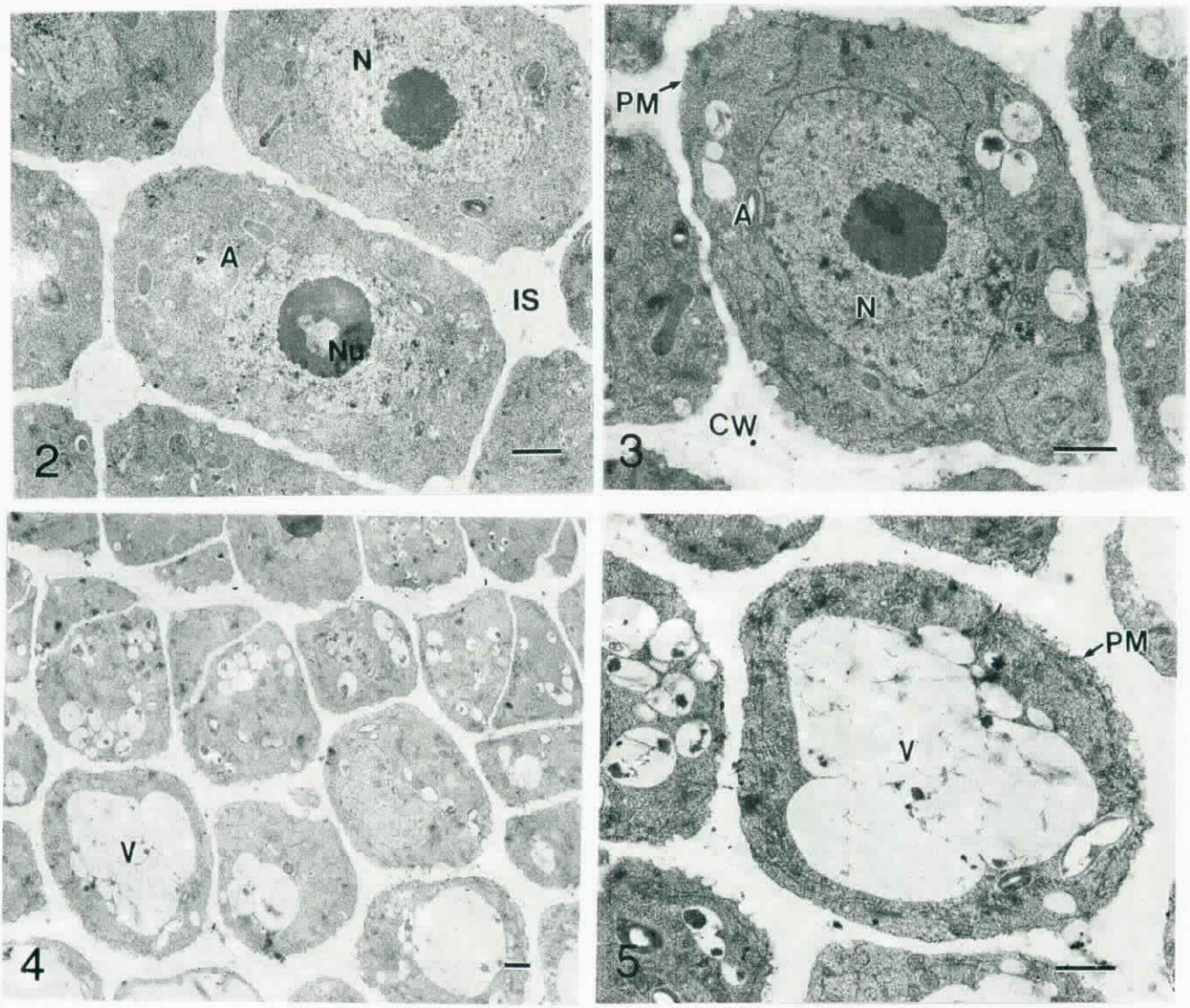
Table 2. *Effect of various compounds on the activity of different fractions of acid phosphatase*

