THE EFFECT OF SALT STRESS ON THE BETAINE ALDEHYDE DEHYDROGENASE IN SPINACH

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Abstract: The growth, betaine aldehyde dehydrogenase, malate dehydrogenase, phosphatase and peroxidase of spinach have been measured over a range of salinities. A slight decrease in fresh weight of the shoot was found with increasing salinity above 100 mM NaCl; at 300 mM NaCl, the fresh weight growth of the root, or the cotyledon was drastically inhibited. Tissue enzyme activities were studies throughout the experimental range. Betaine aldehyde dehydrogenase was restricted to the shoot of spinach, not detected in the root or the cotyledon. Betaine aldehyde dehydrogenase increased significantly at 150 mM NaCl, and 3-fold increase at 300 mM NaCl. However, fresh weight growth, malate dehydrogenase, alkaline phosphatase showed the similar inhibition at a salinity higher than 150 mM NaCl. Peroxidase of salinized spinach was stimulated slightly.

It was suggested that the increased betaine aldehyde dehydrogenase of salinized spinach may reflect the betaine biosynthetic pathway accelerated somehow by an incremental salt stress.

INTRODUCTION

Betaine accumulation has been reported in the plants of some families, e. g. Chenopodiaceae, Gramineae, when plants were subjected to an adverse environmental condition (e. g. water or salt stress) (Storey, Ahmad and Wyn Jones, 1977). Betaine levels were high ($>10\ \mu$ mole g fr. wt^{-1}) in the shoot of the halophytes at low salt conditions, lower values ($1\text{-}10\ \mu$ mole g fr. wt^{-1}) were found in semi-resistant glycophytes and none detected in the sensitive glycophytes. In the two resistant groups betaine accumulated to higher levels following NaCl stress (Storey and Wyn Jones, 1977). Betaine accumulation was also a metabolic response to water or salt stress among certain wild and cultivated Gramineae, including barley and *Spartina* (Hanson and Nelsen, 1978; Wyn Jones and Storey, 1978). This stress-induced betaine accumulation was considered to be a favorable adaptation to the outside reduced water potential. One of the physiological role of betaine was considered to be as an osmoticum that effectively reduced the water potential of the cell, and/or as a protectant against enzyme inactivation (Paleg *et al.*, 1981; Wyn Jones, 1979).

Based on the studies with mammalian system (Glenn and Vanko, 1959; Rothschild and Guzman-Barron, 1954; Wilken *et al.*, 1970), and on the *in vivo* feeding of redioactive precursors to green leaves (Delwiche and Bregoff, 1958; Hanson and Nelsen, 1978; Hanson and Scott, 1980), it was suggested that betaine was synthesized successively from glycine, serine, ethanolamine, choline and betaine aldehyde. The radiotracer evidence suggested that phosphoryl base intermediate or phosphatidyl base intermediate was involved in the synthesis of choline and betaine in the salinized sugar beet leaves, or barley leaves respectively (Hitz *et al.*, 1981; Hanson and Rhodes, 1983). Little was known about the enzyme responsible for the metabolism of betaine in plants. Betaine aldehyde DH was the last enzyme for betaine biosynthesis, some characteristic properties of the betaine aldehyde DH in spinach leaves and its no substantial change in the water-stressed leaf disks of spinach was described

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in our previous paper (Pan *et al.*, 1981).

It was reported that the moderately salt tolerant chenopod, spinach, which accumulated betaine when subjected to an incremental salt stress had little effect on growth (Coughlan and Wyn Jones, 1980). Besides that, spinach has been proved to be a particularly suitable species for organell and biochemical work. There is no paper concerning with the comparison of enzyme involved in betaine metabolism with the enzymes involved in other metabolism in the salinized plants.

In the current study, spinach seedlings were salinized by an incremental salt stress, and the effect of salt stress on betaine aldehyde DH, malate DH, phosphatase, and peroxidase activity of spinach, particularly in the shoot part was described.

MATERIALS AND METHODS

Materials

Spinacea oleracea L. was obtained from T. SAKATA & CO., Japan.

Growth conditions

Spinach seeds were soaked in running water for 5 hrs, then germinated in the vermiculite for 10 days, grown at a 10 h/14 h light/dark cycle (light intensity 15000 lux), 29°C light/25°C dark, no humidity control. After 10-14 days, seedlings were transfered into a 41 ½ strength Hoagland's solution, pH 6.5-6.8 (20-30 seedlings/container). The plants used for experiments were 3 weeks old.

