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The effects of salt stress on acid phosphatase activity of *Zea mays* seedlings

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Absrract. NaCl salt stress was imposed on 4-day-old seedlings of *Zea mays* for one to three days. The growth of salt-stressed seedlings was inhibited, and the total acid phosphatase activity was slightly reduced. Multiple acid phosphatases having different molecular weights were revealed by 5 to 20% acrylamide gradient gel electrophoresis. The high molecular weight (MW. 190,000) acid phosphatase increased in different parts of the salt-stressed seedlings. The role of this promotive effect of salt stress on the high-molecular-weight acid phosphatases of *Zea mays* seedlings is discussed.

Key words: Acid phosphatase; Salt stress; *Zea mays*

Introduction

Acid phosphatase (EC 3.1.3.2.) is an important hydrolytic enzyme which is widely distributed in plants and often occurrs in multiple forms (Yamagata *et al.*, 1979; Mizuta, 1980) differing in molecular size, substrate specificity, and electrophoretic behavior. Water stress can cause increased acid phosphatase activity in the leaves of cowpeas (Takaoki, 1968), cotton (Vieira-Da-Silva, 1969) and wheat (Barrett-Lennard *et al.*, 1982); phosphorus deficiency also increased acid phosphatase activity in various plants (Dracup *et al.*, 1984; Barrett-Lennard *et al.*, 1982). However, not all isoenzymes can be affected equally by the same environmental changes. In wheat, water stress caused increase in one of the acid phosphatase isoenzymes (Barrett-Lennard *et al.*, 1982), but in cultured tobacco, phosphorus deficiency caused an increase in one of the extracted phosphatases (Katsuyi and Sato, 1977). It was reported previously that incremental salt stress had a promotive effect on the acid phosphatase activity of hydroponically grown spinach leaves, particularly on that of high-molecular-weight (MW. 300,000) acid phosphatase (Pan, 1987).

In this paper, we report that salt stress had a slightly inhibitory effect on total acid phosphatase activity in hydroponically grown *Zea mays* seedlings, but had a specific promotive effect on its high-molecular-weight acid phosphatase.

Materials and Methods

Growth of Plants

Seeds of *Zea mays* L. var. Tainan 11 were surface-sterized in 1% NaOCl for 1 h, then were soaked in running water overnight. Four-day-old seedlings were used as experimental material. Seedlings were grown in half-strengh Hoagland's solution, in which 0, 150, or 300 mM NaCl was

added. Seedlings were grown with a 12-h photoperiod at 30°C day/25°C night temperature.

Enzyme Preparation

After the experimental period, healthy seedlings were dissected into shoot, root or endosperm parts, then frozen individually in liquid nitrogen. Each parts were ground with a motar and pestle in grinding medium (2 ml/g) containing 0.15 M Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol. The tissue extract was filtered through two layers of cheesecloth, and centrifuged at 10,000 xg for 30 min. The supernatant was taken for measuring acid phosphatase activity, protein content, or for electrophoresis.

Assays of Acid Phosphatase Activity

The assay medium consisted of 100 mM sodium acetate buffer (pH 5.0), an appropriate amount of enzyme and 5 mM *p*-nitrophenol phosphate in a final volume of 1.0 ml. The reaction was initiated by adding 0.1 ml of substrate to the medium at 35°C and after 30 min incubation was terminated by the addition of 4.0 ml of 0.3 M Na_2CO_3. The amount of *p*-nitrophenol liberated in the assay medium was determined spectrophotometrically at 400 nm, taking $4.0\times10^3\ M^{-1}\ cm^{-1}$ as the molar extinction coefficient for *p*-nitrophenol.

Protein Determination

Protein content was determined by Bradford's method (1976), using bovine serum albumin as a reference protein.

Analytical Disc Gradient Gel Electrophoresis

Disc gradient gel electrophoresis was performed at pH 8.3 on 5 to 20% polyacrylamide gradient gels. Similar amounts of protein from the control or the salt-stressed samples were taken for electrophoresis. The sample was run at 100 V constant voltage and stained for protein with Coomassie Brilliant Blue R. For staining acid phosphatase activity, the slab gel was incubated in a 100 ml solution containing 0.2 M sodium acetate buffer