Compound added (0.5 mM)	Relative activity of different fractions (%)		
	Soluble	IB*	CB*
Control	100± 5.1*	100± 3.1	100± 7.2
Fe-EDTA	113± 2.1	129± 3.0	100± 2.7
$CaCl_2$	101± 3.5	107± 6.6	96± 4.1
$Mgcl_2$	119± 2.6	108±14.3	125± 5.0
$CuSO_4$	43± 3.1	26± 2.5	73± 3.3
$HgCl_2$	12± 0.3	22± 6.1	53± 0.5
KH_2PO_4	97± 4.0	81± 6.4	103±11.8
NaF	54± 0.4	67± 2.9	85± 6.4
Na_2MoO_4	23± 1.0	18± 1.1	37± 1.5
NH_4NO_3	81±16.1	79± 6.4	97± 9.0
$CoCl_2$	107±17.0	95± 9.9	131±10.6
$ZnSO_4$	72± 1.9	53± 3.3	74± 5.8
$MnSO_4$	152±13.5	163± 9.8	99±14.2
H_3BO_3	98± 7.4	90± 4.0	109± 5.1

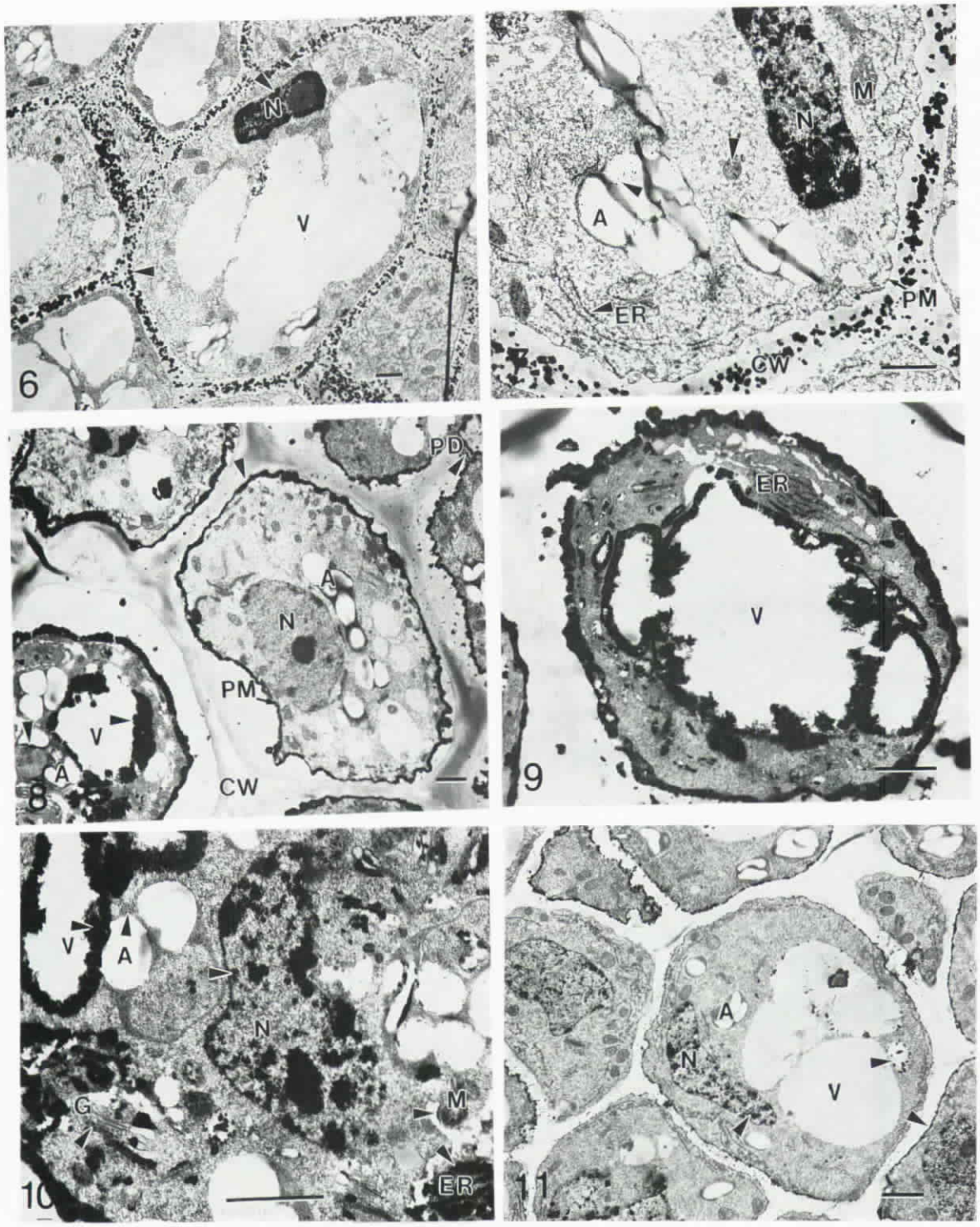
*IB, Ionically bound fraction; CB, Covalently bound fraction.
+ Mean ± standard deviation (N=3).