The NaCl concentration was raised every 3 d by increments of 50 mM up to 200 mM NaCl and therefore brought to a final 300 mM NaCl. The bathing solution were changed every 3 d.

Experimental analysis

Every 3 d throughout the experiment, triplicate samples of 5-10 plants were taken and dissected to the shoot, the root and the cotyledon. After the fresh weight of each part was measured respectively. Each set of samples was frozen in liquid nitrogen and stored in a freezer until enzyme preparation.

The frozen tissus was ground with an appropriate amount of grinding medium containing 0.15 M Tricine-NaOH, pH 7.5, 0.25 M sucrose, and 2 mM dithiothreitol with a pestle and motar. Afterward the extract was filtered through a two-layered cheesecloth. This homogenate was centrifuged at 10,000 g for 20 min. This supernatant was measured betaine aldehyde DH, malate DH, phosphatase, and peroxidase.

Betaine aldehyde DH Activity was determined as previously described (Pan *et al.*, 1981). Fluorescence measurements were made with a Turner model 112 fluorometer attached to a recorder.

Malate DH activity, the reaction mixture contained in 3 ml: K-P0$_4$ buffer pH 7.5, 260 μ mole; NADH, 0.75 μ mole; OAA, 0.6 μ mole, was incubated for 1 min at 30°C, the reaction was initiated by the addition of enzyme. The change in absorbance at 340 nm was followed.

Phosphatase activity was determined as described in the paper (Li and Chan, 1981). Alkaline phosphatase activity was measured by the release of 100 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, and 10 mM p-nitrophenyl phosphate in a volume of 1.0 ml. The reaction was initiated by the addition of the enzyme and terminated by adding of 4.0 ml of 0.6 N Na_2CO_4. The absorbance at 400 nm of the mixture was measured spectrophotometrically by using a control lacking enzyme as a blank. Acid phosphatase activity was measured by the same procedure except that the reaction mixture contained 100 mM Na-acetate, pH 5.0, instead of 100 mM Tris-HCl, pH 8.5.

Fig. 1. The external feature of spinach at the end of experimental period.
(A) Control, plants were grown at Hoagland's solution throughout the experiment.
(B) Treatment, plants were grown at incremental NaCl stress.

