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Heat Shock Proteins of Sorghum [*Sorghum bicolor* (L.) Moench] and Pearl Millet [*Pennisetum glaucum* (L.) R.Br.] Cultivars with Differing Heat Tolerance at Seedling Establishment Stage

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ABSTRACT

The production of heat shock proteins was compared in sorghum and pearl millet genotypes differing in seedling establishment characteristics under heat stress. Two major heat shock proteins (hsps) of apparent mol. wt. 65 kD and 62 kD were seen in all the genotypes of sorghum tested when the incubation temperature of the 40 h seedlings was altered from 35 °C to 45 °C for 2 h. Under identical conditions, pearl millet genotypes showed more hsps and the apparent mol. wt. of these ranged from 30–70 kD. The hsp bands were more prominent in whole seedlings and roots as compared to plumules. Differences in the production of hsps were seen in sorghum and pearl millet genotypes with contrasting heat tolerance at seedling establishment stage but the significance of these needs to be studied further.

Key words: Heat shock proteins, sorghum, genotypic differences.

INTRODUCTION

Heat shock proteins (hsps) are synthesized in many organisms in response to a rapid shift to a higher temperature. The response of different organisms varies with respect to the optimum temperature needed to induce hsps and the number and nature of proteins synthesized (Schlesinger, Ashburner, and Tissieres, 1982). Some similarities have been observed in the pattern and type of hsps produced among a variety of organisms ranging from bacteria to mammalian cells (Barnett, Altschuler, McDaniel, and Mascarenhas, 1980; Bascyzynski, Walden, and Atkinson, 1982; Schlesinger *et al.*, 1982). The heat shock response is very rapid and hsp synthesis occurs as early as 20 min after exposure to high temperature (Bascyzynski *et al.*, 1982; Key, Lin, and Chen, 1981). The maximum response is obtained in the range of 40–45 °C for many plant species (Altschuler and Mascarenhas, 1982*a*; Key *et al.*, 1981; Nover, Scharf, and Neumann, 1983) but both the threshold temperature for the optimum production of hsps and the critical limiting temperature when the hsp production is stopped, vary with species (Altschuler and Mascarenhas, 1982*b*; Nover *et al.*, 1983). Though the precise role of hsps is not known, thermotolerance has been postulated as an essential function of these proteins (Mitchell, Moller, Peterson, and Sarmiento, 1979; Key, Kimpel, Lin, Nagao, Vierling, Czarneeka, Gurley, Roberts, Mansfield, and Edelman, 1985).

Inhibition of seedling emergence is a common problem in sorghum and pearl millet in the semi-arid tropics where the soil temperatures can reach as high as 50 °C (Soman, Jayachandran, and Bidinger, 1987; Soman and Fussel, personal communication). A few genotypes have been identified from these two cereals at ICRISAT, using both laboratory and field screening methods (Soman and Peacock, 1985), which can emerge at high soil temperatures (45–50 °C). In this paper, we have looked at differences, if any, in the production of heat shock proteins, using both

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sorghum and pearl millet genotypes with contrasting seedling establishment characteristics under heat stress.

MATERIALS AND METHODS

Measurement of the percentage inhibition of protein synthesis

Sorghum seeds were cut into half and the half-seeds containing the embryo portion were treated essentially as described by Ougham and Stoddart (1986). Ten half-seeds were imbibed for 16 h in buffer (Tris–HCl, pH 7·5, 20 mol m^{-3}, $MgCl_2$, 5·0 mol m^{-3} and chloramphenicol 0·03 mg cm^{-3}) containing 1·0 μCi (37 kBq) of [U-^{14}C]-amino acid mixture (Amersham, UK) at 35 °C or 45 °C. At the end of incubation, the seeds were washed free of radioisotope in distilled water, blotted dry and homogenized in 1·0 cm^3 extraction buffer (as above but without chloramphenicol) using a mortar and pestle. The homogenate was centrifuged at 15 000 rev. min^{-1} in an Eppendorf centrifuge for 5 min. The supernatant was decanted and an aliquot was spotted on to a nitrocellulose membrane filter (0·45 μm) over which was placed a glass fibre filter (GF/A, Whatman) in a Millipore filtration manifold. Non-incorporated radioactivity was removed by washing with distilled water and the discs were counted for radioactivity in Beckman LSC (Mans and Novelli, 1960). The results are expressed as d min^{-1} of ^{14}C-amino acid incorporated per embryo-containing half-seed at 35 °C or 45 °C. The percentage inhibition of protein synthesis at 45 °C was calculated from the d min^{-1} incorporated at 35 °C and 45 °C.

