STUDIES ON ACID PHOSPHATASE IN RICE TISSUES

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Abstract: Acid phosphatase in different tissues of rice (*Oryza sativa*) was studied with polyacrylamide gel electrophoresis and enzyme assay. The zymogram of acid phosphatase showed different isozyme patterns in roots, leaves, seedlings and calli. One of the medium mobility isozymes, cell-wall-containing enzyme, commonly occurred in all tissues. Some isozymes had tissue-specificity. The optimal pH of acid phosphatase was around 5. Enzyme activity was strongly inhibited by $HgCl_2$, $(NH_4)_4Mo_7O_{24}$, NaF, $CuSO_4$ and KH_2PO_4. In germinating seeds, the increasing activity of acid phosphatase related with transportation of phosphates and store food had been suggested.

INTRODUCTION

Acid phosphatase (EC 3. 1. 3. 2) is an important hydrolytic enzyme, for hydrolyzing monophosphate esters, and widely distributed in plants (Mizuta and Suda, 1980; Yamagata, *et al.*, 1979). It has been suggested that it may be involved in defense, autolysis, cell wall transformation, maintenance of phosphate pool, fruit ripening and seed germination (Fernandez, *et al.*, 1990).

Enzyme activity and isozyme patterns of acid phosphatase in plants tissues were usually affected by the developmental stages of tissues. In pea seed, the activity of acid phosphatase increased during germination, as well as isozyme changed (Johnson, *et al.*, 1973); in root, high ratio of magnesium/calcium in soil enhanced the activity of acid phosphatase in two *Alyssum* species (Gabbrielli, *et al.*, 1989); in cultured tobacco cells, zinc ions had differential inhibition on multiple forms of acid phosphatase (Pan, *et al.*, 1987).

In the present study, acid phosphatase activity and its isozyme patterns in different tissues of rice plant were examined.

MATERIALS AND METHODS

1. Plant materials

Seeds of rice (*Oryza sativa*) were surface sterilized in 2% NaOCl solution for 1 h, rinsed in running water for a period of time, germinated on wet tissue paper and incubated at 37°C for 2 days. Seedlings were transfered to hydroponic culture under illumination period of 16 h in light and 8 h in darkness at 30°C and 24°C, respectively, and collected at different intervals.

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2. Enzyme preparation and assay

Plant tissues, frozen in liquid nitrogen, were homogenized in blender with 0.05 M tris-HCl buffer (pH 7.5) containing 0.007% (v/v) beta-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 at 8000 rpm and supernatant was discarded. Pellet was dissolved in small amount of 0.05 M tris-HCl, dialyzed overnight, then centrifuged at 8000 rpm for 10 min. Collected supernatant was the enzyme solution. Only enzyme extract of milky seed was passed through a DEAE-cellulose column.

The method of enzyme assay was similar to that used by Hooley (1984). Reaction mixture contained 0.1 N sodium acetate (pH 5) and 0.05 M disodium *p*-nitrophenyl phosphate. The effects of pH on the activity of acid phosphatase were performed in the series of sodium acetate buffer. Reaction was held 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 (1951).

3. Polyacrylamide gel electrophgoresis

Electrophoresis was carried out with 7.5% polyacrylamide gel (pH 8.8) as a running gel, 5% polyacrylamide (pH 6.8) as a stacking gel, and electrode buffer containing 0.38 M glycine (pH 8.3). Gel thickness was 0.75 mm. Voltage was set at 150 volt in first half an hour and then increased to 200 volt. While the tracing dye arrived at the bottom of the gel, the gel were removed and put into the stainer containing 0.2 M sodium acetate buffer (pH 5.0), 0.1% fast red TR salt and 5 mM $MgCl_2$. After 3 h, the gel was destained with distilled water and dried.