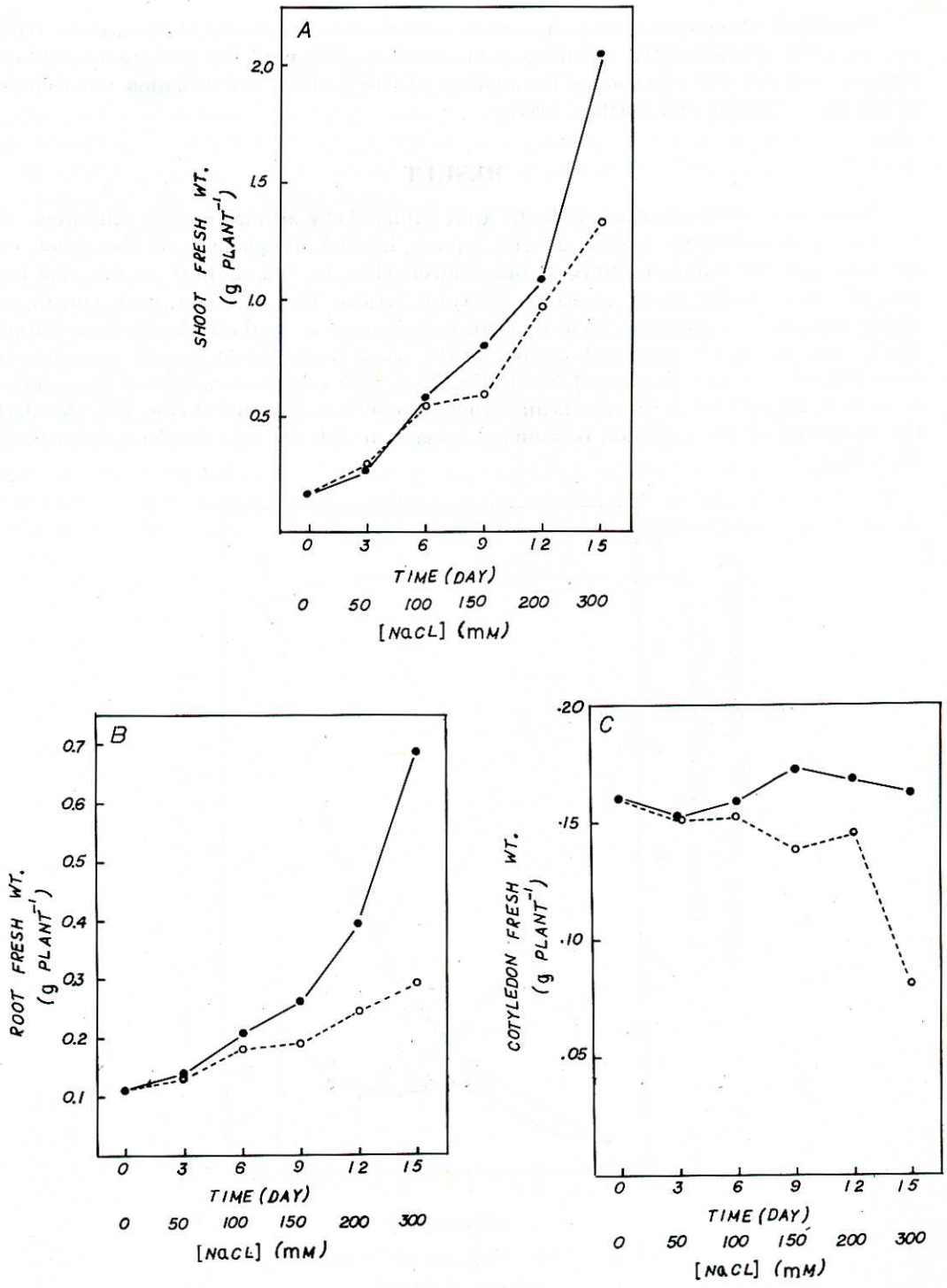

Fig. 2. The effect of incremental NaCl concentration on the growth of spinach. Control (●—●); Treatment (○---○).
(A) The shoot part. (B) The root part. (C) The cotyledon part.

Peroxidase activity, the reaction mixture contained in 1.0 ml: 0.1 M Na-acetate buffer pH 5.8, 1 mM pyrogallol and enzyme was incubated at 30°C until the background appeared zero, the reaction was initiated by the addition of 0.01% H_2O_2, the oxidation was followed at 470 nm. (Chance, and Maehly, 1955).

RESULT

In this experiment, spinach seedlings were salinized by an incremental salt stress. At the end of experimental period, a very similar, healthy morphology of the shoot was obtained from the salinized plants as the controls (Fig. 1). In contrast to this, the root part of the salinized plants appeared brownish, visible loss of turgor, and growth was highly inhibited. A slight succulent response was observed at a salinity higher than 150 mM NaCl. A more or less consistent decline in the shoot fresh weight growth was observed from 150 mM NaCl up to 300 mM NaCl (Fig. 2A), The root fresh weight of the salinized spinach at 300 mM NaCl was drastically reduced to 50% of the control (Fig. 2B). Similarly the senescence of the cotyledon of salinized spinach at 300 mM was accelerated drastically (Fig. 2C).