The substrate specificity of these three fractions are different. Among the substrates tested, fructose-1,6-phosphate is the only suitable substrate superior to p-NPP for assaying soluble fractions of acid phosphatse, whereas p-NPP is suitable for acid phosphatase in ionically and covalently bound fractions (Table 1).

The activity of acid phosphatase can be affected by various ions. As shown in Table 2, enzyme activities in different fractions were affected differently by different compounds. The addition of Ca^{++}, Mg^{++}, Fe^{++}, Co^{++}, NO_3^-, BO_3^-, and $H_2PO_4^-$ to the reaction mixture did not have a distinct effect on enzyme activity. Manganese ions showed a significant enhancement of enzyme activity in the soluble fraction and ionically bound fraction. Ions of copper, mercury, zinc, molybdate, and floride decreased enzyme activity in all three fractions. Mercury had the severest effect, and its effect on soluble and ionically bound fractions was more pronounced than on the covalent bound fraction. A similar effect on different fractions of acid phosphatase was found by the addition of molybdate ions to the reaction medium. Sodium floride, and inhibitor of



Figs. 2–5. Different cell types in root cap of rice. 2, Cells in subepidermal region of root meristematic. 3, Cells in central column of root cap. 4, Cells in subperipheral region of root cap. 5, Cells with vacuolar fusion and mucilage (arrowheads) in peripheral region of root cap. All bars in 1 μm. A, Amyloplast; CW, Cell wall; ER, Endoplasmic reticulum; G, Golgi body; Is, Intercellular space; M, Mitochondrion; N, Nucleus; PD, Plasmodesmata; PM, Plasmalemma; V, Vacuole.



Figs. 6–11. Acid phosphatase detected in root cap of rice. 6, Lead deposits (arrowheads) in cells of central column of root cap. 7, Lead deposits (arrowheads) occurring in nucleus, plasmalemma and cell wall. 8, Lead deposits (arrowheads) in cells of subperipheral regions of root cap. 9, Lead deposits accumulated in vacuole of peripheral cells in root cap. 10, Lead deposits (arrowheads) accumulated in different cell autophagic cell of root cap. 11, Decrease of lead deposits in extranuclear region of NaF treated cells. All bars in 1 μm.

acid phosphatase, showed about 46% inhibition of enzyme activity in the soluble fraction, however, only 33% and 15% inhibition was observed in the ionically and covalently bound fraction, respectively.

As shown in Figures 2 and 3, cells in the meristem and cap column adjacent to meristematic tissue all have prominent nuclei, dense cytoplasm, starch-containing plastids, numerous and tiny vacuoles, and a high volume ratio of nucleus to cell. Plasmodesmata between two adjacent cells in the central and subperipheral regions of root cap are also observed (Figs. 2,4). However, during specimen preparation, root cap cells more easier plasmolyze than meristematic cell. In root cap cells, vacuole size increase by vacuolar fusion is depended on cell locality, from the central column to the peripheral regions. Cells in the peripheral root cap are more loosely arranged and have a large, central vacuole, and the cytoplasm and organelles occupy the outside region adjacent to the cell wall (Fig. 5).

Cytochemical study shows that lead was deposited at sites of acid phosphatase. Acid phosphatase detected in the root cap also varied with cell locality (Figs. 6,7). Almost all nuclei, the cell wall, and intercellular spaces show enzyme activity; however, the presence of their quantity in lead deposits gradually increase from inside to outside regions. In the cap column, the above three are the major sites of enzyme locality and the minors are amyloplast, mitochondria, Golgi body and endoplasmic reticulum (Fig. 8). In addition, vacuoles in cells of the peripheral root cap accumulate large amounts of acid phosphatase (Fig. 9). Lead deposits are often detected in plasmodesmata and its vicinity. Almost all organelles of the dying and free cells near the surface of root cap are occupied by lead deposits. The detectable sites of acid phosphatase includes: extracelluar matrix, cell wall, plasmalemma, Golgi complex, plastid, mitochondrion, network-like endoplasmic reticulum, and nucleus (Fig. 10). As shown in Figure 11, activities of acid phosphatase of the cell wall and extranuclear organelles in NaF-treated sections of root cap decreased significanty. However, enzyme activity in nucleus did not show distinguishable blockage by NaF treatment.