RESULTS

Electrophoretic analysis of acid phosphatase was shown in Fig. 1. It revealed that root and leaf had the similar isozyme patterns except A11 and A12 only appearing in leaf, and A4 in root (Fig. 2). A11, A12 and A4 can be recognized as tissue specific isozymes. The appearance of large molecule A2 isozyme nearly occurred after the differentiation of root and shoot. Here was no any direct evidence to support it was synthesized de novo or came from modification. Milky seeds passed through DEAE-cellulose column owned five peaks of acid phosphatase activity (Fig. 3). Similar result was obtained with electrophoretic analysis (Fig. 4). At different germinating stages various isozyme patterns of seedlings were observed (Fig. 5). It is worthy to note the isozyme pattern changed when seeds germinating from 3 days to 5 days, although the number of isozymes were the same. Dry seeds had the least number of bands (Figs. 1, 5). Both the cells of suspension culture and calli showed the presence of A1 band isozyme which had not been detected in the other plant tissues. However, suspension cultured cells had distinct band of A3 and calli cells had A4.

As shown in Figure 6, the activity of acid phosphatase in germinating seeds increased corelatively with prolonging time of incubation beyond two days. However, the higher enzyme activity in unimbibed and one-day imbibed seeds did

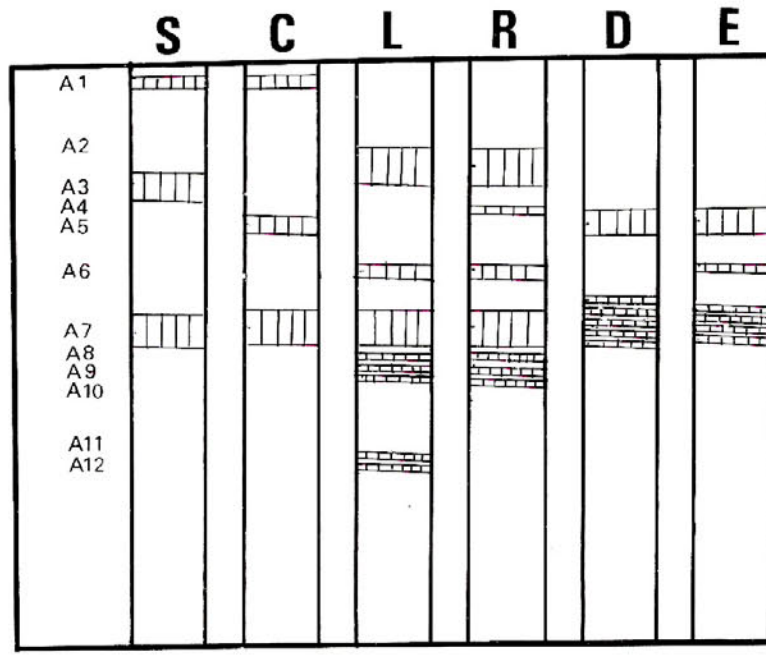

Fig. 1. Diagram of acid phosphatase pattern in rice cultured cells, tissues and germinating seeds. S, suspension cultured cells; C, calli; L, leaf from 25-day-old seedlings; R, root from 25-day-old seedlings; D, 3-day-old seedling; E, 5-day-old seedling.

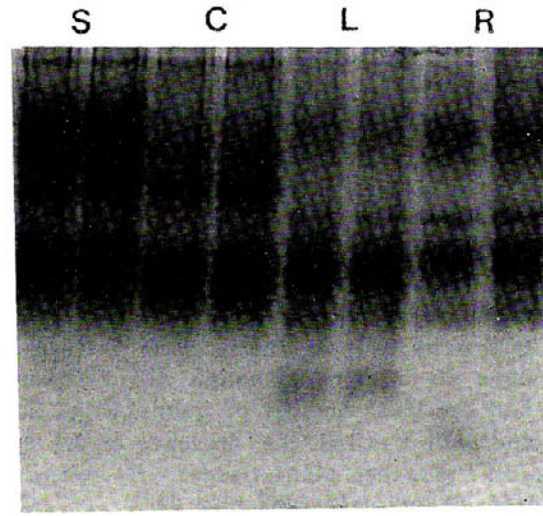

Fig. 2. Zymogram of acid phosphatase in rice cultured cells and tissues. S, suspension cultured cells; C, calli; leaf from 25-day-old seedlings; R, root from 25-day-old seedlings.

