

INFLUENCE OF NaCl ON SOME BIOCHEMICAL ASPECTS OF TWO SORGHUM VARIETIES

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Abstract

Effect of NaCl on two enzymes viz., α -amylase and protease and the metabolic sequence induced by them in two varieties of sorghum differing in salt tolerance were studied during germination and seedling growth. The increase in salt concentration of the medium resulted in the decrease of α -amylase and protease in both the varieties, Giza-10 (tolerant) and IS-4087 (Susceptible), which was more pronounced in the latter. Decrease in concentration of reducing and non-reducing sugars, slower mobilization of reserve protein and reduced levels of amino acids were observed with increase in salinity levels. The salt tolerance of variety Giza-10 can be attributed to its capability to mobilize higher levels of sugars and reserve proteins during germination.

Introduction

Sorghum an important crop of arid regions is not only a source of food for humans but also provides fodder to animals. As salinity and drought are associated in arid regions, it is important to select cultivars tolerant to both these conditions.

Plants are generally most sensitive to salinity during germination and early seedling growth (Carter, 1975). Even in obligate halophytes reduced salinity is necessary for germination (Chapman, 1975). Most of the work on the effects of salinity on sorghum is restricted to germination and fresh weight of plants whereas studies on salinity induced metabolic changes during germination are scarce. Genotypic differences between salt sensitive and tolerant plants may be studied with respect to a number of physiological and biochemical parameters to develop rapid screening method for salt tolerance (Levitt, 1972; Epstein, 1972). Genotypic variability in relation to salinity has also been reported for sorghum (Taylor *et al.*, 1975; Ogra & Bajjal, 1978). This paper describes the effect of NaCl induced salinity on the activity of α -amylase and protease; changes in carbohydrate and mobilization of protein during early seedling growth in two cultivar of sorghum.

Materials and Methods

Seeds of two varieties of sorghum, viz. Giza-10 (tolerant) and IS-4087 (susceptible) were surface sterilized with 10% solution of sodium hypochloride for 5 min washed thoroughly in sterile distilled water and transferred on filter papers in 14 cm Petri dishes. Ten ml of saline solutions were used containing 50, 100 and 150 meq/l NaCl. Four replicates

were kept in a completely randomized design. The seeds were allowed to germinate in dark at $25 \pm 2^\circ\text{C}$. Fresh samples were collected randomly at every 24 h after sowing for the assay of α -amylase, protease, protein and total amino acids. Parallel samples were dried at $70 \pm 2^\circ\text{C}$ in an oven for the estimation of carbohydrates.

α -amylase: Ten seedlings were extracted with 0.2M citrate buffer (pH 5.5), centrifuged at 10,000 g and the supernatant used for assay of enzymes according to the method outlined by Chisspeels & Varner (1967) and the activity expressed as mg of starch hydrolyzed/g fresh weight/h.

Protease: Ten seedlings were homogenized in a mortar and pestle, extracted with cold 1% NaCl in 0.2 M phosphate buffer (pH 7.5) and centrifuged at 12000 g for 30 min. One ml of supernatant was incubated at 50°C with 5 ml of 1% casein solution in 0.2 M sodium phosphate buffer (pH 6.0). The reaction was terminated after 60 min with 1 ml of 40% TCA (Ainouz *et al.*, 1972). The proteolytic activity was measured at 570 nm in TCA soluble fraction after reaction with Folin phenol reagent (Lowry *et al.*, 1951).

Reducing and non-reducing sugar: The reducing sugars were determined in an aliquot of 80% alcoholic extract of dry sample according to the method of Nelson (1944) as modified by Somogyi (1952). The total reducing sugar was determined from the same alcoholic extracts after its digestion with HCl. The non-reducing sugars were calculated according to the method of Loomis & Shull (1937).