Discussion

Ueki and Sato (1977) pointed out that the acid phosphatase had a broad optimal pH at 6.0-7.0 in cultured tobacco cells. However, in suspension cells of *Nicotiana otophora*, the pH optimum of acid phosphatase was at pH 4.0. In this study, acid phosphatase in rice root had a similar pH optimum at 5.0. The range of optimal pH for acid phosphatase has been shown to vary with species, varieties, organs and developmental stages (Baker and Taken, 1973 ; Park and Van Etten, 1986).

Manganese has been reported to be a component of acid phosphatase purified from soybeans (Fujimoto *et al.*, 1977). In this study, $MnSO_4$ significantly enhanced the activity of soluble and ionically bound isozyme of acid phosphatase. However, it did not have the distinct effect on covalently bound one. The activity of acid phosphatase in pea hypocotyl was not affected by $MgCl_2$, $CaCl_2$, $CoCl_2$, and H_3BO_3 but was strongly inhibited by $ZnSO_4$, $CuSO_4$, NaF, and $NH_4MO_7O_{24}$ was also found (Mizuta and Suda, 1980). In culture tobacco cells, acid phosphatase was inhibited by KH_2PO_4 and NaF, and the addition of orthophosphate to medium resulted in the suppression of the release of phosphate into extracellular region. Inorganic phosphate has been suggested to be a competitive inhibitor and NaF a non-competitive inhibitors of acid phosphatase (Ueki and Sato, 1977). In this study, no effect of 0.5 mM KH_2PO_4 on the activity of acid phosphatse may be the result of one of the following reasons: without high enough concentration of phosphate added; or without enough sensitivity of isozyme in rice.

Cellular and subcellular localization of acid phosphatase are still in consistency. The localization and isozyme patterns of acid phosphatase varied with species, tissues, developmental stages of plants and outside stress (Hall and Sexton, 1977; Pan, 1985). Acid phosphatase is mainly distributed in the cell wall and the nucleus in cultured cells of carrot, apple and tobacco (Halperin, 1969; Hislop *et al.*, 1979; Pan *et al.*, 1987). Halperin (1969) demonstrated that only in senescent cells was acid phosphatase localized in the nucleus. In peripheral cells of rice root cap, the frequent occurrence of lead deposit in the nucleus as well as other cell organelles was also found in this study. This may indicate that these cells may die soon. However, in cells of the central column of the root cap, the nucleus is disshape and also had high activity of acid phosphatase. Similar positive result in detection of acid phosphatase in some cells of the root meristem was also observed (Chen *et al.*, 1990). No matter what kinds

of tissue the cells are in, nuclei with acid phosphatase activity are smaller and in shapes other than spherical. The physiological meaning is still unknown.

The inhibitory effect of NaF on the activity of acid phosphatase varied with different isozymes in pea hypocotyl (Mizuta and Suda, 1980). The effect of NaF on acid phosphatase varied between plant species (Ueki and Sato, 1977; Mizuta and Suda, 1980; Pan *et al.*, 1987). Sodium fluoride has often been used as an inhibitor of control experiment in cytochemically localization of acid phosphatase. However, in rice root cap, most extranuclear isozyme of acid phosphatase was blocked by NaF treatment, whereas only nuclear one was not distinctly affected.

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酸性磷酸酶在水稻根冠的次細胞定位

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利用電子顯微鏡技術以 Gomori's 試劑測定酸性磷酸酶在水稻根冠的分布所在。超微構造顯示酸性磷酸酶的次細胞分布隨細胞在根冠所處的位置不同而有差異，在冠柱內酸性磷酸酶主要出現於細胞核細胞壁及細胞間隙，少量分布於澱粉體粒線體高爾基體及內質網，根冠周邊的漸趨脫落細胞中幾乎所有的胞器都有酸性磷酸酶被檢出，其中液胞更有大量的酸性磷酸酶聚集。