LOCALIZATION OF ACID PHOSPHATASE IN RICE TISSUE

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Subcellular localization of acid phosphatases by the method of Gomori showed that they mainly distributed in cell wall, nucleus and vacoule in callus tissue. In greening calli ready to differentiation, acid phosphatases were also found in plastid. In leaf, they were also present in mitochondria, Golgi and cytoplasm. While in root except meristematic tissue, acid phosphatases mainly appeared in cell wall.

I. INTRODUCTION

The role of acid phosphatase in metabolism is still unknown. Even though localization of this enzyme has been studied in many different plant tissues for a long time (Charvat and Esau, 1975; Ciarrocchi *et al.*, 1981; Dauwalder *et al.*, 1969; Vithanage, 1984) there aren't any direct evidence to sufficiently document the exact reaction it involved. Some investigator suggested it is concerned with some functions as follow: 1) autolysis of cytoplasm; 2) digestion of primary wall; 3) formation of secondary wall; 4) secretion; 5) transportation of sugar and nutrient reserves; 6) differentiation of plastid, etc.. Not only that, but also involved in several developmental stage: 1) germination of seed (Rossi *et al.*, 1981); 2) differentiation of plant tissues (Bentwood and Cronshaw, 1976); 3) senescence of plant tissues (Baker and Tadakazu, 1973).

The subcellular localization of acid phosphatase in plant cell had been observed by many studies (Gahan *et al.*, 1979; Hall and Davie, 1971; Pan *et al.*, 1987). The enzyme often occurred in cell wall, vacuole, and sometimes also existed in endoplasmic reticulum, Golgi body, plastid, mitochondrion, nucleus, nucleolus, and even in cytoplasm. The various distribution of acid phosphatase in plant cell was related with physiological state which varied with developmental stage; example for cell division, differentiation or senescence. Simultaneously not only the cells of different tissues but also the cell types in the same tissue all have their own distributed location of acid phosphatase.

In roots and leaves, the characteristics of acid phosphatase have been documented by several reports (Berjak, 1972; Hall and Butt, 1967; Parida and Mishra, 1980). But only few scientists payed attention to investigate the distribution of acid phosphatase in different tissues of root and leaf.

In this study, subcellular localization of acid phosphatase in different rice tissues was emphasized.

II. MATERIALS AND METHODS

1. Plant materials

Rice seeds (*Oryza sativa* L. cv Tainung No. 67) were surface sterilized in 2%

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NaClO solution for 1 h, and were rinsed in running water for a period of time. The seeds were planted on vermiculite and maintained in culture room at 26°C Plant materials were collected as follow: 1) dry seeds were sterilized and had no any more treatment; 2) seeds were allowed to imbibe for three days in water; 3) seedlings of 5, 11, 23, and 30 day cultures; 4) milky seeds of outside growing plants.

2. Enzyme preparation

Plant tissues, frozen in liquid nitrogen, were homogenized and blended with 0.05 M tris-HCl buffer (pH 7.5) containing 0.007% (v/v) β-mercaptoethanol. The homogenate was strained through gauze and centrifuged at 8000 rpm for 10 min. The supernatant was brought to 70% saturation with ammonium sulfate, centrifuged as above and supernatant was discarded. Pellet was dissolved in a small amount of 0.05 M Tris-HCl buffer (pH 7.5) containing 0.007% β-mercaptoethanol, and dialyzed overnight, then centrifuged at 8000 rpm for 10 min. Collected supernatant was the enzyme solution (Yamagata *et al.*, 1979). Only enzyme extract of milky seed was passed through a DEAE column (Pan, 1985).

3. Enzyme assay and protein determination

The assay method was similar to that used by Hooley(1984). Reaction mixture contained 0.1 N sodium acetate (pH 5.0), and 0.05 M *p*-nitrophenyl phosphate disodium (as substrate). Reaction went on at 37°C for 20 min. Then 0.6 N Na_2CO_3 was added to stop reaction. The absorbance of 400 nm was measured by spectrophotometer. One unit was the amount of enzyme that liberated 1 μmole *p*-nitrophenol per min. The quantity of protein was determined by Lowry's method (Lowry *et al.*, 1951).

4. Glutaraldehyde treatment

(1) *In vivo*

Half a gram of rice calli was respectively treated with 2.5% and 5% of glutaraldehyde in 0.1 M phosphate buffer (pH 7.0). The period of treatment was 2, 4, 6, 12, 16, 20, 28, 32, 36, 40 and 44 h. Then, the tissues were washed with phosphate buffer (pH 7.0) for 10 min, homogenized in 0.05 M Tris-HCl buffer (pH 7.5) at 4°C and centrifuged at 8000 rpm for 10 min. The enzyme activity of supernatant was detected.