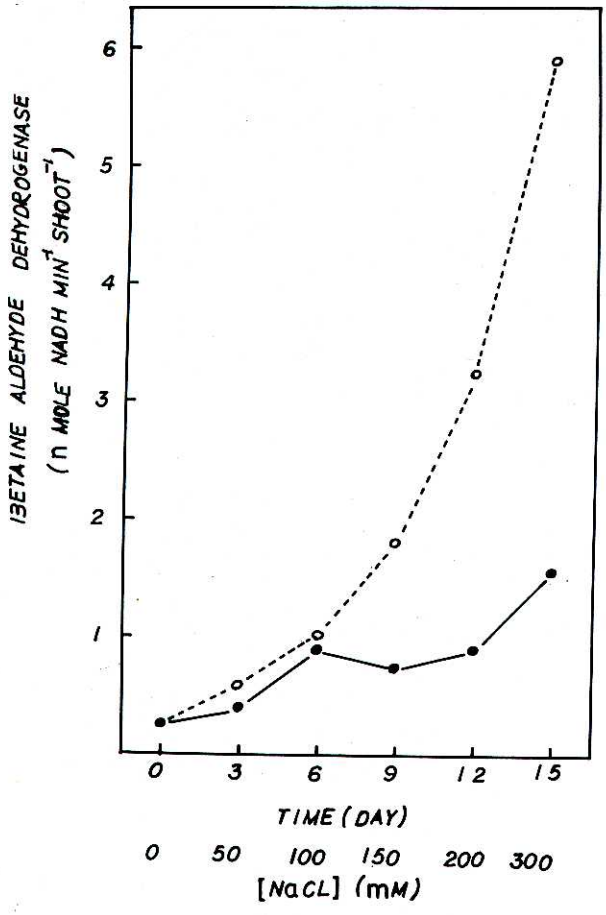

Fig. 3. The effect of incremental NaCl concentration on the betaine aldehyde DH of the shoot of spinach. Control (●—●); Treatment (○---○).

Fig. 3 showed the betaine aldehyde DH of the shoot of spinach in the course of incremental salt stress. In the control plants, betaine aldehyde DH activity were 0.28-1.6 n mole NADH min.$^{-1}$ shoot^{-1}; in the experimental plants, betaine aldehyde DH increased greatly in response to the increasing external salinity (final levels, mean$\pm$s. d., n=3, 5.89$\pm$1.64 n mole NADH min.$^{-1}$ shoot^{-1} at 300 mM NaCl). In contrast, there was no detectable this enzyme activity was found in the root or the cotyledon of both the control and the salinized plant throughout the experimental period.

For comparison with betaine aldehyde DH, malate DH was studied, representing the ordinary oxidative pathway. In consistent to the fresh weight growth, malate DH, alkaline phosphatase or acid phosphatase (not cited) activity in the shoot of the salinized plant was lower steadily than the controls at a salinity higher than 150 mM NaCl. (Figs. 4, 5). Fresh weight growth of the salinized plant was reduced to 73% of the control at 150 mM NaCl and 65% of the control at 300 mM NaCl. Similarly, malate DH of the salinized plant at 150 mM NaCl was reduced to 73% of the control and 39% of the control at 300 mM NaCl (Fig. 4). Alkaline phosphatase of the salinized plant was 56% of the control at 150 mM NaCl and 43% of the control at 300 mM NaCl (Fig. 5). A very similar result was also obtained from the root or the cotyledon of spinach in this experiment. This result indicated that the higher salinity in the growth medium had inhibitory effect on the fresh weight growth, and the activity of malate DH or alkaline phosphatase isolated

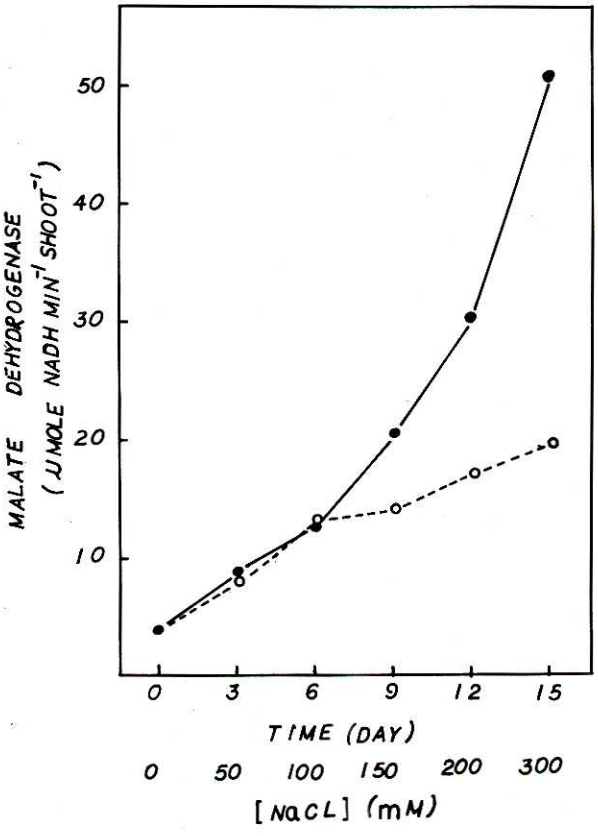

Fig. 4. The effect of incremental NaCl concentration on the malate DH of the shoot of spinach. Control (●—●) Treatment (○---○).

