PHYSIOLOGICAL STUDIES ON THE EFFECT OF SALINITY ON SORGHUM I. CHANGES IN ALPHA-AMYLASE AND ACID PROTEASE DURING SEEDLING GROWTH

R.K. Ogra and B.D. Baijal

Department of Botany, Agra College, Agra

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Summary

Influence of salinity on alpha-amylase and acid protease and the biochemical changes induced were investigated in two varieties of sorghum, differing in their salt tolerance during germination. Increase in the salt concentration of the external medium, resulted in the decrease of alpha-amylase and acid protease in both the varieties i.e. T. 8B (tolerant) and M.P. Chari (susceptible). The decrease was comparatively more in the susceptible variety, resulting in delayed mobilization of food reserves. The salt tolerance of variety T. 8B is attributed to its better malting capacity during germination.

Introduction

The most important problem associated with saline habitats is that of seed germination. Generally plants are most sensitive to salinity during germination or early seedling growth (Carter, 1975); germination even of obligate halophytes, takes place under conditions of reduced salinity (Chapman, 1975). Most of studies on the effect of salinity on sorghum are restricted to per cent germination and fresh weight studies while very little work is available regarding the metabolic changes induced by salinity during germination.

Levitt (1972) and Epstein (1972) have drawn attention to genotypic differences between salt tolerant and salt sensitive plants in respect to a number of physiological and biochemical parameters. Their observations seem more pertinent in an effort to develop repaid screening methods for salt tolerance, as the specific capabilities of organisms depend on the synthesis of appropriate enzymes which in turn is gene controlled. Sufficient genetic variability in relation to salinity exists in sorghum (Taylor *et al.*, 1975; Ogra and Baijal, 1978) as in other agricultural crops.

Keeping this in view and the fact that germination and seedling growth are intimately connected with crop growth, an attempt was made to study the activity of certain hydrolytic enzymes (alpha-amylase and acid protease) in two cultivars of sorghum (differing in salt tolerance) grown at varying salinity levels, alongwith the subsequent changes in carbohydrates and nitrogen fractions during early seedling growth.

Materials and Methods

Two varieties of sorghum T8B (Tolerant) and M.P. Chari (susceptible) were selected for the present study (Ogra and Baijal, 1978). After sterilizing and thorough washing, the seeds were transferred on moist filter paper in sterilized petridishes. Deionized water was used as control and for treatments, the seeds were irrigated with salt solutions of 4, 8, 12 and 16 mmhos/cm Electrical Conductivity (EC) using NaCl and $CaCl_2$ in equimolar concentrations. The petriplates were placed in dark at 35°C $\pm$2.

Fresh samples of seedlings were drawn every 24 hours after sowing for the assay of alpha-amylase and acid protease. Parallel samples were dried at 70°C $\pm$2 in an oven for the estimation of different carbohydrate and nitrogen fractions.

Alpha-amylase

For alpha-amylase, the seedlings were extracted with 0.2 M citrate buffer, pH 5.5, in a mortar and pestle. The homogenized samples were centrifuged at 10,000 Xg and the supernatant used as enzyme preparation. The assay was carried out according to Chrispeels and Varner (1967) and the activity expressed as mg. of starch utilized per hour per gm of fresh weight.

Acid Protease

Protease was extracted from the seedlings in 0.2M phosphate buffer pH 7. The homogenate obtained after crushing was centrifuged at 12,000 xg and supernatant used as enzyme after dialysis against the same buffer for 24 hours at 40°C. The assay was carried out at pH 3.6 using bovine albumin as substrate and the activity has been expressed as μg of tyrosine released/gm fresh wt./hr. (Garg and Virupaksha, 1970).

Reducing and Non-reducing sugars

The reducing sugars were estimated from the alcoholic extracts (80%) of the dry samples, according to the method of Nelson (1944) as modified by Somogyi (1952). The total sugars were determined from the same alcoholic extracts, after its digestion with HCl as total reducing sugars. The non-reducing sugars were calculated according to Loomis and Shull (1937).