(2) *In vitro*

Half a ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) was added to equal ammount of enzyme solution extracted from rice calli, incubation time was 2, 4, 6, 12, 20, 24, 28 and 32 h. After incubation, samples were taken to detect the enzyme activity immediately.

5. Electron Microscopy

The root and the leaf of rice were cut into 0.5 mm, and was prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) at 4°C for 2 h, then washed with cooled phosphate buffer overnight. Then material was washed with 0.1 M acetate buffer (pH 7.0) for 1 h (15 min/time), and in Gomori's solution containing 0.1 M sodium acetate buffer (pH 5.0), 3.0 mM *p*-nitrophenyl phosphate, 3.6 mM lead nitrate, and 0.05 M sucrose at 37°C shaking water bath for 90 min.

Control was carried as follows: 1) no substrate; 2) no lead nitrate; 3) added 0.01% NaF. Then, material was washed with 0.1 M sodium acetate buffer (pH 7.0) for 1 h and postfixed with osmium tetraoxide for 2 h. After been washed with phosphate buffer 4 times, the samples were dehydrated with a graded series of alcohol, and embedded in Spurr's resin. Ultrasection was cut on LKB ultramicrotome V with diamond knife. The section was doubly stained with uranyl acetate and lead citrate, then observed with Hitachi H-600 electron microscope at 75 KV.

III. RESULTS

1. Effect of glutaraldehyde on activity of acid phosphatase

Treated with glutaraldehyde at the concentration of 2.5% and 5%, acid phosphatase activity in callus cells showed no obvious decline with time. Even if the time increased up to 44 hours, the glutaraldehyde had little inhibitory effect on enzyme activity. There still passing 90% of enzyme activity was preserved although the effect of 5% gluteraldehyde was more than that of 2.5%. We, concluded that at general level of concentration and duration for fixation, glutaraldehyde didn't affect apparently on acid phosphatase activity in cells of tissue block. While acid phosphatase activity of the extracted enzyme solution decreased with the increasing duration of glutaraldehyde treatment. Especially in the first 2 hour, the effect was most conspicious. As in vivo, 5% of glutaraldehyde had larger effect on activity of acid phosphatase. Through 40 hours, the preserved activity was below 50% (Fig. 1).

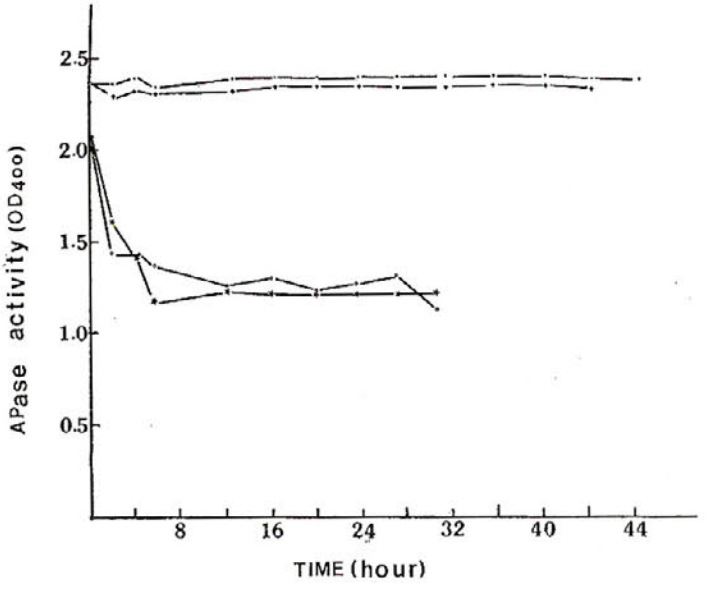

Fig. 1. Effect of Glutaraldehyde on activity of Acid phosphatase.
A: In vivo; B: In vitro; —■— A1: 2.5%; —+— A2: 5.0%; —★— B1: 2.5%; —○— B2: 5.0%.

2. Localization of acid phosphatase

(1) *Callus cell*

In some callus cells, acid phosphatase was simultaneously found in nucleus, vacuole, plasmalemma, and cell wall (Fig. 2A). But in other cells, the enzyme activity only distributed in nucleus (Fig. 2B) or plasmalemma (Fig. 2C). In nucleus, enzyme product expressed as granular distribution (Fig. 2D). But in cell wall, sometimes enzyme product spreaded in pieces (Fig. 2E). It was also found that acid phosphatase distributed in middle lamella and plasmodesmata (Fig. 2F).

