



## Senescence of rice leaves XXV. Changes of acid phosphatase activity

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**Abstract.** The changes of acid phosphatase (Apase) activity in detached and intact leaves of rice seedlings during senescence were investigated. Apase activities in freshly excised leaves and excised leaves that had been incubated in darkness for 4 days were monitored with pH optimum of 5.0. Both freshly excised and senescent detached leaves exhibited activity towards several phosphomonoesters. In general, about 90% of Apase activity was soluble and 7% was associated with 500 g pellet. Apase was present in detached and intact leaves throughout senescence. There seems to be a direct correlation between senescence and increase of Apase activity in intact leaves, but not in detached leaves. It seems unlikely that Apase may constitute a marker for senescence of rice leaves.

**Key words:** Acid phosphatase; Leaf senescence; Light; *Oryza sativa*.

### Introduction

Acid phosphatase (Apase, E. C. 3.1.3.2) is widely distributed in higher plants. The role of Apase in plant cells is not altogether clear. It has been suggested that Apase may be involved in the onset and development of senescence (De Leo and Sacher, 1970a). However, there are conflicting reports on the changes of Apase activity during leaf senescence. Depending on plant species, decreased, increased or constant activity of Apase during leaf senescence has been demonstrated (Baker and Takeo, 1973; De Leo and Sacher, 1970a,b; Parida and Mishara, 1980; Wyen *et al.*, 1971). The present investigation was conducted to determine the changes of Apase activity during senescence of both detached and intact leaves of rice seedlings.

### Materials and Methods

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were grown as previously described (Kao, 1980). In experiments with intact leaves, leaf samples (3 cm from tip) were collected from the third leaves of seedlings at 10, 12, 14 and 16 days after planting. For the experiments with detached leaves, the apical 3-cm segments excised from the third leaves of 10-day-old seedlings were used. A group of 10 segments were floated on a Petri dish containing 10 ml of distilled water. Incubation was carried out at 27°C in darkness or the light provided with fluorescent lamps (16.7 Wm<sup>-2</sup>).

Apase was extracted and assayed following the method of Pan (1987) with some modifications. Leaf segments, weighing about 60 mg, were homogenized in prechilled mortar and pestle with 8 ml of cold 50 mM of Tris-maleate buffer (pH 7.0) with 13 mM mercaptoethanol at 4°C. The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The resulting clear supernatant fractions were used directly for enzyme assay. The reaction mixture contained 0.6 ml of 100 mM acetate

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buffer (pH 5.0) with 13 mM mercaptoethanol, 0.1 ml of 200 mM *p*-nitrophenyl phosphate (pNPP), and 0.3 ml of enzyme extract. Reactions were carried out at 37°C for 10 min, and terminated by adding 4.0 ml of 0.3 M sodium carbonate. The amount of *p*-nitrophenol liberated in the assay medium was determined spectrophotometrically at 400 nm. One unit of Apase activity is defined as the amount of enzyme that liberated 1  $\mu$ mol of *p*-nitrophenol per min. In experiments where substrate specificity was examined, the method of Kar and Mishara (1975) was adopted. The reaction was terminated by adding 1.0 ml of 20% (v/v) perchloric acid. After the precipitate was removed by centrifugation, an aliquot was taken for determination of inorganic phosphorus (Yoshida *et al.*, 1972). One unit of enzyme activity is defined as the amount of enzyme that liberated 1  $\mu$ mol inorganic phosphorus per min. For the experiments of Apase distribution, subcellular fractionation was carried out by successive centrifugations of the extract at 500, 2,000, 10,000 and 30,000 g. Each pellet was suspended in extraction medium. Apase activity was then determined.

Chlorophyll was extracted and determined as described previously (Kao, 1980) and expressed as  $A_{665}$  per 10 segments.

## Results

Apase activity in crude extract of rice leaves was linear with time (Fig. 1). The pH optimum for freshly excised and senescent detached leaves was 5.0 (Fig. 2). Michaelis constant was 1.9 mM (data not shown). Apase from both freshly excised and senescent detached leaves exhibited activity towards several phosphomonoesters (Table 1). Vanadate and molybdate, but not azide, showed strong inhibition of Apase activity (data not shown).

The senescence of rice leaves was followed by measuring the decrease of chlorophyll. Figure 3 shows the time course of chlorophyll and Apase activity of detached leaves. Under dark condition, a decrease in chlorophyll level was evident at 2 days after leaf detachment, whereas Apase activity increased immediately after excision. Chlorophyll level was much higher in detached leaves incubated in the light than in the dark. However, light did not suppress or only slightly suppressed the rise of Apase activity.