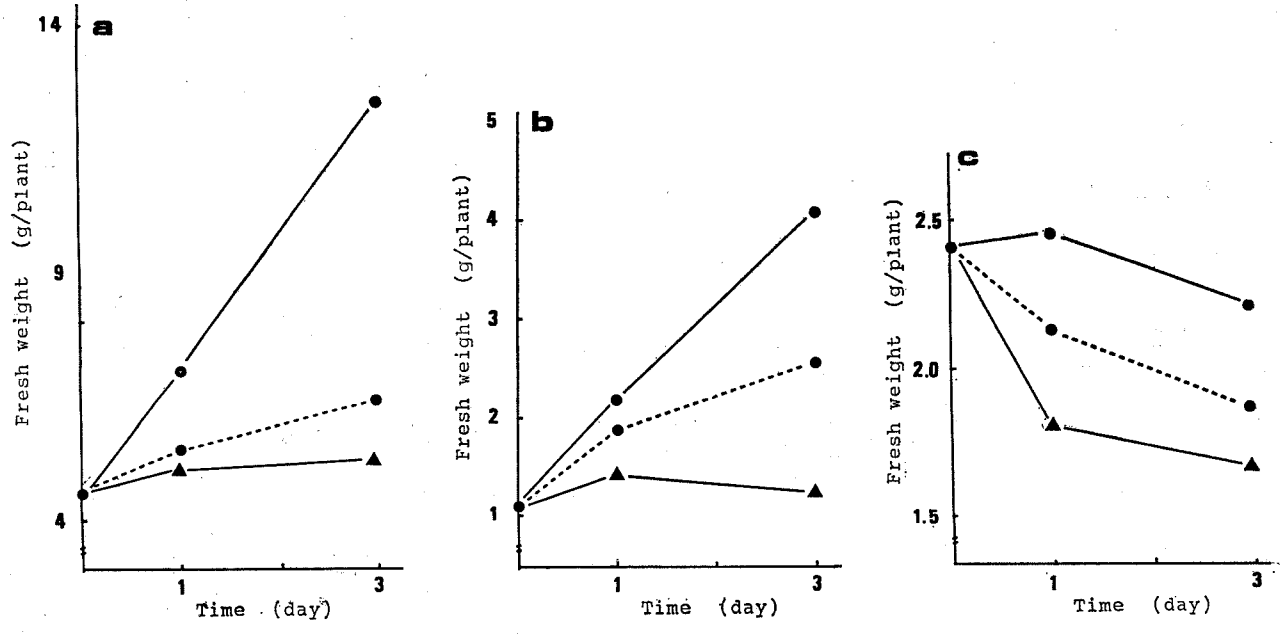

Fig. 1. The effect of salt stress on the growth of *Zea mays* seedlings after treatment.
Control (●—●—●), 150 mM NaCl (●---●---●), 300 mM NaCl (▲—▲—▲), a: the shoot part, b: the root part, c: the endosperm part.