Total and soluble nitrogen, Proteins

Total nitrogen was estimated as described by Snell and Snell (1955). For soluble nitrogen the dry samples were extracted with 80% ethanol (three times in 24 hrs) and the nitrogen determined in the supernatant as for total nitrogen. Method of Lowery *et al.* (1951) was followed for the determination of total proteins, after their precipitation with Trichloroacetic acid (Thimann and Laloraya, 1960).

RESULTS

Carbohydrate metabolism

Alpha-amylase, non-reducing and reducing sugars : The activity of alpha-amylase increased during germination. The increase was maximum after 48 hours in both the varieties. The activity decreased with increasing concentration of the salts (Fig. IA-B). Variety M.P. Chari showed maximum decrease in the alpha-amylase with increase in salinity, so that on the 4th day seedlings grown at 16 EC had as much as 58% reduction in the activity over that of the control. On the other hand T.8B did not show any appreciable decrease in the activity even upto 12 EC (Fig. 1B). On the 4th day it showed only 21% decrease at 16 EC, over its control.

The level of both reducing and non-reducing sugars increased with age of the seedlings. The amount was maximum in control samples and decreased with increase in salinity (Fig. 1A & B). In the susceptible variety M.P. Chari, the level of reducing sugars was maximum on the 3rd day in seedling growing in control and 4 EC salinity level and non-reducing in control only wheareas, in other treatments, the increase was still in progress. This is due to delay in germination, with increasing salinity. Variety T.8B showed higher turnover of non-reducing sugars than M.P. Chari (Fig. 1B-b and 1A-b). This was true at higher salinity levels also. Reducing sugars also exhibited similar trend.

Nitrogen Metabolism

Protease, total nitrogen, soluble nitrogen and proteins : The proteolytic activity increased with age of the seedlings. The increase was maximum after 2nd day and continued to increase on the 4th day also. Like alpha-amylase the activity of protease also decreased with increase in the salt concentration of the external medium. On the 4th day variety M. P. Chari exhibited maximum decrease (65.53% over control) in activity as compared to the tolerant one T. 8B (32.31% over control) at 16 EC salinity.