Chlorophyll level and Apase activity in the third

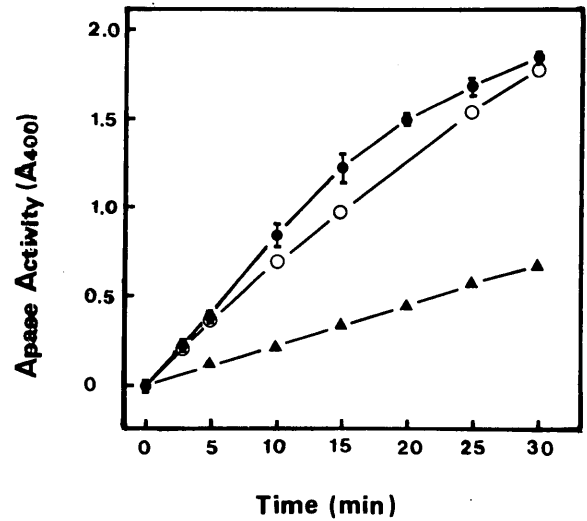


Fig. 1. Effects of reaction time and volume of enzyme extract on Apase activity. Reaction mixture contained 0.5 ml (●) 0.3 ml (○) or 0.1 ml (▲) enzyme extract.

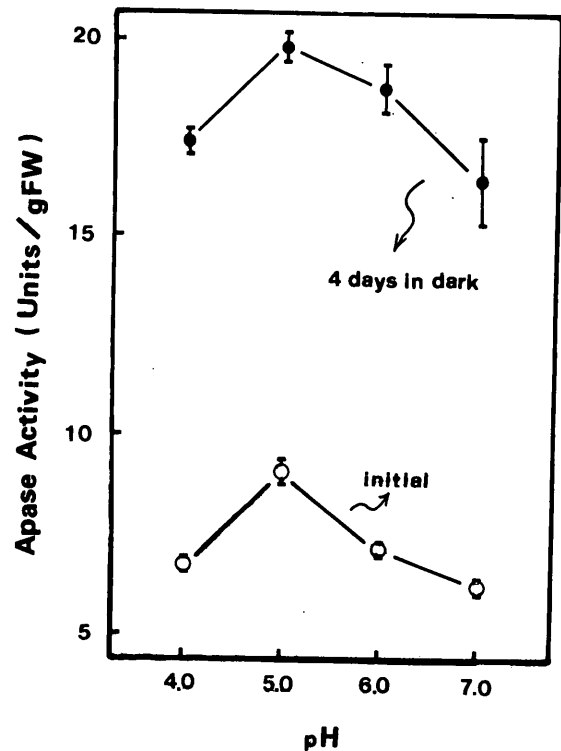


Fig. 2. Effect of pH on Apase activities in crude extract of freshly excised leaves (initial) and excised leaves which had been incubated in darkness for 4 days (4 days in dark). Aliquots of enzyme extract were incubated with buffer (60 mM final concentration). The buffers for pH 4.0-5.0 and pH 6.0-7.0 are acetate and Tris-maleate, respectively.

**Table 1.** Activity of Apase with various substrates. Enzyme extract was prepared from freshly excised leaves (initial) and excised leaves which had been incubated in darkness and the light for 4 days (4 days in dark and 4 days in light, respectively). The assay mixture contained 5 mM of respective substrates

Substrate	Enzyme units/gFW		
	Initial	4 days in dark	4 days in light
Adenosine 5'-triphosphate	8.9±0.1	17.8±0.2	18.7±0.2
Adenosine 5'-diphosphate	7.8±0.2	15.8±0.3	16.6±0.3
Sodium pyrophosphate	6.2±0.2	13.5±0.3	14.0±0.2
pNPP	5.1	14.9±0.2	12.0±0.4
β-Glycerolphosphate	1.8	5.9±0.2	6.3
Glucose 6-phosphate	1.2	4.8±0.1	4.6±0.1
Adenosine 5'-monophosphate	0.8	2.7	2.6±0.1
Phosphorylethanolamine	0.7±0.1	2.2±0.1	2.2

