

Induction of acid phosphatase in detached rice leaves under stress conditions

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Abstract. The effect of water stress, sorbitol and NaCl on acid phosphatase (APase) activity in detached rice leaves was investigated. APase activity was increased in detached rice leaves treated with methyl jasmonate (MJ), water stress, sorbitol, and NaCl. The induction of APase activity under water, osmotic and salt stresses, and MJ was not accompanied by a decrease in phosphorus level. These results suggest that the induction of APase under stress conditions and MJ may have a cause other than phosphorus deficiency. Evidence is presented to show that stress-induced APase activity is due to de novo APase synthesis and is unlikely to be mediated through MJ.

Keywords: Acid phosphatase; Methyl jasmonate; *Oryza sativa*; Osmotic stress; Salt stress; Water stress.

Introduction

Acid phosphatase (APase, EC 3.1.3.2) is widely distributed in plants. It has long been recognized that APase activity in plants typically increases when plants become phosphorus (Pi) deficient. The increase in APase activity correlates with a low level of Pi in numerous species and plant parts (Barrett-Lennard et al., 1982; Duff et al., 1989; Goldstein et al., 1989; Lefebvre et al., 1990; Ueki and Sato, 1977). Salt, water, and osmotic stresses have also been reported to increase APase activity (Barrett-Lennard et al., 1982; Pan, 1987; Szabo-Nagy et al., 1992). It has been demonstrated that drought induced an increase in APase activity without exerting significant changes in the Pi level (Barrett-Lennard et al., 1982). Szabo-Nagy et al. (1992) also demonstrated that the induction of APase under osmotic and salt stresses was not accompanied by a decrease in Pi level. Recently, we reported that methyl jasmonate (MJ) markedly induced APase activity in detached rice leaves under light conditions and this induction is not caused by a decrease in Pi level (Yeh et al., 1995). It is not known whether the mechanism of APase activity induced by water, salt, and osmotic stresses is similar to that of MJ.

This study was designed to examine whether a low level of Pi can serve as a signal for the induction of APase under water, salt, and osmotic stresses in detached rice leaves. We also compared the effect of MJ and stresses on the isoenzymes of APase in detached rice leaves.

Materials and Methods

Rice (*Oryza sativa*, cv. Taichung Native 1) seedlings were grown in hydroponic culture as described previously

(Chen et al., 1990). The apical 3 cm of the third leaves of 12-day-old seedlings were used for the experiments. Ten segments of rice leaves were floated on 10 ml of test solution in a Petri dish. Incubation was conducted under light conditions ($40 \mu\text{mol m}^{-2} \text{s}^{-1}$). Water stress was applied by a previously described method (Kao, 1981). Groups of ten leaf segments were weighed and exposed to the vapor above a solution of 0.5 M NaCl.

For enzyme extraction, leaf segments were homogenized in a prechilled mortar and pestle with cold 50 mM Tris-Mes buffer, pH 7.0, containing 13 mM mercaptoethanol at 4°C. The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The resulting clear supernatant was used directly for APase assay. APase activity was assayed using *p*-nitrophenyl phosphate as substrate (Huang and Kao, 1991). One unit of APase activity is defined as the amount of enzyme which liberates 1 μmol of *p*-nitrophenol min^{-1} . Pi was extracted with perchloric acid (2.5%) and determined by spectrophotometric method of Yoshida et al. (1972). All data for control, NaCl and sorbitol treatments were expressed on the basis of fresh weight, whereas those for water stress treatment were on the basis of initial fresh weight.

Native PAGE was performed using a modification of the method of Davis (1964). Final acrylamide monomer concentrations in the slab gels were 7.5% (w/v) for the separating gel and 5% (w/v) for the stacking gel. Samples were run at a constant current of 20 mA at 4°C. Following electrophoresis, APase were visualized at room temperature using 0.1% Fast red TR salt and 1% α -naphthylphosphate in 5 mM MgCl_2 and 200 mM sodium acetate (pH 5.0).

Results and Discussion

Effect of NaCl and sorbitol on APase activity in detached rice leaves under light conditions is shown in Fig-

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ure 1. NaCl at 0.4 M significantly increased APase activity. No further increase in APase was observed when NaCl was increased to 0.6 M. Increase in APase activity by sorbitol was observed only at 0.6 M.

Figure 2 shows the changes with time in APase activity in detached rice leaves treated with sorbitol, water stress, and NaCl. In control leaves, APase activity remained unchanged during 24 h of incubation. Increase in APase activity in detached rice leaves by sorbitol, water, and NaCl stresses was observed at 12, 12 and 24 h, respectively, after the start of treatment. Clearly, APase activity in detached rice leaves was induced by water, salt, and osmotic stresses. These results are in general agreement with those reported by Barrett-Lennard et al. (1982), Pan (1987), and Szabo-Nagy et al. (1992).