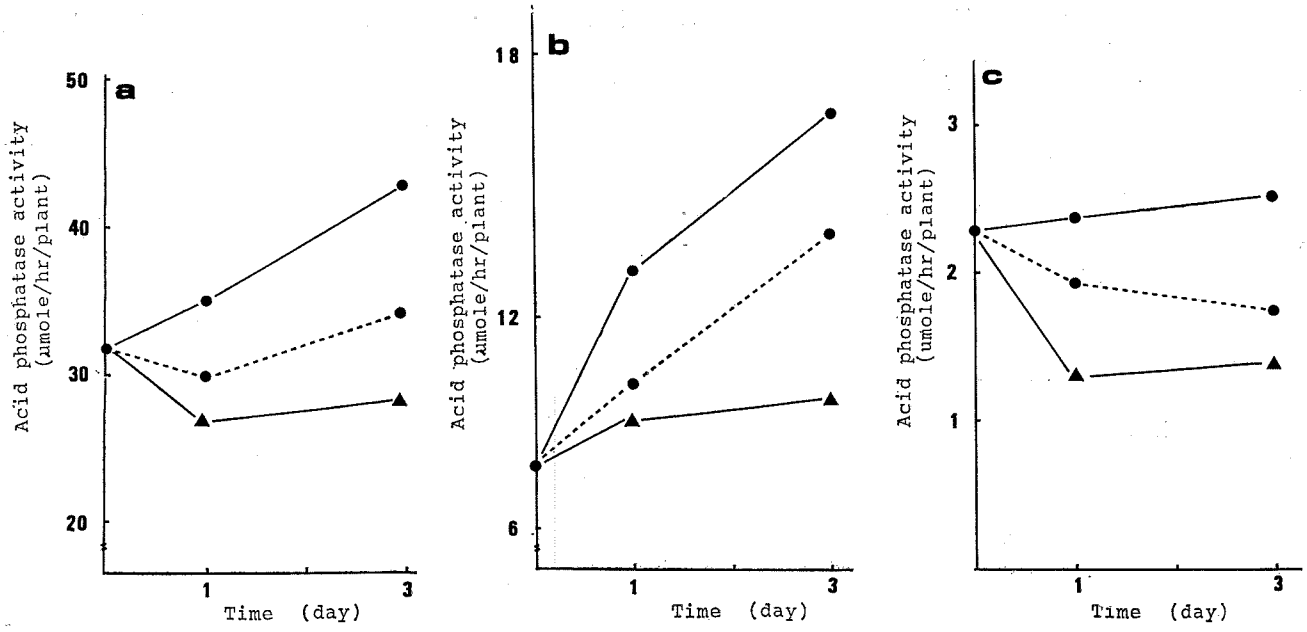

Fig. 2. The effect of salt stress on the acid phosphatase activity of *Zea mays* seedlings after treatment. Control (●—●—●), 150 mM NaCl (●---●---●), 300 mM NaCl (▲—▲—▲), a: the shoot part, b: the root part, c: the endosperm part.

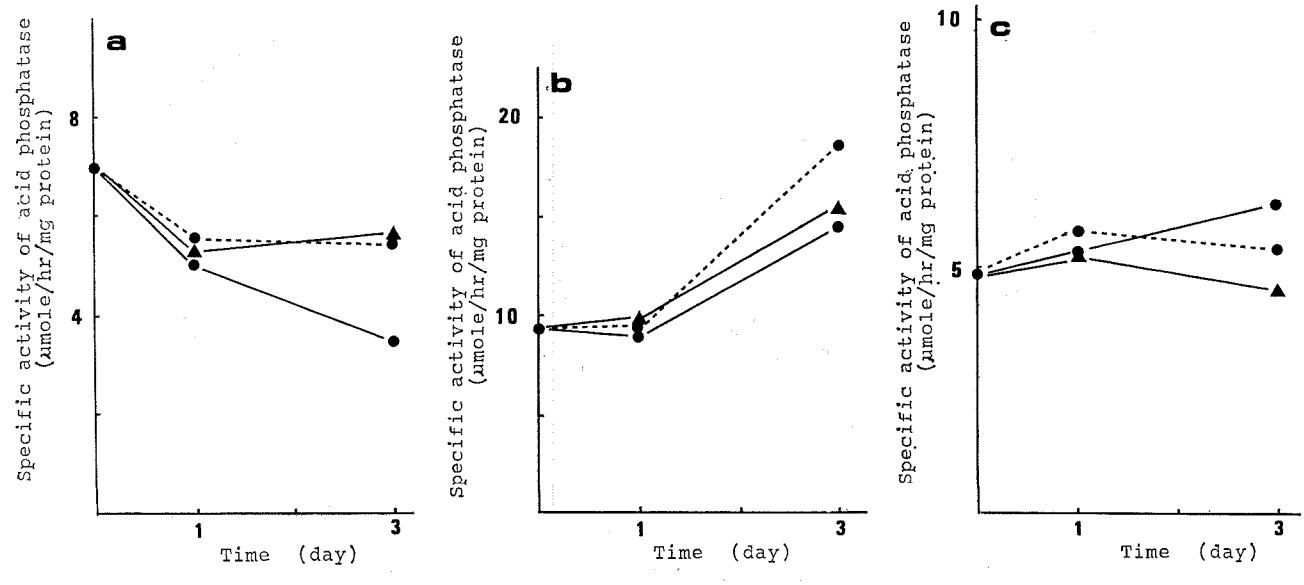

Fig. 3. The effect of salt stress on the specific activity of acid phosphatase of *Zea mays* seedlings after treatment. Control (●—●—●), 150 mM NaCl (●---●---●), 300 mM NaCl (▲—▲—▲), a: the shoot part, b: the root part, c: the endosperm part.

(pH 5.0), 0.1% α-naphthyl phosphate, 0.1% Fast red TR salt, and 5 mM $MgCl_2$ for 3 h at 30°C (Cullis and Kolodynska, 1975). Disc gradient gel electrophoresis was also used to estimate the approximate molecular weight of acid phosphatase (Lambin and Fine, 1979). The standard proteins were thyroglobulin (MW. 669,000), ferritin (MW. 440,000), catalase (MW. 232,000), and lactate dehydrogenase (MW. 140.000).