FIG. 1—A M.P. CHARI

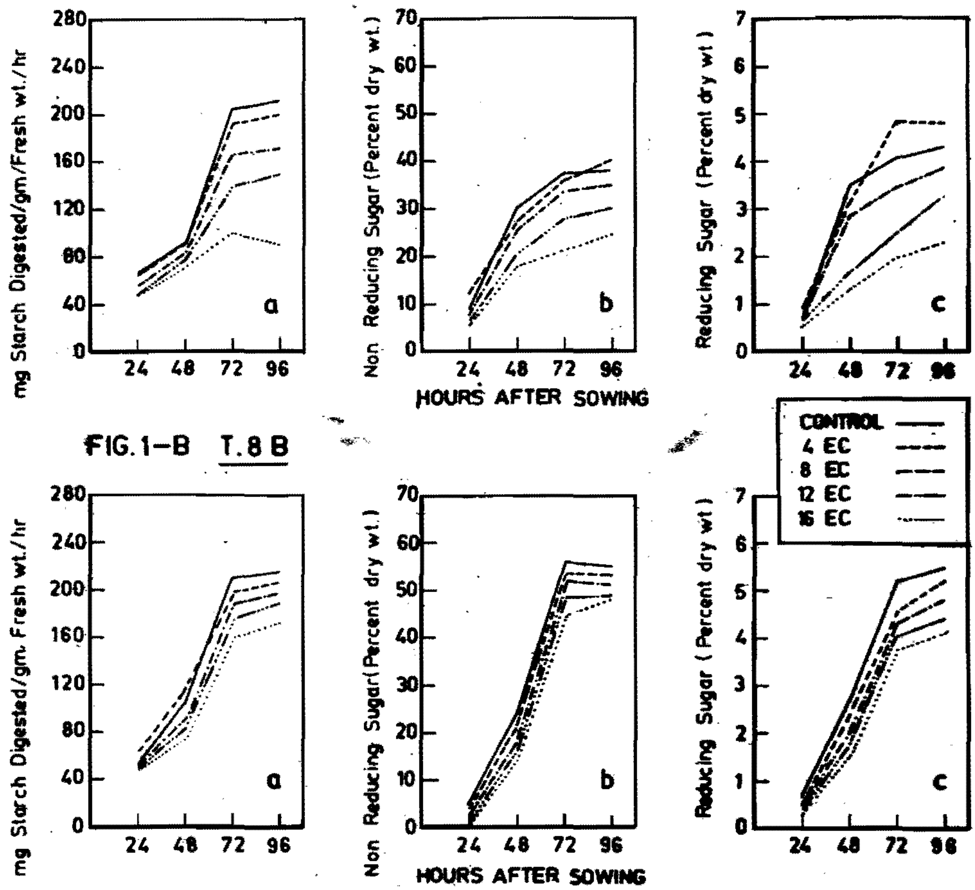

Fig 1, A-B. Influence of salinity on changes in (a) amylase (b) Non-reducing sugars and (c) Reducing sugars of M. P. Chari and T. 8B, during germination.

It can be seen that the inhibition in both alpha-amylase and protease due to salinlty, becomes more and more distinct with the increase in the age of seedlings in the susceptibte variety M. P. Chari (Fig. 1 & 2).

The data for total nitrogen did not show any significant difference with treatment or age of the seedlings in both the varieties (data not given).

Variety M. P. Chari had higher protein levels (10-11%) than the T. 8B (7-8%) at 24 hours after germination. But thereafter, M. P. Chari showed gradual depletion of reserve proteins with increase in salinity, so that at 96 hours only 6.5% proteins were left in the control, as compared to 9.3% in seedlings grown at 16 EC level. Variety T. 8B showed better mobilization of reserve proteins even at 16 EC level so that on the 4th day, it had 25% depletion over first day as compared to 11.6% in

M. P. Chari. The data for soluble nitrogen of both the varieties shows almost a parallel trend with the proteolytic activity. M. P. Chari had higher level of soluble nitrogen as compared to T. 8B (Fig. 2A & B).