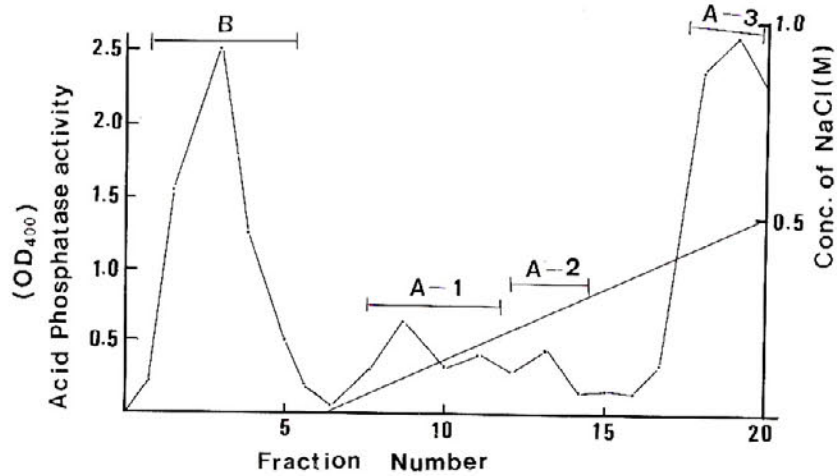

Fig. 3. Fractions of acid phosphatase in milky seed of rice. B, fraction before NaCl gradient added. A-1, fraction I; A-2, fraction II; A-3, fraction III.

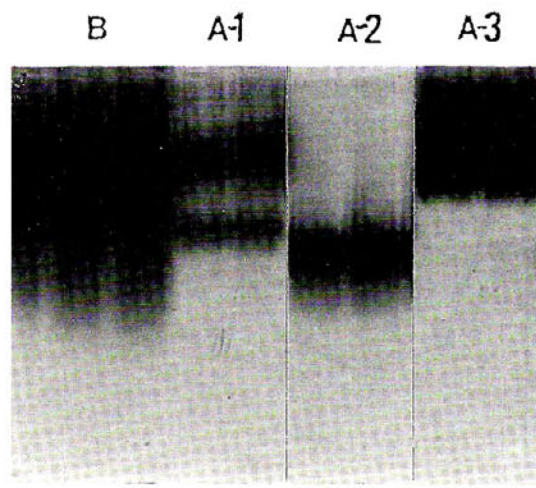

Fig. 4. Zymogram of acid phosphatase in rice milky seed. B, fraction before NaCl gradient added; A-1, fraction I; A-2, fraction II; A-3, fraction III.

occur and it might be explained by that the pre-existing enzyme in seeds was diluted after imbibition of water from medium. Further study is needed.

The optimal pH of acid phosphatases both in root and leaf of rice was around 5, and however, the enzymes in different organs showed different responses to pH ranges, broad in leaf and narrow in root (Fig. 7). Having the effects on the activity of acid phosphatase, thirteen kinds of tested salts could be divided into four groups; low promotion, non-effect, low inhibition and high inhibition (Fig. 8). $MgCl_2$, and $CoCl_2$ occupied the ability of low promotion. $CaCl_2$, KCl, Na_2-EDTA and $MnSO_4$ had no significant effect on the activity of acid phosphatase. $KMnO_4$,

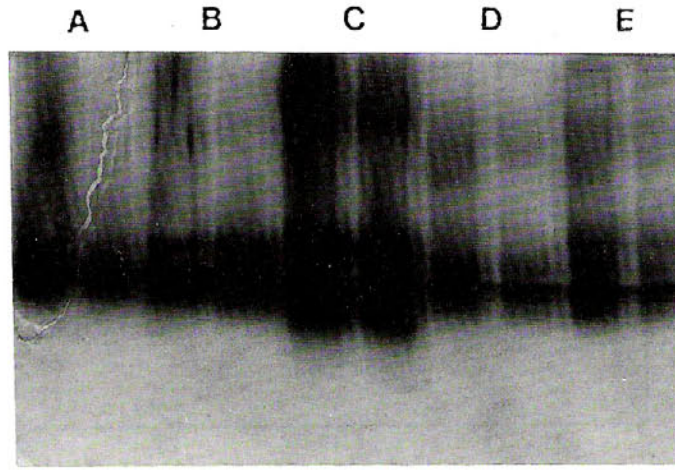

Fig. 5. Zymogram of acid phosphatase in rice germinating seeds collected in different intervals. A, dry seed; B, 11-day-old seedlings; C, 23-day-old seedlings; D, 3-day-old seedlings; E, 5-day-old seedlings.