Plant materials and incubation conditions

Sorghum bicolor (L.) Moench. lines IS 17820, IS 23077, IS 3762, and IS 305, with good seedling emergence under heat stress (at 45 °C), and IS 14960, IS 2146, and IS 83, with poor emergence, were selected. For pearl millet [*Pennisetum glaucum* (L.) R.Br.), IP 14000 and IP 14085, with good seedling emergence, and IP 3120 and IP 3374, with poor emergence, were chosen (Soman and Peacock, 1985). Seeds were surface sterilized for 20 min in sodium hypochlorite solution (1% available chlorine), placed on pre-soaked Whatman No. 1 filter paper in Petri plates and germinated in darkness at 35 °C for 24 to 72 h. Then the endosperm was separated from the sorghum seedling and six such seedlings were pre-incubated for 2 h in 2·0 cm^3 of heat shock (HS) buffer containing Tris–HCl, pH 7·5, 50 mol m^{-3}, sucrose 1%, and $MgCl_2$ 5·0 mol m^{-3} at 35 °C. In the case of pearl millet, the seedlings were not separated from the endosperm portion as they were too small and so there was no pre-incubation step at 35 °C to recover from any injury shock. At the end of the pre-incubation, the solution was decanted and 2·0 cm^3 of fresh HS buffer was added, followed by 20 μCi (740 kBq) of [^{35}S]-methionine (1746 Ci $mmol^{-1}$) or 5·0 μCi (185 kBq) of [^{14}C]-leucine (282 mCi $mmol^{-1}$). The incubation was carried out in the dark at 45 °C, and the controls were set at 35 °C for 2 h. At the end of incubation, the solution was decanted and the seedlings were washed with 1·0 mol m^{-3} non-radioactive methionine or leucine, followed by two washings with cold water. Seedlings were either taken as such or divided into roots, embryo portion and plumules for homogenization.

Preparation of samples

The samples were homogenized with 1·0 cm^3 cold extraction buffer (Tris–HCl 50 mol m^{-3}, pH 7·5, β-mercaptoethanol 0·04%) in a precooled (4 °C) mortar and pestle. The homogenate was centrifuged at 15 000 rev. min^{-1} in an Eppendorf centrifuge for 6 min. The supernatant was decanted and spun again for 3 min. Protein was estimated by the method of Lowry, Rosebrough, Farr, and Randall (1957), after precipitating the proteins with 10% TCA and dissolving the final washed pellet in 1N NaOH. Incorporation of [^{35}S]-methionine into protein was determined by the method described by Mans and Novelli (1960).

Electrophoretic detection of ksps

An aliquot of the supernatant was treated with Tris, sodium dodecyl sulphate, glycerol and bromophenol blue according to the method of Laemmli (1970), and electrophoresis was carried out on polyacrylamide slab gels (12·5%) containing SDS. Samples were loaded either on the basis of equal amount of proteins or counts. Both radioactive and non-radioactive molecular weight markers were run alongside. The radioactive markers used were phosphorylase b, 92·5 kD; Bovine serum albumin, 69 kD; ovalbumin, 46 kD, carbonic anhydrase, 30 kD and lysozyme, 14·3 kD. The gels were stained with Coomassie Blue R-250. After destaining, they were soaked in Amplify (Amersham) for 15 min. These gels were then dried on Whatman 3 MM paper under vacuum, and fluorography was carried out by exposing the dried gels to X-ray films (MP) in an intensifying screen at −70 °C.

Chemicals

X-ray film, [^{35}S]-methionine and radioactive mol. wt. markers were obtained from Amersham, UK. The photographic chemicals and intensifying screens were from Kodak, USA. The non-radioactive mol. wt. markers were from Bio-Rad. All other chemicals were from Sigma Chemicals Co. USA.

RESULTS AND DISCUSSION

Poor germination of seeds at high temperature is related to decreased protein synthesis in the embryo of these seeds and the sensitivity of embryo protein synthesis to temperature shows considerable genetic variation (Riley 1981; Ougham and Stoddart, 1985). Sorghum genotypes with contrasting seedling emergence patterns at high temperature (Soman and Peacock, 1985) were selected and the sensitivity of embryo protein synthesis at 45 °C was determined. The data (Table 1) indicated that IS 3762 and IS 305, with good seedling establishment characteristics showed less percentage inhibition of protein synthesis at 45 °C (20–30%) compared to the genotypes with poor establishment characteristics, IS 2146 and IS 83 (40–70%). Similar results were obtained with other contrasting genotypes which were used in the study on hsp synthesis. A similar study could not be conducted in pearl millet because of the small seed size and the selection criteria were mainly based on the seedling emergence data of Soman and Peacock (1985).

TABLE 1. *Per cent inhibition of embryo protein synthesis at 45 °C in genotypes with contrasting seedling emergence characteristics under heat stress*

The values given are the mean ± s.e. of five independent determinations.