Results and Discussion

Figure 1 shows the inhibitory effects of salt stress on the growth of each part of *Zea mays* seedlings. Four-day-old *Zea mays* seedlings were imposed to either 150 mM or 300 mM NaCl for one or three days. Higher concentration and the longer exposure of seedlings to NaCl causes more reduction of growth. The inhibitory effect of salt stress was also reflected in the protein content of each part of *Zea mays* seedlings (data not shown). This is consistent with the inhibitory effect of stress on the growth of most glycophytes (Polyjakoff-Mayer, 1982; Pan, 1985). Compared to its effect on growth (Fig. 1), a lesser inhibitory effect of salt stress on total acid phosphatases per plant part was observed in *Zea mays* seedlings (Fig. 2). However, a slightly promotive effect of salt stress on the activity of total acid phosphatase expressed on a per fresh weight or a per mg of protein basis was observed in shoots and roots (Fig. 3). Since the protein content of stressed tissue was lowered, the higher acid phosphatase activity might be due to the high resistance of the pre-existent acid phosphatase to stress-induced degradation or due to stress-stimulated new acid phosphatase synthesis.

The zymogram pattern of acid phosphatase in the 10,000 xg soluble fraction showed at least three bands following gradient disc polyacrylamide gel electrophoresis. A similar zymogram acid phosphatase was found in shoot, root or endosperm tissue of *Zea mays* seedlings, and this pattern was not significantly changed under the experimental salt-stressed condition (Fig. 4). The most distinct band

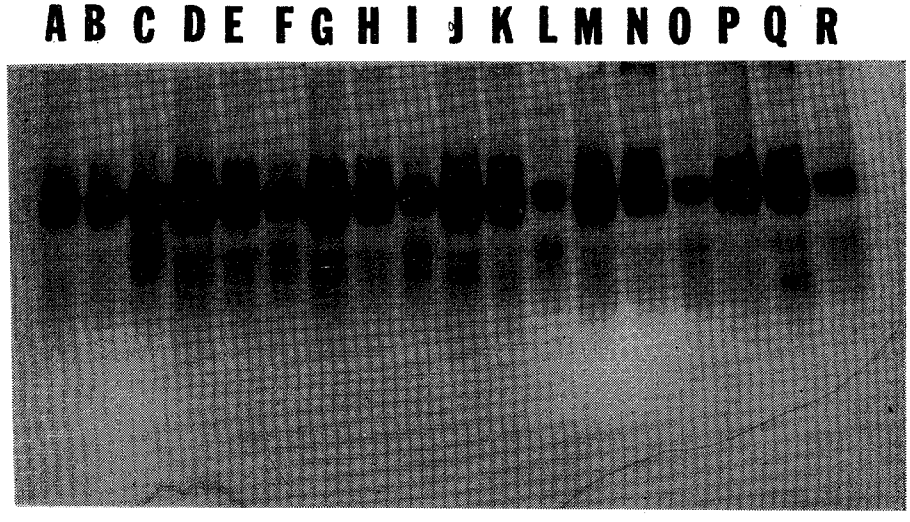

Fig. 4. The zymogram of acid phosphatase of *Zea mays* seedlings exposed to different salt stress conditions.
A to I: 5-day-old seedling; J to R: 7-day-old seedling; A, J: shoot part, no salt treated; B, K: root part, no salt treated; C, L: endosperm part, no salt treated; D, M: shoot part, 150 mM NaCl treated; E, N: root part, 150 mM NaCl treated; F, O: endosperm part, 150 mM NaCl treated; G, P: shoot part, 300 mM NaCl treated; H, Q: root part, 300 mM NaCl treated; I, R: endosperm part, 300 mM NaCl treated.