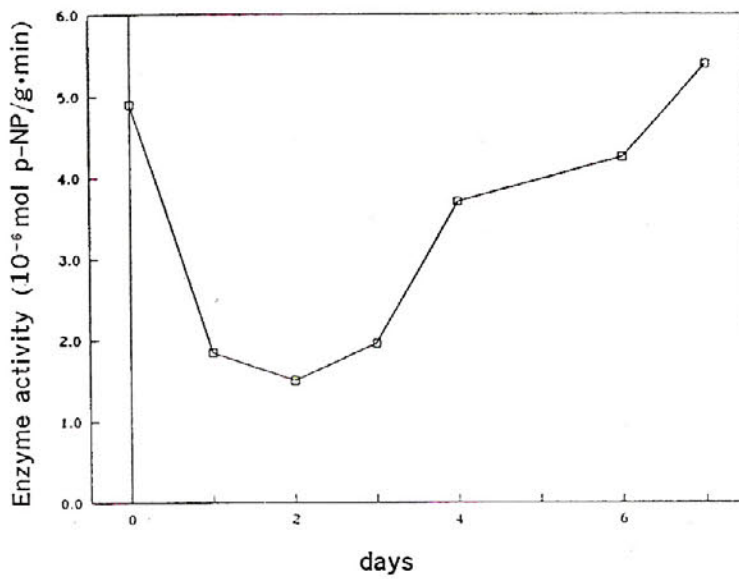

Fig. 6. Activity of acid phosphatase in germinating seeds.

$HgCl_2$ and $(NH_4)_6Mo_7O_{24}$ belonged to the group of strong inhibition. However, the different degree of inhibitory effect of $CuSO_4$, K_2HPO_4, NaF and $ZnSO_4$ was related to the concentration of individual salts in the tested solution, strong inhibitory effect in higher concentration and low inhibition in lower concentration. The inhibitory effect of $HgCl_2$ and $KMnO_4$ was worth to mention because mercury salts were often recognized as a denaturing agent for enzyme and $KMnO_4$ as strongly oxidizing agent for killing germs. It also showed that different status of manganese in salts occupying different effect on acid phosphatase activity.

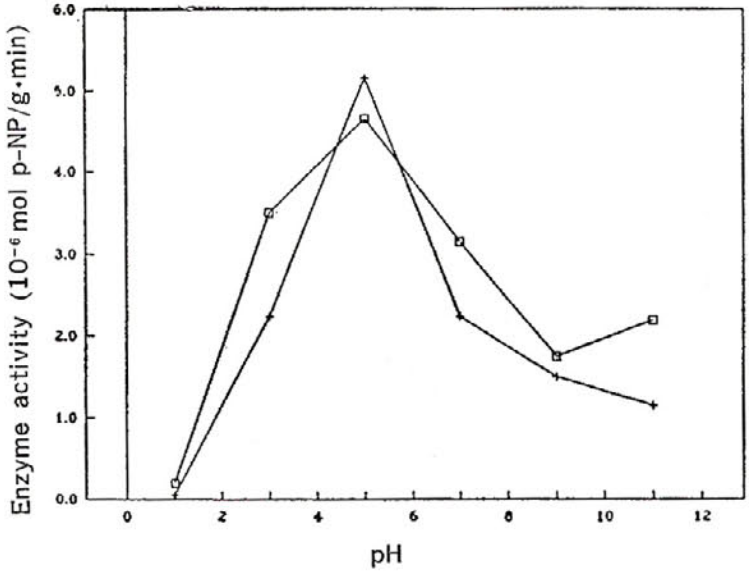

Fig. 7. The optimal pH of acid phosphatase in root (□); and in leaf of rice and in leaf (+) of 14-day-old rice seedlings.