Protein and total amino acid: Ten seedlings were ground in 0.1 M NaCl solution (Ainouz, 1970) in the ratio of 1:10 (w/v) in a mortar and pestle and filtered through nylon cloth. The filtrate was precipitated with equal volume of 10% TCA and centrifuged at 1000 g for 5 min. The pellet was re-suspended in 0.1 N NaOH and the protein was then estimated by the method of Lowry *et al.*, (1951). The supernatant was assayed for total amino acid by the method of Moor & Stein (1948).

Results

Salinity even at the lowest level had pronounced effect on all the parameters studied (Figs. 1 and 2). There was a slow increase of α -amylase activity up to 48 h followed by a sharp increase up to 72 h. The activity decreased with the increase in salt concentrations (Fig. 1a). The effect of salinity was more pronounced in seedlings of variety IS-4087, where after 96 h the α -amylase activity was only 28% of the control at 150 meq/l NaCl salinity level, whereas, it was 70% in Giza-10.

The level of both reducing and non-reducing sugars followed the pattern of α -amylase. The amount was maximum in control with exception of 50 meq/l NaCl treatment and decreased with increase in salt concentration (Fig. 1 b,c). In seedlings of both the va-

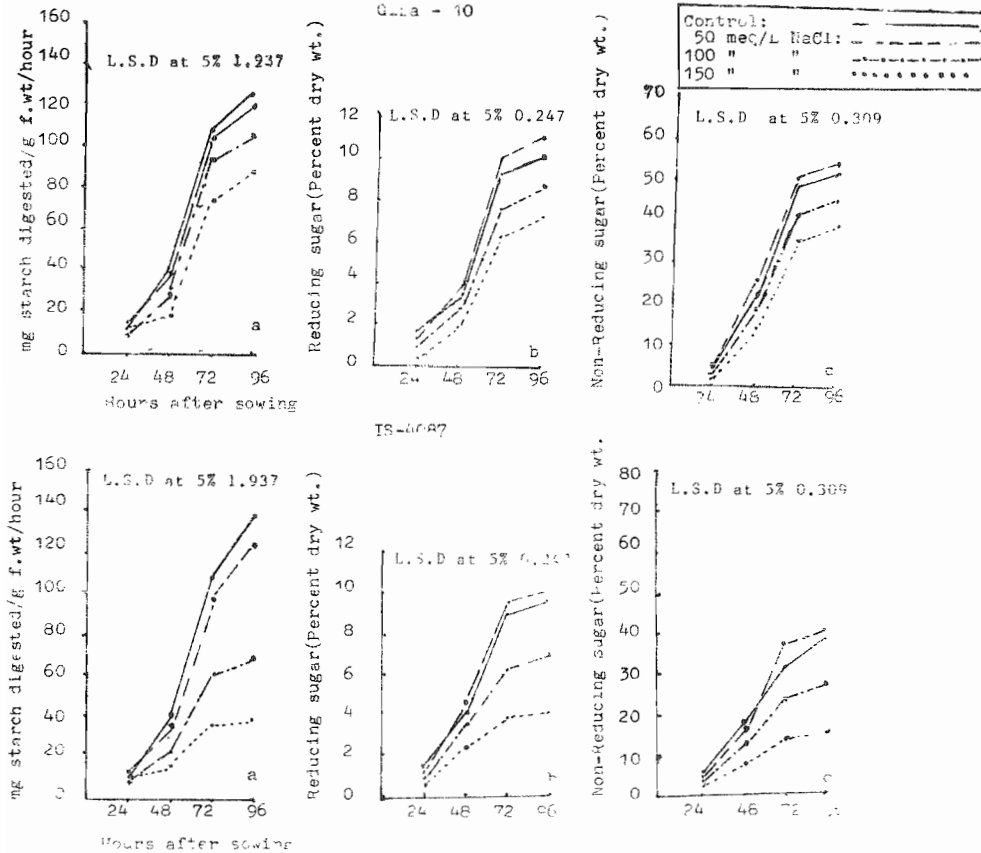


Fig. 1. Effect of different concentrations of NaCl of changes in (a) α -amylase (b) reducing sugars (c) Non-reducing sugars of varieties Giza-10 and IS-4087 during germination.

rieties, the level of sugar increased gradually up to 48 h and then showed a sharp increase up to 72 h. At 150 meq/l NaCl salinity, the amount of reducing and non-reducing sugars at 96 h in variety IS-4087 were 40% and 41% of the control, whereas the corresponding value for Giza-10 were 63% and 70%.

