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HEAT SHOCK RESPONSES IN SORGHUM

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The changes in the levels of ∝-amylase, peroxidase, proteins and electrophoretic pattern of proteins and isoperoxidases in *Sorghum bicolor* seedlings growing under the influence of sodium fluoride, ethephon and putrescine with or without thermal stress have been investigated. The study has revealed that ∝-amylase, some specific isoperoxidases and low molecular weight proteins are involved in the thermal tolerance of the plants.

Keywords : .Thermal stress; ∝-amylase; Peroxidases; Protein profile; *Sorghum bicolor*.

Introduction

Plants growing under arid and semi-arid conditions are subjected to extremes of climatic conditions. The most readily observable effect of heat stress is the expression of heat shock protein (hsp)[1]. Induction of hsp accompanies the changes in the level of several enzymes and metabolites. In the present study, changes in the activities of ∝-amylase and peroxidase and electrophoretic pattern of peroxidases and proteins have been investigated in *Sorghum bicolor* seedlings subjected to high concentration of a salt (Sodium fluoride), a bioregulator (ethephon) and a polyamine (putrescine) with or without thermal stress.

Materials and Methods

Seeds of *Sorghum bicolor* (L.) Moench. (Syn : *Sorghum vulgare* Pers.) cv. SPV-96 surface sterilized using 0.1 percent mercuric chloride were germinated in 9.0 cm petridishes lined with filter paper discs containing 10 ml of distilled water (control) or test solutions and incubated in growth chamber at 30±2°C. Test solutions contained NaF (4.76×10^{-3} mol/1) or Ethephon (960 mg/1) or putrescine (5×10^{-3} mol/1). After one week, half the seedlings of each treatment were incubated at 50±2°C for one hour and returned to growth chamber. Biochemical analyses were made after 24 hours of thermal stress. Results are averages of two replicates and expressed as percent increase/decrease over controls. Crude enzyme extract was prepared by homogenizing plant material in pre-chilled mortar and pestle in phosphate buffer (0.05M;pH 7.0) and centrifuging it at 20,000 rpm in refrigerated centrifuge for ten minutes. The supernatant thus collected was used for protein estimation[2], peroxidase assay[3], ∝-amylase assay[4], polyacrylamide gel electrophoresis (PAGE) for isoperoxidases[5] and SDS-PAGE for proteins[6]. The isoperoxidases were visualized using guaiacol and H_2O_2[7] and proteins by coomassie brilliant blue R-250[8]

Results and Discussion

Soluble protein contents increased in all the seedlings subjected to all the three

treatments at room temperature (Table-1). Thermal stress singly or in the presence of ethephon/putrescine decreased them significantly. However, the reduction was more marked in ethephon treated seedlings. Fluoride salt abated the inhibitory effect of thermal stress. ∝-amylase activity decreased invariably under the influence of fluoride salt, ethephon and putrescine. Thermal stress alone and along with other treatments increased the enzyme activity by two to four fold. Peroxidase activity was not significantly affected by any of the treatments at root temperature, but was enhanced in the seedlings subjected to different treatment and thermal stress. Thermal stress alone reduced the enzyme activity.

Thermal stress alone or along with other treatments repressed a low molecular weight protein and induced a group of four fast migrating polypeptides-characteristics of hsp (Fig.1). Thermal stress alone or in combination with NaF, ethephon or putrescine specifically induced isoperoxidase II and repressed the form IV. Thermal stress along with other treatments intensified the isoperoxidases V and VIII. The isozyme XI was induced under all the three treatments while XII by NaF and putrescine only at room temperature. Hyperthermia repressed isoperoxidase XI as well as XII.

It is evident from the results that amylase activity increased significantly under thermal stress despite the fact that protein synthesis was severely retarded. The continued/increased activity of the enzyme under hyperthermia reflects the ability of the plants to cope up with adverse conditions. Induction of specific isoperoxidases (II, V & VIII) coupled with increased enzyme activity and appearance of some low molecular weight proteins (hsp) also constitute the cellular macromolecules coping up with hyperthermal conditions. Changes in protein profile under stress conditions is a common phenomenon[9,10]. The thermal stress

Table 1. Changes in biochemical parameters (percent increase/decrease over control) in response to different treatments in *Sorghum bicolor*.

Parameters	Proteins		∝-amylase		Peroxidase	
Treatments	RT	TS	RT	TS	RT	TS
Water	100	58	100	225	100	46
NaF	186	109	86	215	99	152
Ethephon	140	25	89	444	92	349
Putrescine	183	33	77	223	106	411

RT= room temperature; TS= thermal stress.

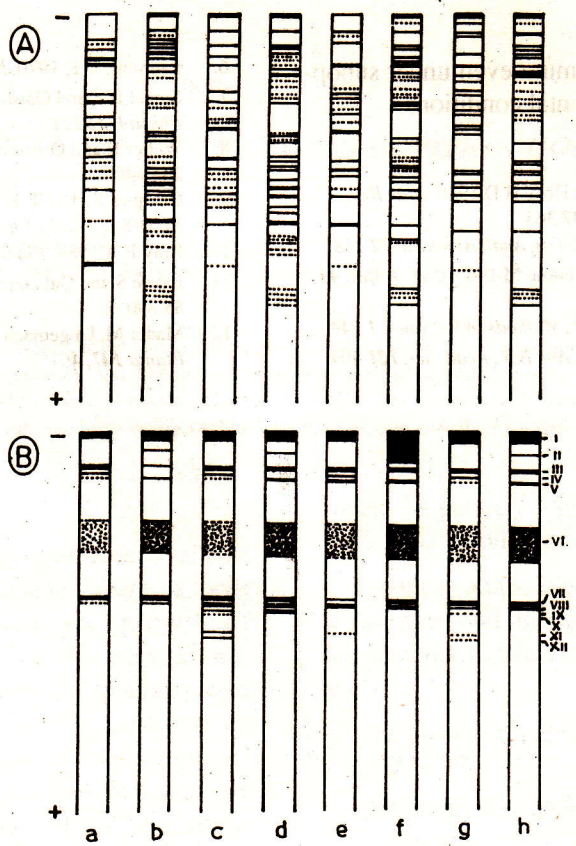

Fig. 1. SDS-PAGE profile of proteins (A) and isoperoxidase pattern (B) of *Sorghum bicolor* seedlings subjected to different treatments.

a=control; b=thermal stress (TS); c=NaF; d=NaF + TS; e=ethephon; f= ethephon + TS; g= putrescine; h= putrescine + TS

also antagonises the stimulatory effect of bioregulant and polyamine on protein synthesis.

Peroxidase activity is inversely related with growth[11] and its repression under hyperthermia indirectly suggests the better performance of the crop at higher temperature. The increase in the peroxidase activity at higher temperature in the presence of NaF, ethephon and putrescine could be accounted by the appearance of an anodic form (VIII). Anodic isoperoxidases are involved in the process of lignification[12] a process which may contribute to the mechanical strength and/or water conduction. On the basis of these results, it can be concluded that these molecules are an integral part of the tolerance devices of the plants and maintain the metabolic

activity at maximum even under suboptimal environmental conditions.

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