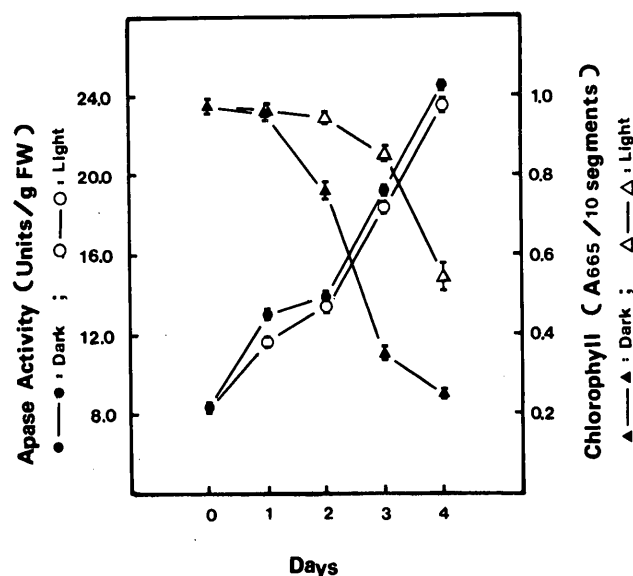


Fig. 3. Effects of light and dark on the chlorophyll content and Apase activity of detached rice leaves.

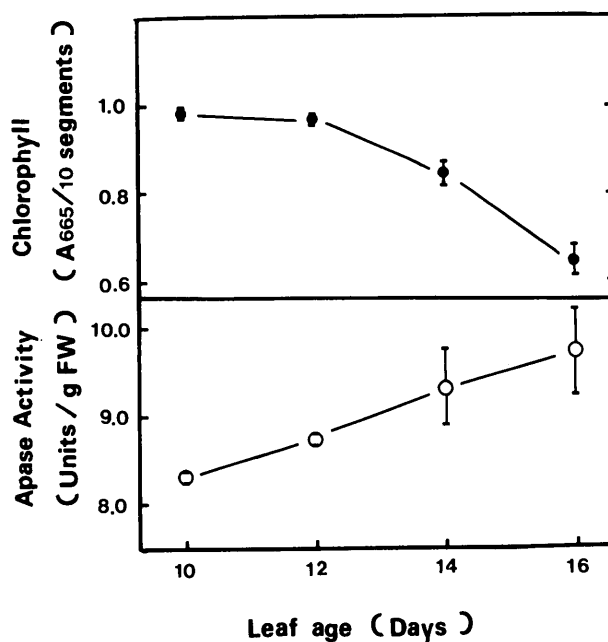


Fig. 4. Chlorophyll content and Apase activity in intact leaves of rice seedlings during senescence.

leaves from 10- to 16-day-old seedlings are showed in Fig. 4. Chlorophyll level in intact leaves decreased with increasing age. However, Apase activity increased with increasing age. When 10-day-old greenhouse-grown seedlings were transferred to continuous light or darkness, decrease of chlorophyll level and increase of Apase activity were significantly retarded by continuous light (Fig. 5).

Tables 2 and 3 show Apase activity in subcellular fraction during senescence of rice leaves. In general,

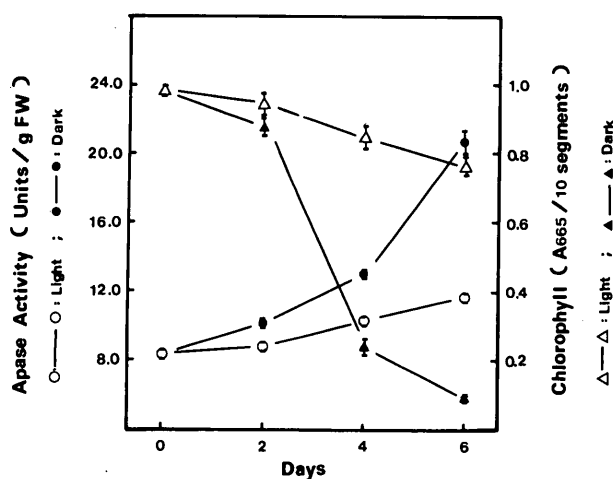
about 90% of Apase activity was soluble and 7% was associated with 500 g pellet. These observations indicate that Apase is mostly soluble enzyme but suggest that part of the Apase activity is associated with easily sedimentable structures. Apase activity in all fractions increased during senescence of detached leaves (Table 2). However, no increase in Apase activity in 10,000 and 20,000 g fractions was observed during senescence of intact leaves (Table 3).