(2) *Greening callus cell*

Transfered to differentiating medium after 14 days, the cell of greening calli contained large amount of starch grain accumulated in plastid (Fig. 3A). Gomori's positive reaction existed in plastid (Fig. 3B). Moreover, enzyme activity also distributed in nucleus, vacuole, cell wall and plasmalemma (Fig. 3C). In vacuole, we found enzyme product clearly, too (Fig. 3D).

(3) *Leaf cell*

Treated with Gomori's solution, even without double staining, particles of electron dense were also found in leaf cells (Fig. 4A). In mesophyllous cells, strong activity of acid phosphatase was found in plasmalemma (Figs. 4B, 4C, 4D, 4F). However, the enzyme product also distributed in vesicle finally fused to plasmalemma (Fig. 4C), vacuole (Fig. 4D), nucleus (Fig. 4B), mitochondrion (Fig. 4B), Golgi body (Figs. 4B, 4F) and cytoplasm (Fig. 4E). Subcellular location of acid phosphatase could vary with different cells (Figs. 4B, 4D, 4E).

(4) *Root cell*

After Gomori's reaction, the particles of electron-dense distributed in a concentration gradient from outside of root (Fig. 3E). Meristematic cells were different from the cells of elongation region. Lead salt precipitation also observed in nucleus and mitochondrion (Fig. 3F), but no enzyme product deposited in amyloplast.

IV. DISCUSSION

Gilder and Cronshaw(1974) had elucidated glutaraldehyde could preserve acid phosphatase in plant cell. However, others had regarded it was inhibitor of this enzyme, (Anderson, 1967; Janigan, 1955). Our data showed that the former was more supported. In our opinion, prefixation with glutaraldehyde was a necessary step for cytochemical study of acid phosphatase.

The distribution of acid phosphatase in callus tissue was almost in cell wall, vacuole and nucleus (Fig. 2A). Similar result had been observed in culture cell of carrot (Halperin, 1969), apple (Hislop, *et al.*, 1979) and tobacco (Pan, *et al.*, 1987). Halperin (1969) demostrated the situation that acid phosphatase located in nucleus only happened in senescencing cell. The distribution of acid phosphatase in nucleus is not so often as in cell wall and vacuole, so Hall (1977) suggested the enzyme product locating in nucleus was an artifact. While in order to identify that the lead precipitation in nucleus was reacted from the activity of acid phosphatase, Deltour *et al.* (1981) isolated nucleus particularly and took it to detect the enzyme activity. Finally, it was shown that high activity of acid phosphatase exactly existed in nucleus.

Abbleviation:

C: Cytoplasm; CL: Chloroplast; G: Golgi body; M: Mitochondrion; N: Nucleus; Nu: Nucleolus; P: Plasmodesmata; PL: Plastid; S: Starch grain; V: Vacuole; VE: Vesicle; W: Cell wall.