FIG. 2—A M.P. CHARI

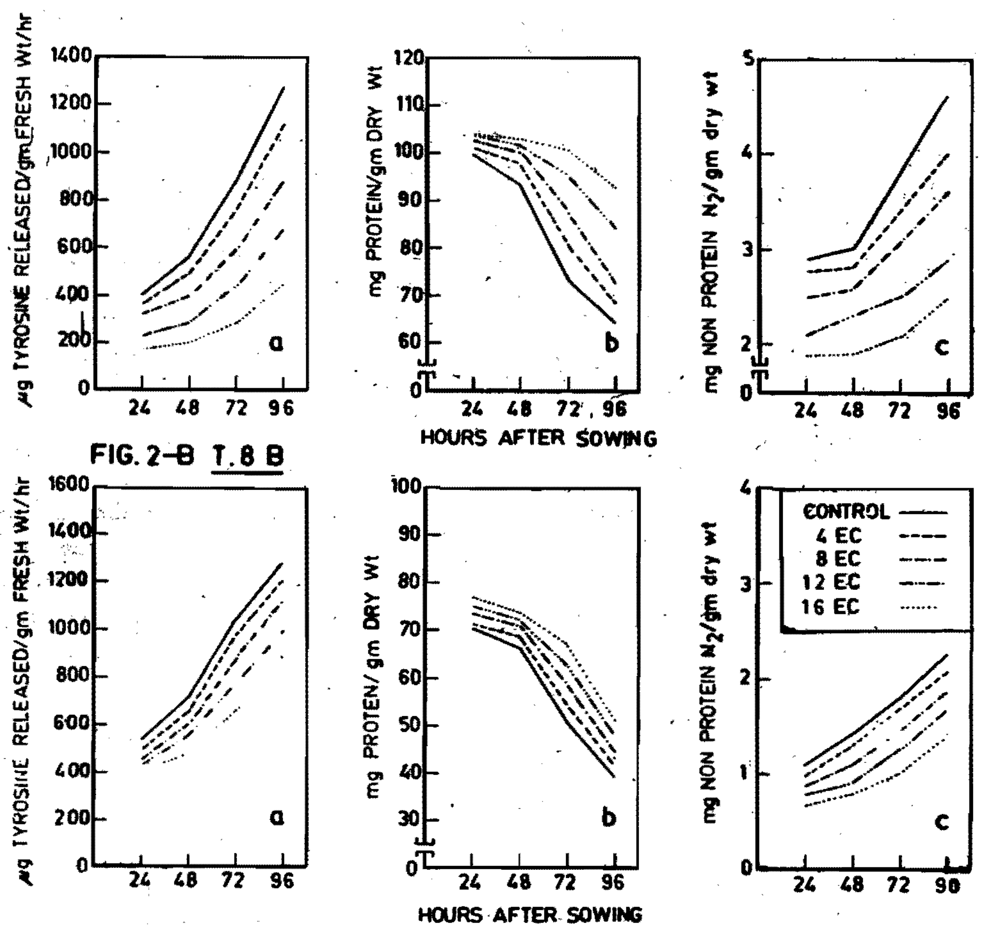

Fig 2, A-B. Influence of salinity on changes in (a) Acid protease (b) Proteins and (c) Nonprotein nitrogen of M. P. Chari and T. 8B, during germination.

DISCUSSION

The results indicate, that variety T. 8B showed better adaptiblity of the hydrolytic enzymes to salt stress, while variety M. P. Chari showed good response only upto 8 EC salinity level. The two varieties differed in their root and coleoptile growth under salt stress conditions also (Ogra and Baijal, 1978). The loss in dry weight in the seedlings during germination is retarded, mainly due to slower water uptake, with increasing salinity, resulting in the seeds and hence their mobilization (Ramana and Rama Das, 1978).

The depressed growth of coleoptile and root may be attributed to the delay in hydrolysis and mobilization of reserves with increasing salinty. Salt stress had inhibitory effect on both alpha-amylase and acid protease, which was more pronounced in the susceptible variety M.P. Chari. Sarin and Narayanan (1968) and Ansari *et al.* (1977) have also reported a decrease in alpha-amylase with salinity in wheat. The decrease being most in the susceptible variety. Both reducing and non-reducing sugars increased with age of the seedlings, but decreased with increasing salinity treatment, on dry weight basis, as was also observed by Bhardwaj (1958) in wheat and gram. Although both the varieties showed reduction in alpha-amylase activity and the different sugar fractions, they differed in their degree of response to salinity. This was also true of their growth. Variety T. 8B had much higher levels of non-reducing sugars as compared to the variety M. P. Chari, which reflects its better malting capacity. Therefore, the salt tolerance of variety T. 8B can be attributed to comparatively higher levels of reducing and non-reducing sugars, which help in osmotic adjustment to salinity. The salt tolerance during germination as a result of better malting capacity and resultant higher sugar levels, has also been reported by George and Williams (1964) in barley.

The activity of protease in the germinating sorghum seedlings decreased with increasing salinity of the external medium. The susceptible variety M. P. Chari showed maximum inhibition as compared to the tolerant variety T. 8B. Sheoran and Garg (1978) have reported that salinity either reduced, or had no effect on the protease activity in all organs, except the leaves in mung bean during germination and early seedling growth. Rakova *et al.* (1969) observed that in pea roots, sodium salts inhibited the synthesis as well as hydrolysis of basic proteins. Most of these reports are based on leaf proteinases, it appears that the effect of salinity on protease also depends upon the plant part used (Sheoran and Garg, 1978).

The decreased proteolysis caused by salinity resulted in slower depletion of reserve proteins during germination of the seeds and the increase in protein levels with increasing salinity can best be explained on this basis and not as a result of enhanced protein synthesis. This is borne out by the fact, that while tolerant variety T. 8B showed lesser inhibition of protease activity, it also exhibited greater depletion of proteins. Similar results were obtained by Prisco and Viera (1976) in the cotyledons of germinating *Vigna sinensis* seeds, who reported that NaCl caused delay in the breakdown of proteins, more due to inhibition of translocation of hydrolysis products than to inhibition of protease activity. The obvious implication is that inhibition in seedling growth can also be, partly attributed to delayed mobilization of reserve proteins, as proteolysis is probably the primary but essential step towards synthesis of new proteins for seedling growth (Ryan 1973).

From the present investigation it is presumed that the induction of germination and also the activity of various enzymes and the metabolic sequences induced by them are delayed under salt stress conditions and that the salt tolerance depends upon the active osmotic adjustment by the emerging seedlings to the external medium.

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