Protease, protein and total amino acid: The proteolytic activity increased with the age of seedlings in all the treatments. The activity showed a steep increase after 48 h and reached a maximum value at 96 h. The proteolytic activity, however, decreased with increase in salt concentration of the germination media. At 96 h seedlings of variety IS-4087 showed a reduction of 73%, whereas, Giza-10 exhibited a decrease of 31% at 150 meq/l NaCl as compared to control (Fig. 2a).

The seedlings of variety IS-4087 had slightly higher level of protein than Giza-10 (12% and 11%) at 24 h in 0 meq/L NaCl (Control) after germination. The former variety

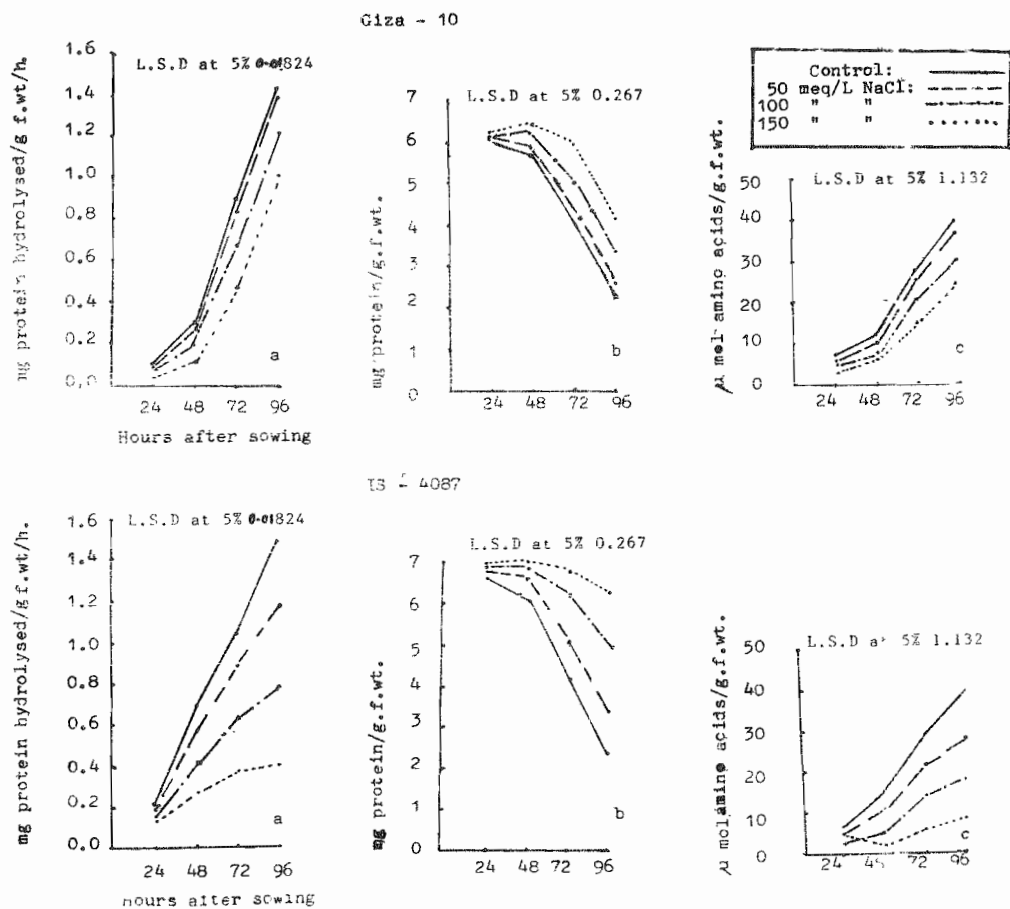


Fig. 2. Effect of different concentration of NaCl on changes in (a) protease (b) protein (c) total amino acids of varieties Giza-10 and IS-4087 during germination.