**Table 2.** *Apase activity in subcellular fraction and total activity in extracts from freshly excised leaves (initial) and excised leaves which had been incubated in the light and darkness for 4 days (4 days in light and 4 days in dark, respectively)*

Pellet obtained by centrifugation at	Initial		4 days in light		4 days in dark	
	Units/gFW	(%)	Units/gFW	(%)	Units/gFW	(%)
10 min 500 g	0.32	(6.8)	1.17	(6.9)	0.96	(5.2)
10 min 2,000 g	0.08	(1.7)	0.15	(0.9)	0.27	(1.5)
30 min 10,000 g	0.08	(1.7)	0.16	(0.9)	0.26	(1.4)
30 min 30,000 g	0.08	(1.7)	0.12	(0.7)	0.19	(1.0)
Supernatant	4.16	(88.1)	15.38	(90.0)	16.90	(90.9)
Total activity	4.72		16.98		18.57	

**Table 3.** *Apase activity in subcellular fraction and total activity in extracts from the third leaves of 10-day-old and 16-day-old seedlings*

Pellet obtained by centrifugation at	10-day-old		16-day-old	
	Units/gFW	(%)	Units/gFW	(%)
10 min 500 g	0.32	(6.8)	0.62	(8.9)
10 min 2,000 g	0.08	(1.7)	0.13	(1.9)
30 min 10,000 g	0.08	(1.7)	0.07	(1.0)
30 min 30,000 g	0.08	(1.7)	0.07	(1.0)
Supernatant	4.16	(88.1)	6.10	(87.2)
Total activity	4.72		6.99	



**Fig. 5.** Effects of light and dark on the chlorophyll content and Apase activity of intact leaves of rice seedlings. Ten-day-old rice seedlings were transferred to continuous light ( $16.7 \text{ Wm}^{-2}$ ) or darkness. Temperature for both light and dark conditions were controlled at  $27^\circ\text{C}$ . For enzyme assay and chlorophyll determination, the apical 3-cm portions of the third leaves were collected at 0, 2, 4 and 6 days after transferring seedlings to the light or darkness.

## Discussion

Apase was present in both detached and intact leaves throughout senescence. The pH optimum and relative activity towards various substrates are generally in agreement with those from other plant sources (Baker and Takeo, 1973; Pan, 1987).

De Leo and Sacher (1971a,b) proposed that an increase in Apase activity was a change common to senescing fruit and leaf tissue. Some of the results of the present study do not support this suggestion. This conclusion is based on the observation that light which markedly retarded senescence of detached rice leaves did not inhibit or only slightly inhibited the increase of Apase. It seems that Apase is not of functional importance in regulating senescence of detached rice leaves.

Conventionally, the biochemical parameters used to indicate senescence of leaves are the decreases in the amount of chlorophyll and protein in the leaves (Thimann, 1980). However, efforts have been made to seek a marker enzyme for leaf senescence (Laurière,

1983). Based on the discussion mentioned above, it seems unlikely that Apase may constitute a marker for rice leaf senescence.

Apase isozymes are known to occur in rice tissues (Huang, 1988; Igaue *et al.*, 1973). Thus, the possibility that certain specific Apase isozyme may constitute a marker of senescence can not be excluded. The possible changes of Apase isozymes in detached and intact rice leaves during senescence are now the subject of further research.

Since the increase of Apase activity in rice leaves is rapid following excision, one may argue that the increase of Apase activity is probably a wound response. When detached leaves were used to study senescence and determine Apase activity, wounding is always a problem. However, in the present study, each long and narrow rice leaf was cut once transversely, the area of wounding was very small. The increase of Apase activity is therefore thought to be an excision-related response. Several lines of evidence indicate that Apase activity in plants typically increases when plants become phosphorus deficient or water deficit (Barrett-Lennard *et al.*, 1982a,b; Besford, 1979). It has been reported that there was a slight but significant increase in the fresh weight of rice leaf segments floated on water (Kao, 1981). It seems unlikely that the rise of Apase activity in detached leaves is resulted from water deficit. Since leakage of phosphate from detached rice leaves has been shown to increase during senescence (Shyr and Kao, 1985), phosphorus deficiency may contribute, at least in part, to the rise of Apase activity in detached rice leaves during senescence.

In intact leaf system, the rise of Apase activity was also found to be associated with the progress of senescence. When greenhouse-grown seedlings were transferred to continuous light or darkness, light treatment which significantly retarded senescence suppressed the rise of Apase activity. It seems that the increase of Apase activity in intact rice leaves has direct relationship to senescence. Slow, natural senescence does not appear to be the same as senescence in detached leaves. This conclusion is further supported by the observations that Apase activity in all subcellular fractions increased during senescence of detached leaves, whereas only soluble Apase activity and Apase activity associated with easily sedimentable structures increased during senescence of intact leaves.

Biswas and Choudhuri (1980) demonstrated that

the induction of senescence of the flag leaves in mature rice plants was preceded by a plentiful transport of phosphorus to the grains, which might result in phosphorus deficiency in flag leaves. We do not know whether the induction of senescence of intact rice seedlings leaves is also preceded by the transport of phosphorus to other young expanding and growing leaves. If it does occur, then phosphorus deficiency could be the trigger resulting in the rise of Apase in intact rice leaves during senescence.