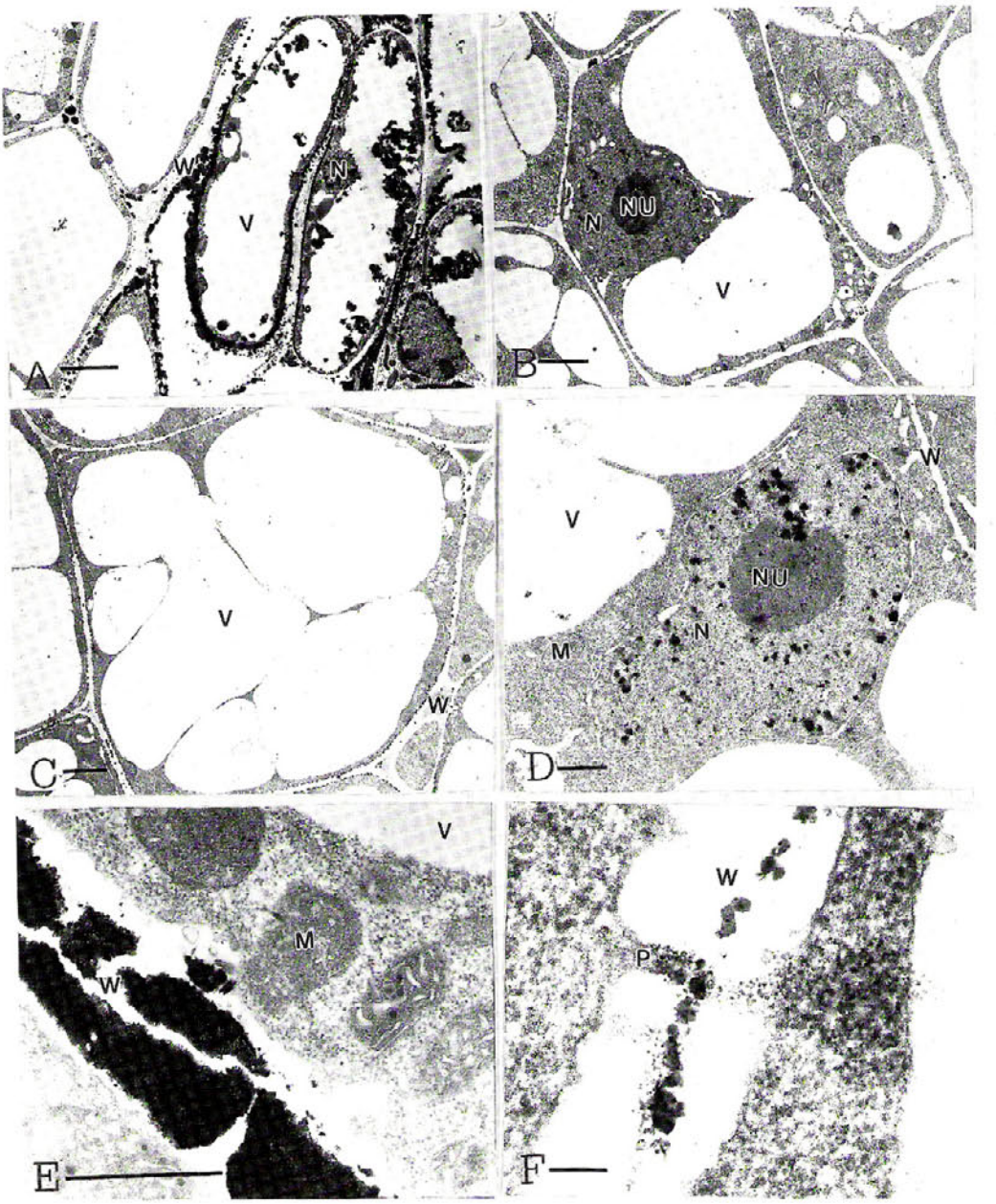

Fig. 2. Subcellular localization of acid phosphatase in rice callus cell.
A→F: Gomori's treatment and double staining.
'Bar' A: 5 μm; B: 3 μm; C: 2 μm; D, E: 1 μm; F: 0.1 μm.