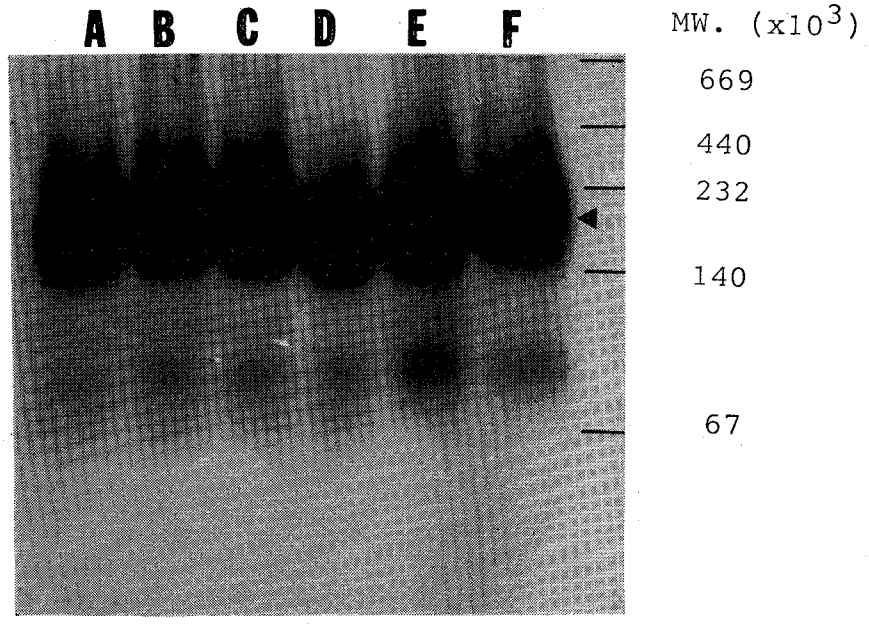

Fig. 5. The zymogram of acid phosphatase in the shoot part of *Zea mays* seedlings exposed to different NaCl stress. A: control, for 1-day treatment, B: 150 mM NaCl treated for 1 day, C: 300 mM NaCl treated for 1 day, D: control, for 3-day treatment, E: 150 mM NaCl treated for 3 days, F: 300 mM NaCl treated for 3 days. (Molecular weights are shown as $\times 10^{-3}$; arrowhead denotes the major acid phosphatase)

was a high-molecular-weight (HMW) acid phosphatase of about 190,000 daltons. (Figs. 5 and 6). This HMW acid phosphatase apparently increased in salt-stressed shoots or roots (Figs. 4 and 5). Whether total acid phosphatase activity was increased or inhibited, the HMW forms always increased. This is true for both spinach leaf discs (Pan, 1985) and spinach leaves from seedlings which were subjected to incremental salt stress (Pan, 1987). Accordingly, it seems to be common in plants that a HMW acid phosphatase is specifically and sensitively increased in stressed tissue. It has been previously reported that a chloroamphenicol-stimulated acid phosphatase in germinating cotton embryos was a HMW form. This chloroamphenicol-stimulated phosphatase activity was greatly inhibited when phosphate was included in the germination medium (Bhargava and Sachar, 1983). Moreover, high phosphatase activity was considered as a biochemical marker of phosphorus deficiency (Barett-Lennard *et al.*, 1982). Unfortunately, the phosphorus content of the stressed

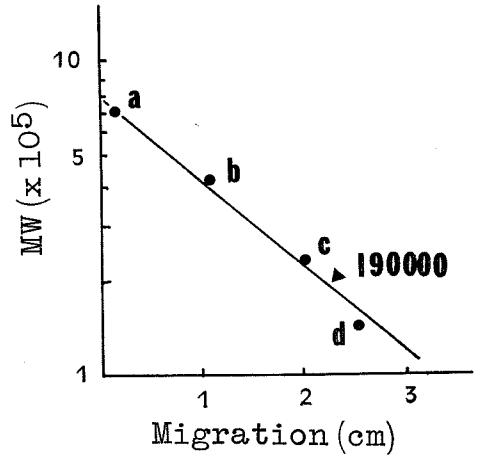

Fig. 6. Molecular weight determination of acid phosphatase by 5 to 20% gradient polyacrylamide gel electrophoresis.
The standard proteins were a: thyroglobulin, MW. 669,000; b: ferritin, MW. 440,000; c: catalase, MW. 232,000; d: lactate dehydrogenase, MW. 140,000.

tissues was not measured to compare with that of the control in this study. The nature of the increased

HMW acid phosphatase activity in stressed tissue is not clear at this time. But, we can not rule out the possibility that stress causes inefficient utilization of phosphate and increased acid phosphatase activity is the result. Alternatively, HMW acid phosphatase may play some positive, physiological function in regulating plants to survive longer under stress conditions. More research will be required to understand this phenomenon.

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鹽分處理對玉米幼苗酸性磷解酶的影響

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鹽分處理玉米幼苗1～3天，處理後植株之生長明顯受抑制，然而其酸性磷解酶總量之變化較微。以5～20% 梯度膠體電泳可將玉米之酸性磷解酶分爲多種。經鹽分處理後，玉米植株各部分中高分子量之酸性磷解酶有增加之趨勢。