### Literature Cited

- Baker, J. E. and T. Takeo. 1973. Acid phosphatase in plant tissue I. Changes in activity and multiple forms in tea leaves and tomato fruit during maturation and senescence. *Plant Cell Physiol.* **14**: 459-471.
- Barrett-Lennard, E. G., A. D. Robson, and H. Greenway. 1982a. Effect of phosphorus deficiency and water deficit on phosphatase activity from wheat leaves. *J. Exp. Bot.* **33**: 682-693.
- Barrett-Lennard, E. G., A. D. Robson, and H. Greenway. 1982b. Partial separation and characterization of soluble phosphatases from leaves of wheat grown under phosphorus deficient and water deficit. *J. Exp. Bot.* **33**: 694-704.
- Besford, R. T. 1979. Quantitative aspects of leaf acid phosphatase activity and the phosphorus status of tomato plants. *Ann. Bot.* **44**: 153-161.
- Biswas, A. K. and M. A. Choudhuri. 1980. Mechanism of monocarpic senescence in rice. *Plant Physiol.* **65**: 340-345.
- De Leo, P. and J. A. Sacher. 1970a. Senescence. Association of synthesis of acid phosphatase with banana ripening. *Plant Physiol.* **46**: 208-211.
- De Leo, P. and J. A. Sacher. 1970b. Control of ribonuclease and acid phosphatase by auxin and abscisic acid during senescence of *Rhoeo* leaf sections. *Plant Physiol.* **46**: 806-811.
- Huang, Y. F. 1988. Acid Phosphatase in Rice. MS Thesis, Department of Botany, National Taiwan University, Taipei, Taiwan, Republic of China.
- Igaue, I., M. Nishio, and F. Kurasawa. 1973. Occurrence of acid phosphatase-isozymes repressible by inorganic phosphate in rice plant cell cultures. *Agric. Biol. Chem.* **37**: 941-943.
- Laurière, C. 1983. Enzymes and leaf senescence. *Physiol. Veg.* **21**: 1159-1177.
- Kao, C. H. 1980. Senescence of rice leaves IV. Influence of benzyladenine on chlorophyll degradation. *Plant Cell Physiol.* **21**: 339-344.
- Kao, C. H. 1981. Senescence of rice leaves VI. Comparative study of the metabolic changes of senescing turgid and water-stressed excised leaves. *Plant Cell Physiol.* **22**: 683-688.
- Kar, M. and D. Mishra. 1975. Inorganic pyrophosphatase activity during rice leaf senescence. *Can. J. Bot.* **53**: 503-511.
- Pan, S. M. 1987. Characterization of multiple acid phosphatases

- in salt-stressed spinach leaves. *Aust. J. Plant Physiol.* **14**: 117-124.
- Parida, P. K. and D. Mishra. 1980. Acid phosphatase and adenosine triphosphatase activity during rice leaf development and senescence. *Photosynthetica* **14**: 431-436.
- Shyr, Y. Y. and C. H. Kao. 1985. Senescence of rice leaves XV. Solute leakage and inorganic phosphate uptake. *Bot. Bull. Academia Sinica* **26**: 171-178.
- Thimann, K. V. 1980. The senescence of leaves. *In* K. V. Thimann (ed.), *Senescence in Plants*. CRC Press, Boca Raton, Florida, pp. 85-116.
- Wyen, N. V., J. Udvardy, and J. L. Farkas. 1971. Changes in the level of acid phosphatases in *Avena* leaves in response to cellular injury. *Phytochemistry* **10**: 765-770.
- Yoshida, S., D. A. Forno, J. H. Cock, and K. A. Gomez. 1972. *Laboratory Manual for Physiological Studies of Rice*. The International Rice Research Institute, Los Banos, Philippines.

## 水稻葉片老化之研究 (二十五) 酸性磷酸水解酵素活性之變化

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本文主要探討台中在來一號水稻幼苗切離與完整葉片在老化過程，酸性磷酸水解酵素活性之變化。不論是由剛切離之葉片，或由老化之切離葉片，所抽取出之酸性磷酸水解酵素。其最適 pH 值均為 5.0，且可分解多種不同之受質。一般而言，約 90% 之磷酸水解酵素為可溶性，而 7% 之活性則存在於 500 xg 之沉澱內。完整葉片之老化與酸性磷酸水解酵素活性之變化有明顯之相關關係。然而二者間之關係不存在於切離葉片內。酸性磷酸水解酵素似乎不能作為葉片老化之標示酵素。