To understand whether stress-increased APase activity in detached rice leaves was induced by a low level of Pi or Pi deficiency, effect of sorbitol, NaCl, and water stress on the level of Pi was determined. As indicated in Figure 3, the level of Pi in detached rice leaves was not reduced by sorbitol, NaCl, or water stress. These results suggest that the induction of APase activity under water, osmotic, and salt stresses is unlikely to be caused by a low level of Pi. Berrett-Lennard et al. (1982) and Szabo-Nagy et al. (1992) reached a similar conclusion. Figure 4 also shows that MJ-induced APase activity is not accompanied by a reduction of Pi level.

Effect of cycloheximide (CHI), an inhibitor of protein synthesis, on the induction of APase activity by MJ, sorbitol, NaCl, and water stress is shown in Table 1. MJ-, water stress-, NaCl-, and sorbitol-induced APase activity was almost completely blocked by CHI, indicating that APase activity induced by stresses and MJ is due to de novo APase synthesis.

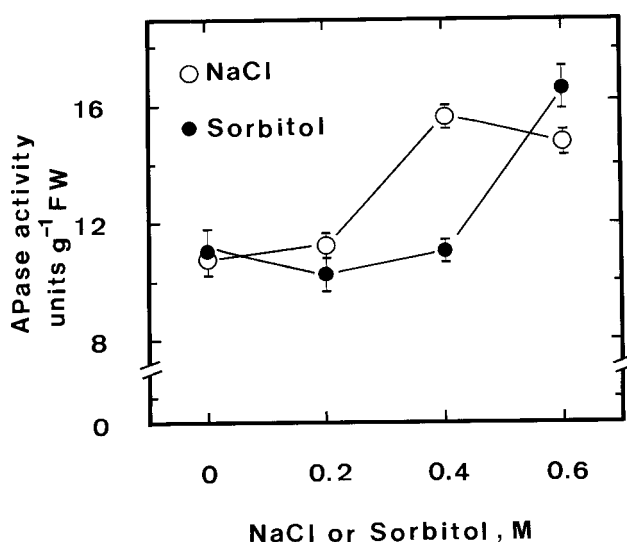


Figure 1. Effect of NaCl and sorbitol on APase activity in detached rice leaves. APase was assayed 24 h after treatment under light conditions. Vertical bars represent standard errors ($n=4$).

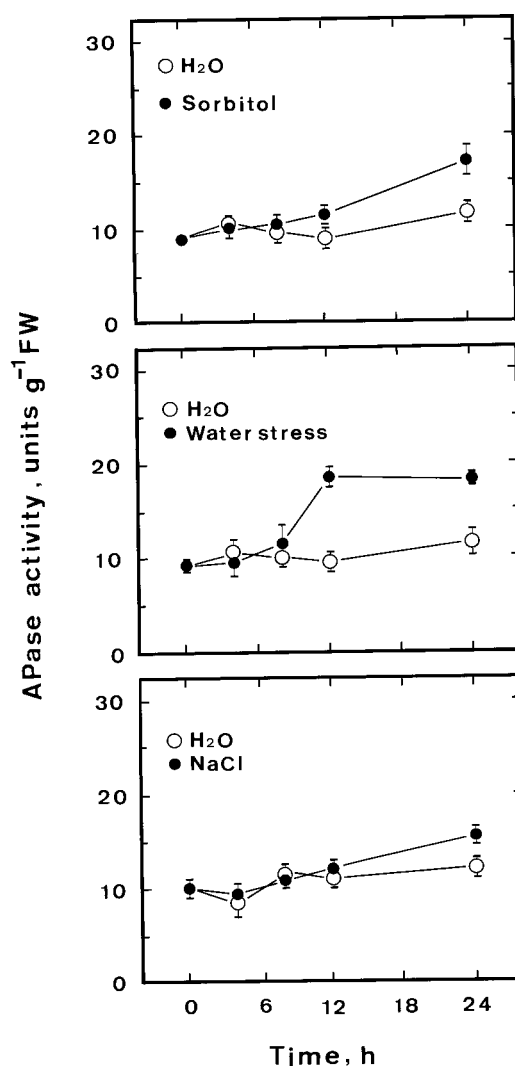


Figure 2. Changes in APase activity in detached rice leaves treated with sorbitol (0.6 M), water stress, and NaCl (0.4 M) under light conditions. Vertical bars represent standard errors ($n=4$).

Table 1. Effect of cycloheximide (CHI, 10 μ M) on MJ-, sorbitol-, NaCl-, and water stress-induced APase activity in detached rice leaves. The concentration of MJ, sorbitol and NaCl was 20 μ M, 0.6, and 0.4 M, respectively. For water stress, detached rice leaves were pretreated with either water or 10 μ M CHI for 6 h. APase activity was assayed 24 h after treatment under light conditions. Vertical bars represent standard errors ($n=4$).

