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SOME PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON SPINACH GROWING ON SALINE SOIL

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Abstract

Rate of respiration and activities of amylase, acid and alkaline phosphatase were compared in glycophytic and halophytic spinach grown on normal and saline soils. Increase in the specific activities of invertase and acid phosphatase associated with the decrease in specific activity of alkaline phosphatase was noticed in halophytic spinach. Diversified behaviour of enzymes in response to the salinity in various halophytes was attributed to their specific internal metabolism depending on the nature of adaptation.

Introduction

Plants growing on saline soils usually suffer from physiological drought and deficiency of some essential mineral elements. Excess of non nutrient salts adversely affect the physiology and biochemistry of cell. Amongst the glycophytes and halophytes there exists a small group of adaptive halophytes which are capable of flourishing in saline environment, their morphology somewhat matches with the former and their physiology-biochemistry come closer to the later group.

Spinach (*Spinacea oleracea* L.) was found growing luxuriantly on saline soil of coconut plantation irrigated with brakish water near Karachi sea coast. The leaves were thick and very saltish to the extent of being unedible. Some physiological and biochemical investigations were therefore undertaken in these plants to elucidate the mechanism of salt tolerance.

Materials and Methods

Respirational Studies :

Fifteen leaf discs of 5 mm diameter were taken separately from the plants grown on normal and saline soils. They were floated on 2.5 ml of 0.2 M phosphate buffer. Rate of respiration was determined in Warburg's respirometer and are expressed in terms of oxygen uptake in microliters (Umbreit *et al.*, 1957).

Enzymatic Studies :

Fresh leaves of Spinach from normal and saline localities were taken and rapidly placed in solid carbon dioxide. Frozen leaves were several times ground in pre-chilled waring blender with fresh acetone previously cooled at —30°C. The resulting slurry was filtered through Buchner funnel and again washed with cold acetone The powder was dried in a vacuum desiccator and stored in deep freeze (Millard & Bonner, 1954).

One gram of acetone powder was shaken vigorously for 15 minutes in 20 ml of .01 M Tris HCl buffer adjusted at pH 7.4. The slurry was centrifuged at 20,000 g, at 0°C. Supernatant was filtered through glasswool to avoid floating debris and the filtrate was dialysed overnight against 0.01 M Tris buffer (pH 7.4). This extract was used in every enzyme assay. The pH of reaction mixture was adjusted at the pH optima for various reactions.

Activity of invertase was estimated by measuring the amount of reducing sugars after enzymatic hydrolysis of sucrose (Nelson, 1944). Sample of 0.5 ml of enzyme extract, 1 ml of 4% Analar sucrose, 1 ml of 0.2 M citrate phosphate buffer, pH 6.4, was taken in a test tube and incubated at 37°C for 20 minutes in a metabolic shaker. The reaction was stopped by plunging the tubes in boiling water for five minutes. One ml of mixed copper reagent was added and the tubes were heated again till boiling. The mixture was cooled and 1 ml of Arsenomoylybdate reagent was added while shaking them rapidly until the evolution of Co_2 was completed. Test tubes were left at room temperature for 15 minutes. Optical densities were recorded against blanks at 500 n.m and compared against the standard curve of glucose.

The activity of acid phosphatase was estimated by measuring the amount of phenolphthalein formed due to anzymatic breakdown of phenolphthalein phosphate which was taken as substrate (Hewitt & Tatham, 1960). To 1 ml of 0.25% (w/v) phenolphthalein phosphate and 3 ml of 0.05 M Tris-HCl buffer, pH 5.4, an amount of 1 ml of enzyme was added and incubated for 15 minutes on metabolic shaker at 37°C. The reaction was terminated with 1 ml of 10% TCA. After five minutes, 2 ml of N NaOH was added and the optical density was recorded at 580 n.m. against reagent blank. Values for phenolphthalein were taken from the standard prepared as above.

The activity of alkaline phosphatase was estimated by measuring the amount of phosphate formed during enzymatic breakdown of sodium Beta-glycerophosphate which was taken as substrate (Dryer *et. al.*, 1957). To 1.5 ml of Sodium Beta-glycerophosphate buffered at 9.3 by Sodium borbate was added 0.5 ml of enzyme extract and incubated at 37°C for 30 minutes. After the incubation, 2 ml of 10% TCA was added to stop the reaction. After five minutes 5 ml of Ammonium metavanadate reagent was added and the reaction mixture was diluted up to 10 ml. Optical density was measured against the blank at 315 n.m. Standard curve was prepared by using potassium dihydrogen phosphate.