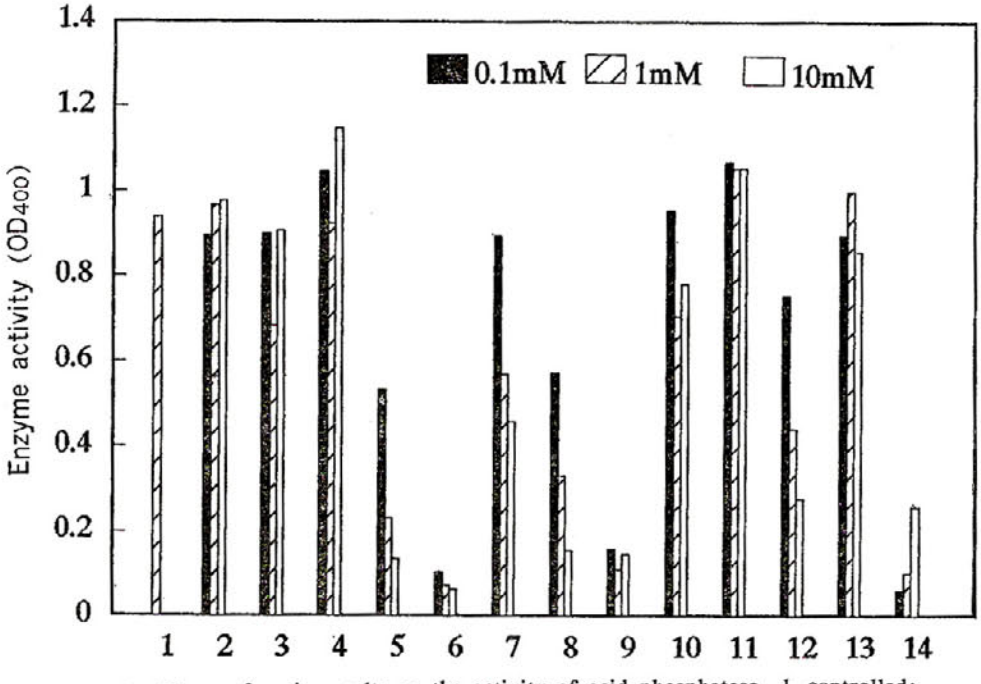

Fig. 8. Effects of various salts on the activity of acid phosphatase. 1, controlled; 2, Na_2-EDTA; 3, $CaCl_2 \cdot 2H_2O$; 4, $MgCl_2 \cdot 6H_2O$; 5, $CuSO_4$; 6, $HgCl_2$; 7, KH_2PO_4; 8, NaF; 9, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$; 10, KCl; 11, $CoCl_2 \cdot 6H_2O$; 12, $ZnSO_4 \cdot 7H_2O$; 13, $MnSO_4 \cdot H_2O$; 14, $KMnO_4$.

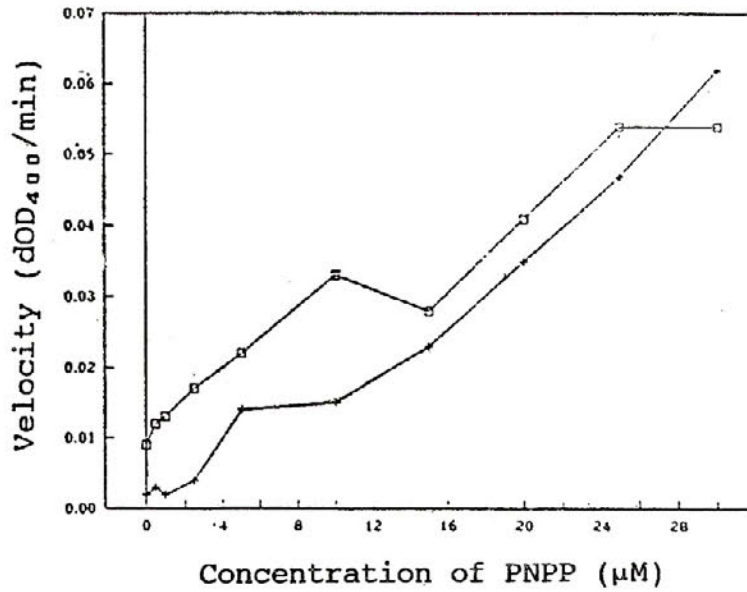

Fig. 9. Kinetics analysis of acid phosphatase in root (+); and in leaf (□) of rice.

Kinetic patterns of acid phosphatases in rice roots and leaves were different (Fig. 9). Acid phosphatase in leaves was easily saturated and its Vmax was at 1.12×10 mol·min^{-1}·g^{-1}. The value of Km at 1/2 Vmax was 8 mM. Its reaction did not completely follow Michaelis-Menten equation and its Hill coefficient was larger than one, i.e., more than one active site in one enzyme and they showed positive cooperation. However, acid phosphatase in roots was not easily saturated and without positive cooperation.