showed a slower depletion of reserve protein under saline conditions (64% in control as compared to 9% at 150 meq/L NaCl). The seedlings of variety Giza-10 showed a faster mobilization of reserve protein even at 150 meq/l NaCl, so that at 96 h 34% of the protein was mobilized as compared to 12% in variety IS-4087 (Fig. 2 b).

The amount of amino acid paralleled the proteolytic activity. It was maximum in control and decreased with increase in salinity. The level of total amino acid increased gradually with time and reached a maximum value at 96 h. The amount of amino acid at 150 meq/l NaCl salinity was only 20% of control in variety IS-4087 and 59% in Giza-10 (Fig. 2 c).

Discussion

The effect of salt stress on hydrolytic enzymes was less in variety Giza-10 as compared to variety IS-4087. Our unpublished data on germination, length of coleoptile and root under salt stress indicated Giza-10 to be more tolerant variety than IS-4087. The effect of salt stress on both α -amylase and protease was more significant in susceptible variety IS-4087 as compared to Giza-10. Similar decrease in α -amylase under salt has also been reported in wheat by Sarin & Narayanan (1968) and Ansari *et al.*, (1977). Both reducing and non-reducing sugars increased with the age of seedlings, but the increase was much less under saline conditions. Bhardwaj (1958) observed similar results in wheat and gram. The salt tolerance of Giza-10 therefore can be attributed to its ability to maintain the integrity of its enzyme system. In addition, ability to maintain higher levels of reducing and non-reducing sugars allows better osmotic adjustment to salinity. Salt tolerance during germination as a result of lesser effect on α -amylase and higher levels of resultant sugars has also been reported by George & Williams (1964) in barley.

The proteolytic activity increased gradually during germination and showed a rapid increase only after 48 h in both varieties, which is similar to the findings of Ramana & Radhakrishanan (1987) in Pearl millet. The activity, however, decreased with the increase in salinity. Variety IS-4087 was more severely effected than Giza-10. Rakova *et al.*, (1969) observed that in pea roots, sodium salts inhibited the synthesis as well as hydrolysis of basic proteins. Sheoran & Grag (1978) reported that salinity either reduced or had no effect on the protease activity in all parts of plant, except in mungbean leaves during germination and early seedling growth. These results are reported on leaf and not in seedlings which may be the reason for inconsistency in results.

The decrease in proteolysis caused by salinity resulted in slower mobilization of reserve protein during germination of seeds, as a result the residual protein level was more with increasing salinity. Giza-10 showed lesser inhibition of protease activity as compared to IS-4087 and therefore greater mobilisation of protein. Prisco & Viera (1979) reported that NaCl caused delay in the breakdown of protein in the cotyledon of *Vigna* seeds. They ascribed it to the inhibition of translocation of hydrolysis products than to the protease activity. However in our experiments a definite inhibition of protease activity was evident. Inhibition in seedling growth can also be partly attributed to delayed mobilization of reserve protein, since proteolysis is probably the primary but essential step towards synthesis of new proteins for seedling growth (Ryan, 1973).

The decrease in protein contents of germinating seedling was accompanied by the accumulation of total amino acid, which may be interpreted as the result of protein hydrolysis (Fig. 2). The results are in agreement with the results obtained with peas (Beevers, 1968) and squash (Wiley & Ashton, 1967). The amount of amino acids decreased

with the increase in salt concentration of the media and variety Giza-10 was comparatively less affected than IS-4087. It would suggest that the initiation of germination, activity of the two hydrolytic enzymes and the metabolic sequence induced by them are delayed under salt stress. The salt tolerance therefore depends upon the active osmotic adjustment by the emerging seedlings to the external medium and a better mobilization of reserve protein.

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