Results

Effects of different pH on the respiration of spinach plants growing on normal or saline soils are shown in the Figure 1. It is evident that oxygen uptake in the plants of saline soil is inhibited by 73.0 60.0 and 76.5 % at pH 5, 6 and 7 respectively. Specific activities of various enzymes are given in Table 1. Enzymes like invertase and acid phosphatase extracted from the plants of saline soil show increase in the specific

TABLE 1. Specific Activities of various Enzymes in Spinach grown on Normal and Saline soils.

Enzymes	Control	Saline
Invertase	204.00	360.00
Acid Phosphatase	25.75	34.40
Alkaline Phosphatase	73.52	35.71

Specific Activities are expressed per mg of protein per 30 minutes. Readings are mean of three raplicates.

activities by 76.4% and 33.5% respectively, whereas that of alkaline phosphate is inhibited by 51.4%. Respective values of the plants grown on normal soil are taken as control.

Discussion

In order to find the reasons for adverse effects of salinitiy it is necessary to search for the answer at subcellular level and study the antagonistic behaviour of sodium towards other cation dependent specific enzymatic reactions. Formation of Acetyl Co-A during respiration which is a magnesium dependent reaction is adversely affected in presence of excessive amounts of sodium (Hiatt & Evans, 1960). Potassium dependent reaction of pyruvic kinase is also inhibited due to sodium (Harold & Evans, 1963). Porth & Poljakeff-Mayber 1971), reported a decrease of ATP-ase as a result of salinity. This is expected to slow down overall oxidative phosphorylation and reduce the rate of respiration (Figure 1). While growing pea on saline media they also noticed a marked increase in activity of acid phosphatase in both the soluble and mitochondrial fraction, though the alkaline phosphatases were not affected significantly. Horovitz & Waisel (1970) found that addition of NaCl in growth medium resulted in stimulation of ATP-ase activities in bean and corn whereas it induced inhibition in the ATP-ase of *Suaeda* and *Atriplex*. Ahmad & Hewitt (1971) while working on *Suaeda fructicosa* grown

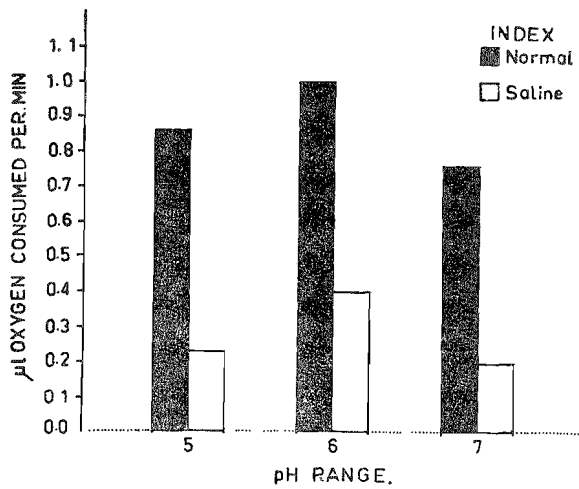

Fig. 1. Effect of different pH levels on the rate of respiration in spinach grown on normal and saline soils.

in saline culture found 60% inhibition in the activity of acid phosphatase and 100% activation in PEP-phosphatase. Ahmad (1972) has also noticed an increase in the activity of pyruvic kinase and decrease in that of acid phosphatase in halophytic over glycophytic species of beet root. In the present investigation, the specific activities of invertase and acid phosphatase were found to increase whereas alkaline phosphatase showed a decrease in spinach growing on saline soil (Table 1). Diversity in these results suggest that there exists some differences in the response of salinity between the species to species depending upon their biochemical and physiological system.

Plants show various types of adaptations for salt tolerance. Some of them develop secretory mechanisms (Pollak & Waisel, 1970), others accumulate salts in their

body and render them insoluble (Ahmad, 1968) and still others develop special kind of protoplasm to overcome salt injury (Strognov, 1962). Hence their enzymes are expected to show some differences depending upon the nature of adaptation. Glycophytes, if grown on the saline soil show poor growth and their enzyme system is badly affected. Adaptive halophytes like spinach, manage to regulate biochemical and physiological activities and flourish in saline habitats. The halophytes seem to have gone through time old gradual process of acclimatization and have developed morphological, biochemical and physiological adaptations which enable them to grow luxuriantly in saline conditions.

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