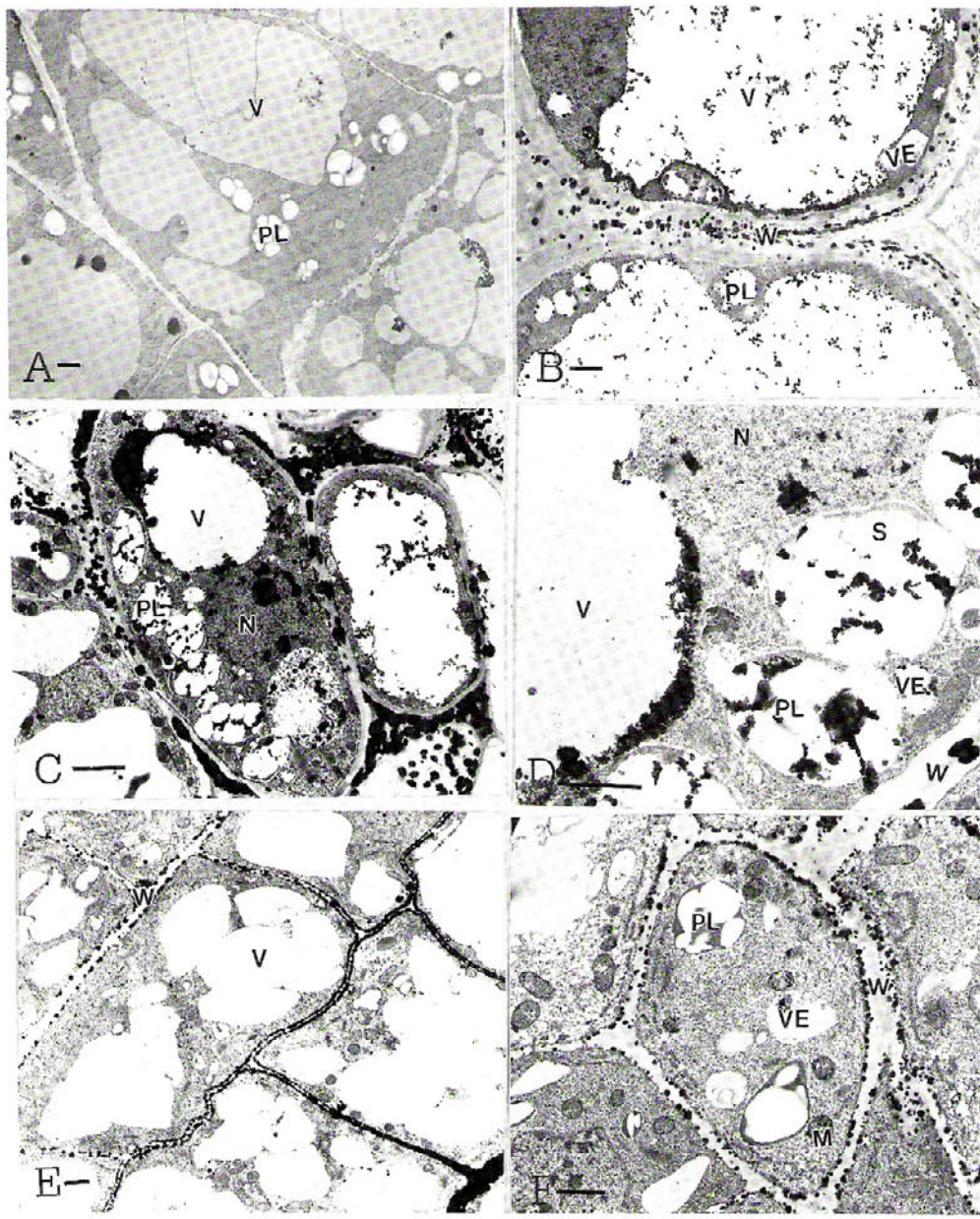

Fig. 3. Distribution of acid phosphatase in callus cell subcultured 14 days in differentiation medium (A→D) and in root cell (E→F).
A: Control treatment; B→F: Gomori's treatment and double staining.
'Bar' all 1 μm.