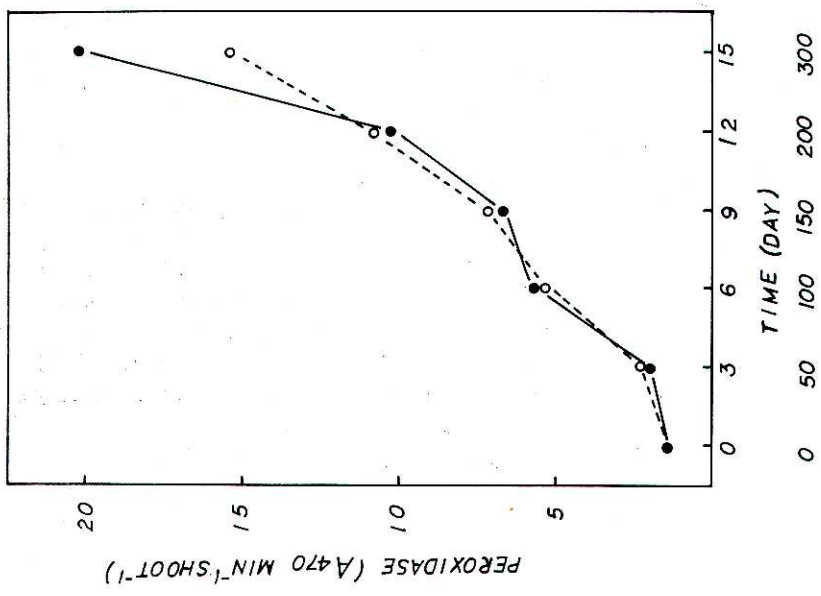

Fig. 6. The effect of incremental NaCl concentration on the peroxidase of the shoot of spinach. Control (●—●); Treatment (○--○).

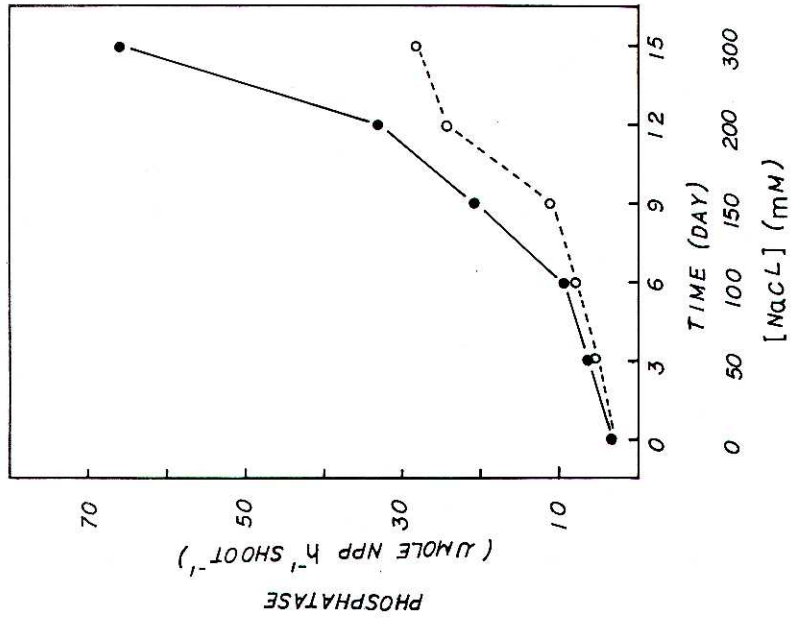

Fig. 5. The effect of incremental NaCl concentration on the alkaline phosphatase of the shoot of spinach. Control (●—●); Treatment (○--○).

from the salinized plant. Fig. 6, it was shown that lower peroxidase activity of the salinized plant was obtained only at 300 mM NaCl (69% of the control). This indicated that the peroxidase activity of spinach was stimulated by the salt in the growth medium. The similar conclusion was also obtained from the data of the root or the cotyledon of spinach (not cited).

Accordingly, malate DH, phosphatase, or peroxidase of spinach responsed to the external salinity was different from that of betaine aldehyde DH.