Treatment	APase activity, units g ⁻¹ FW
Control	10.3 \pm 0.1
MJ	50.1 \pm 2.8
MJ + CHI	11.9 \pm 1.2
Sorbitol	16.0 \pm 1.4
Sorbitol + CHI	8.9 \pm 1.5
NaCl	14.8 \pm 0.1
NaCl + CHI	7.9 \pm 1.5
H ₂ O→H ₂ O	9.9 \pm 0.3
H ₂ O→Water stress	17.5 \pm 0.6
CHI→Water stress	12.0 \pm 1.7

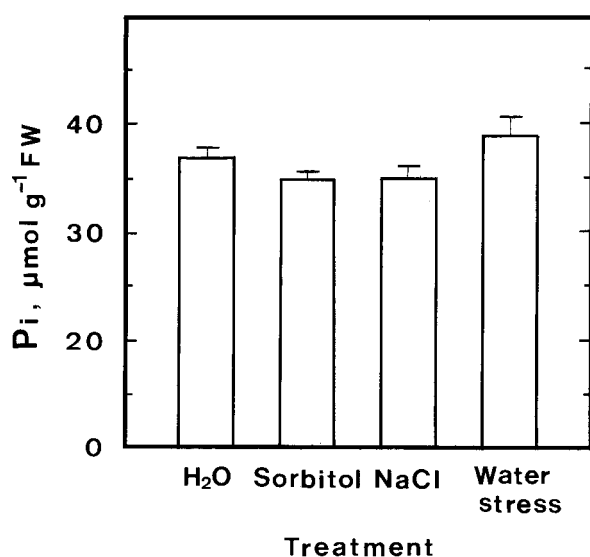


Figure 3. Effect of sorbitol (0.6 M), NaCl (0.4 M), and water stress on Pi levels in detached rice leaves. Pi level was determined 24 h after treatment under light conditions.

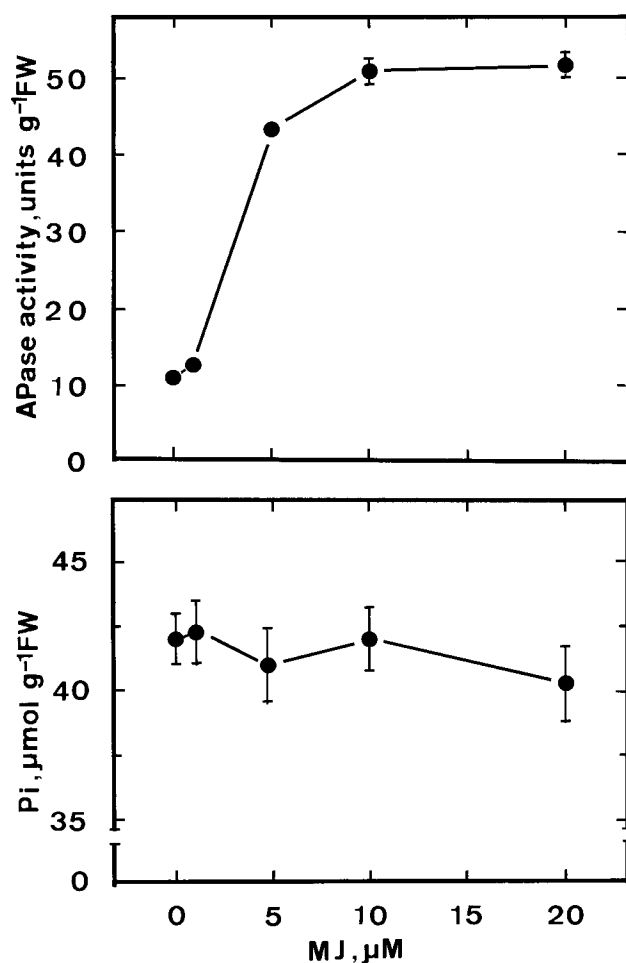


Figure 4. Effect of MJ on APase activity and Pi levels in detached rice leaves. APase activity and Pi level were measured 24 h after treatment under light conditions. Vertical bars represent standard errors ($n=4$).

MJ has been shown to increase APase activity in detached rice leaves under light conditions (Shih and Kao, 1997; Yeh et al., 1995). It has also been shown that sorbitol provoked a marked increase of endogenous jasmonates (Kramell et al., 1995). Thus, it is of great interest to know whether APase activity increased by sorbitol, NaCl, and water stress is similar to that by MJ. When proteins from MJ- and stress-treated detached rice leaves were separated by Native PAGE, a single APase staining band (AP-5) was markedly expressed in MJ-treated leaf preparation, which could not be seen in untreated, water-, NaCl- and sorbitol-stressed leaf preparations (Figure 5). The expression of AP-5 induced by MJ could be significantly inhibited by CHI (Shih and Kao, 1997). These results suggest that MJ likely does not serve as a signal for the induction of APase under osmotic, water, and salt stresses.

Figure 5. APase activity stain of native PAGE. Detached rice leaves were treated with H₂O, MJ (20 μM), water stress, sorbitol (0.6 M), and NaCl (0.4 M) for 24 h under light conditions. Each lane was loaded with 65 μg protein.

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