DISCUSSION

The pattern of isozymes varies with species, tissues, developmental stages of plant and even with subcellular compartment (Mizuta, *et al.*, 1980). There are some different properties between isozymes, may be physical or biological characteritics. One of the biological characteristics is the cell type-specificity (Li, *et al.*, 1970). The isozyme zymograms of acid phosphatase in rice tissues all have one diffused band A7, with low cell type-specificity, so we can find it in various tissues (Fig. 1). Comparing with the study of localization of acid phosphatase (Haung, 1988), we suspected the isozyme A7 distributed in cell wall. Several studies on enzyme localization suggested that the occurance of acid phosphatase in cell wall of higher plants is a common phenomenon (Charavat and Esau, 1975; Hall and Sexton, 1974).

Ueki and Sato (1977) pointed out that S1 isozyme had a broad optimal pH at 6.0-7.0 and S11 had an optimal pH at 6.0, in cultured tobacco cells. In this study, acid phosphatases in roots and leaves had similar optimal pH at 5.0. The ranges of optimal pH for acid phosphatases varied with species, varieties, organs and developmental stages of plants (Baker and Tadakazu 1973; Barrett-Lennard and Greenway, 1982; Park and Van Etten, 1986).

In germinating seeds, the acid phosphatase increasing greatly might be related to sugar and phosphate mobilization. In triticale, Ching *et al.*, (1984) mentioned that the presence of acid phosphatase tremendously related to the content of endogenous substances for synthesizing starch in maize, it is important for the full development of the seedling (Teno, *et al.*, 1987). Cytochemical study showed that acid phosphatase occurred in starch-containing plastids of calli cells during regeneration (Huang and Chen, 1989). In sunflower seeds, acid phosphatase might play the role of supplying inorganic phosphate for the growth of seedlings during germination was suggested (Park and Van Etten, 1986).

Manganese is a component of acid phosphatase purified from soybean had been reported (Fujimoto *et al.*, 1977). It might be reasonable to suggest the promotive effect of manganese salts on the activity of acid phosphatase. However, in this experiment, the addition of $MnSO_4$ did not enhance the enzyme activity, no matter of its concentration at 0.1, 1, or 10 mM. And, $KMnO_4$ did show strongly inhibitory effect and the effectiveness is reversely related to its concentration in solution. In reaction mixture, the concentration of $KMnO_4$ higher than 10 mM resulted in the formation of colloidal precipitation and its result became unreproducible. In pea hypocotyl, the activity of acid phosphatase was not affected by $MgCl_2$, $CaCl_2$, $CoCl_2$ and $CdCl_2$, but was strongly inhibited by $ZnSO_4$, $CuSO_4$, NaF and $(NH_4)_6Mo_7O_{24}$ (Mizuta and Sato, 1980). In cultured tobacco cells, acid phosphatase was inhibited by KH_2PO_4 and NaF, and the addition of orthophosphate to medium resulted in the suppression of releasing phosphate into extracellular region. Inorganic phosphate is competitive and NaF is non-competitive inhibitors of acid phosphatase were suggested (Ueki and Sata, 1977).

As shown in Figure 9, acid phosphatase in root was difficultly saturated in kinetic study. However, acid phosphatase purified from maize scutellum and sunflower seeds did show charaterisrics values of Km and 1/2 Vmax (Palma, *et al.*, 1982; Park and Van Etten, 1986). Several isozymes of acid phosphatase had been electrophoretically detected in this study. Further approach on purified individual isozyme will be continued.

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水稻酸性磷酸酶

黃玉芬 江嘉倩 陳榮銳

摘 要

利用電泳分析法及酵素檢定法探討水稻不同組織中的酸性磷酸酶。同功酸磷酸性酶的類型在根、葉，幼苗及癒合組織都不相同。有些同功酶具組織特異性，一中度移速的同功酶在所有組織裏的細胞壁都有存在。酸性磷酸酶最適酸鹼度是在 5 左右。$HgCl_2$, $KMnO_4$, NaF, $CuSO_4$ 及 $(NH_4)_4Mo_7O_{24}$ 都具很强的抑住酵素活性作用。在發芽種子中酸性磷酸酶被推測與儲藏物質和磷酸鹽的輸送有關。