DISCUSSION

In contrast to the previous work using spinach leaf disks subjected to water stress of −20 bar (Pan *et al.*, 1981), the betaine aldehye DH of the shoot from the salinized plant was significantly higher than that of the control at salinity higher than 150 mM NaCl. In an experiment, water culture plants subjected to the abrupt 300 mM NaCl treatment, the betaine aldehyde DH was much increased to 7-fold of the control (not cited). Although the betaine aldehyde DH of the control *in vivo* was high enough to account for the betaine accumulation in the stressed plant, this elevated activity might reflect that the betaine biosynthesis in the salinized spinach was activated or accelerated somehow. Betaine aldehyde DH was restricted to the shoot of spinach, not detected in the root or the cotyledon of the control or the stressed plant. Therefore, it was concluded that the completion of betaine biosynthesis occurred primarily in the leaves of spinach. This was consistent with the report of Hanson group (Ladyman *et al.*, 1980).

Hanson and coworkers have carried out leaf feeding erperiment in severely water-stressed barley plants, and concluded that the last step of betaine aldehyde to betaine was not acclerated by the stress condition (Hanson and Scott, 1980). This implied that betaine aldehyde DH responsible for betaine aldehyde to betaine was not induced to a higher level in the water-stressed barley. This disprepancy to the present data could be explained by Wyn Jones's proposal: there was a possible significant difference in the betaine biosynthetic pathway in barley and spinach (Coughlan and Wyn Jones, 1982). Wyn Jones *et al.* reported that in the salinized spinach, the labeling of betaine from C^{14}-serine was twice as great in the salinized as in the control, but the incorporation rates of C^{14}-choline or C^{14}-ethanolamine were identical in the control and the salinized leaves. It indicated tentatively that the conversion of serine to ethanolamine was a rate-limiting step in the betaine biosynthetic pathway. In their work, there was no data to show the incorporation rate of betaine aldehyde to betaine in the salinized spinach.

It was reported that the kinetics of the enzymes isolated from the salinized plants differed from the kinetics of enzymes isolated from the control plants (PEP carboxylase, invertase and malate DH) (Hawker and Walker, 1978; Kalir and Pojakoff-Mayber, 1981; Besford, 1979). In this present result, no such positive conclusion can be drawn for the betaine aldehyde DH or malate DH.

Phosphatase activity in plants typically increased when plants became phosphorus deficient. For example, when growth was limited by P, phosphatase activity increased in leaves of tomato (Besford, 1979), cucumber and wheat (Barrett-Lennard *et al.*, 1982), in whole *Spirodela oligorrhiza*. The present result showed that no apparent increased phosphatase activity was found in the salinized plants (Fig. 5). Therefore, no severe disturbance occurred in the major metabolism of salinized spinach, this suggested that this experiment condition having little effect on the growth was suitable for the study of betaine biosynthesis.

Among many biochemical changes associated with the exposure of plants to harmful

conditions. Increased peroxidase activity has been cited as an indicator of physiological stress, an elevated peroxidase level was induced by cold in alfafa (Gerloff *et al.*, 1967), pea (Highkin, 1967), and tobacco (De Jong *et al.*, 1968), drought in cotton and by salt stress in legumes. But, no parallel was found between growth responses and changes in peroxidase activity in 11 cultivars of Brassica in fresh water and saline medium (Stevens *et al.*, 1978). The present data in Fig. 6, suggested that salination in spinach had increased peroxidase activity in the shoot. But, this stimulation was not so obviously as betaine aldehyde DH, which was closely associated to betaine accumulation.

The mechanism of increased betaine aldehyde DH activity in the salinized plants needs to be further studied.

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鹽分處理菠菜對其 Betaine Aldehyde Dehydrogenase 之影響

潘 素 美

摘 要

菠菜幼苗培養於水耕溶液，逐步增加溶液中食鹽的濃度。由 50 mM NaCl 至 300 mM NaCl 處理期間，分別測定對照組與處理組之植株地上部及根系之鮮重量，Betaine aldehyde DH，Malate DH，Phosphatase 及 Peroxidase 之活性。結果顯示 betaine aldehyde DH 只分佈於植株地上部，且於植株接受了 150 mM NaCl 處理後，酵素活性有明顯增加，至 300 mM NaCl 處理後，此酵素活性增加 3 倍左右。然而鮮重量，Malate DH，Alkaline phosphatase 活性却於鹽分高過 150 mM NaCl 時，已有抑制現象。此情形同時發生於植株地上部及根系。

由此結果討論菠菜植株於鹽分處理後，betaine aldehyde DH 之活性增加，反映了 betaine 生物合成步驟之增加，因此作爲菠菜以 osmoreglation 來抗鹽的一種機制。