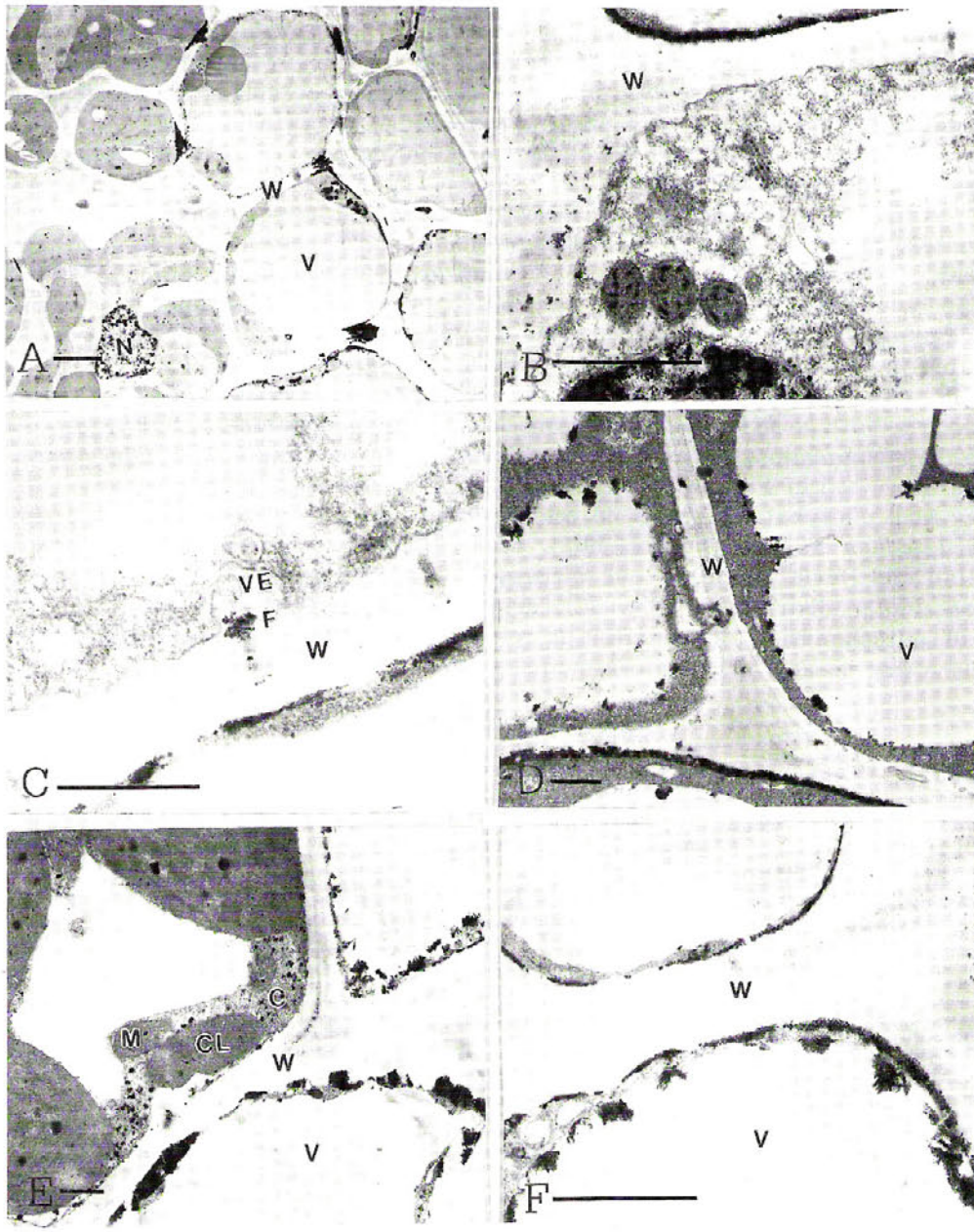

Fig. 4. Subcellular localization of acid phosphatase in rice leaf cell.
A: Gomori's treatment without double staining;
B→F: Gomori's treatment and double staining.
'Bar' A: 2 μm; B→F: 0.5 μm.

It had been reported that starch heavily accumulated in the area utimately forming shoot primodia of tobacco calli cultured in differentiating medium (Thorpe and Murashige, 1970). Futhermore, a significant finding that 1.5 fold increment in acid phosphatase occurred in shoot forming regions of rice callus (Saka and Maeda, 1974). Simultaneously, observing with light microscope, Patel and Berlyn (1982) discovered that acid phosphatase deposited in cytoplasmic granule of pinus shoot primodia tissue. Our observation agreed with them (Figs. 3C, 3D), and Srinivasan and Mullins (1980) considered the present of acid phosphatase was related with organ formation.

Agreeing with Pesacreta's observation (1986), in mesophyllous cell, acid phosphatase distributed in cell wall, plasmelemma, and vacuole (Figs. 4B, 4D). Besides that, in our study, heavy deposition of lead granules also found in nucleus (Fig. 4B). The fact that acid phosphatase distributed in nucleus, mitochondrion, Golgi body and plasmalemma of parenchyma and companion cell of phloem tissue was vestigated in toacco by Bentwood and Cronshaw (1976), also in rice by our observation (Fig. 4B). Charvat and Esau (1975) observing the xylem tissue of *Phaseolus vulgaris*, both vesicles and cytoplasm have acid phosphatase activity. According to their suggestion, the cell of Fig. 4C was going on forming secondary wall, and the other cell of Fig. 4E was at the situation of autolysis probably.

Acid phosphatase located in cell wall and vacuole of root cell was reported in *Lepidium sativum* (Berjak, 1972), barley (Hall and Sutt, 1967), and *Zea mays* (Hall and Davie, 1971). In our observation, except the cell of elongation tissue had similar distribution (Fig. 3E), acid phosphatase was also found in nucleus and mitochondrion of meristmatic cell. Although starch accumulated in plastid, there was no acid phosphatase in it.

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水稻組織酸性磷酸酶的定位

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摘　要

臺農67水稻組織經 Gomori 溶液處理，藉電子顯微鏡觀察酸性磷酸酶的次細胞分布，癒合組織中酸性磷酸酶分布於細胞、細胞核及液胞中。只於分化培養基14天已綠化之組織，除了前提各胞器外，於綠化期間，大量含有澱粉粒的色素體內，也具有高活性的酸性磷酸酶，在葉中，酸性磷酸酶也出現於粒線體、高爾堤體及細胞質中，而根部分生組織內，分布較廣，如細胞核、細胞壁及粒線體等，在延長部的細胞則主要出現於細胞壁內。