Genotype	% Inhibition of protein synthesis
IS 3762	24·11 ± 4·68
IS 305	30·28 ± 12·24
IS 83	44·62 ± 11·29 %CV 25·8
IS 2146	70·11 ± 8·08

The genotypes of sorghum and pearl millet selected as mentioned above were used to look at hsp synthesis. The fluorograms showed the presence of two major hsp bands of apparent mol. wt. 62 kD and 65 kD with a greater percentage incorporation of radioactivity into these proteins at 45 °C than at 35 °C in sorghum seedlings of all the genotypes (Fig. 1a, b). The migration of these two bands was so close that at times they were hardly separated in some of the experiments. These protein bands were more pronounced in the fluorogram of whole seedlings (Fig. 1a) and excised roots (Fig. 1b) compared to those of plumules (figure not shown) as evident from the intensity of the bands. One of the reasons for this difference could be that the total uptake and incorporation of [^{35}S]-methionine was found to be high in roots and least in plumules. In addition to the above mentioned hsp bands, hsps with apparent mol. wts of 46 and 37 kD were present in IS 17820 (lane 6, Fig. 1a) and IS 23077 (lane 8 Fig. 1a) respectively at 45 °C but not at 35 °C and were not found in other cultivars suggesting a genotypic difference in hsp production. These differences were seen more clearly in whole homogenates of seedlings (Fig. 1a) than in roots (Fig. 1b).

In most of the studies (Schlesinger *et al.*, 1982) hsps are newly synthesized and are not present in the control tissues, which are generally incubated at 25 °C or 30 °C. In our experiments, the two hsps are also present in the control tissues incubated at 35 °C in the different genotypes suggesting that the hsps are produced at a temperature of about 35 °C, and this has been verified in another experiment in which controls incubated at 30 °C did not show the 62 or 65 kD protein (Fig. 2). In this experiment, more hsps were seen at 45 °C compared to the control incubated at 30 °C which was in contrast to the control incubated at 35 °C (Fig. 1). This appears to be similar to the observation made by Cooper and Ho (1983) who also noticed that the pattern of protein synthesis was different when the shift in temperature was from 25 °C to 45 °C compared to that from 25 °C to 40 °C. We selected 35 °C as the control because in most of the sorghum-growing areas of the semi-arid tropics, a normal soil temperature of 35 °C is prevalent and the problem of seedling establishment becomes acute only at higher temperatures (≥45 °C).

The pattern of proteins synthesized is also found to vary with the incubation conditions: for example Cooper and Ho (1983) observed as many as ten hsps in excised root tissue of maize, and these differed from those in intact roots. Such differences were seen between tissues (leaf versus root) and also within the same tissue at different stages of growth (Cooper, Ho, and Hauptman, 1984). We also observed some genotypic differences in the number of hsps (lanes 2 and 4 versus lanes 6 and 8 in Fig. 1a) and the amount (intensity of bands) of major hsps

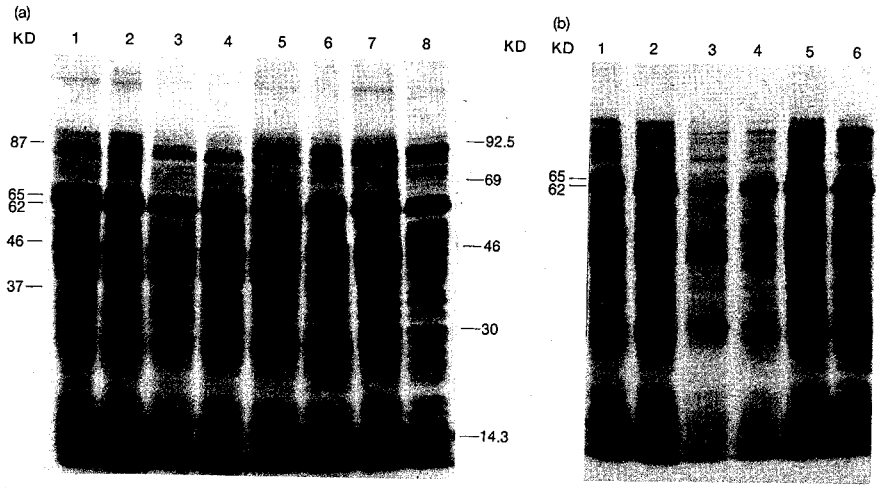

FIG. 1. Pattern of heat shock protein synthesis in sorghum seedlings. Fluorograms of SDS–polyacrylamide slab gel of proteins extracted from (a) whole seedlings and (b) roots after incubation of sorghum seedlings at 35 °C or 45 °C with ^{35}S-methionine. Samples were loaded on the basis of equal counts. The apparent mol. wt. distribution in kD is indicated on the left and hsps are denoted by the bars. (a) lane 1, 3, 5, and 7, 35 °C; lane 2, 4, 6, and 8, 45 °C; lane 1 and 2, IS 83; lane 3 and 4, IS 14960; lane 5 and 6, IS 17820; lane 7 and 8, IS 23077. (b) lane 1, 3, and 5, 35 °C; lane 2, 4 and 6, 45 °C; lane 1 and 2, IS 83; lane 3 and 4, IS 17820; lane 5 and 6, IS 23077. The mol. wt. of the radioactive standards used are indicated on the right.

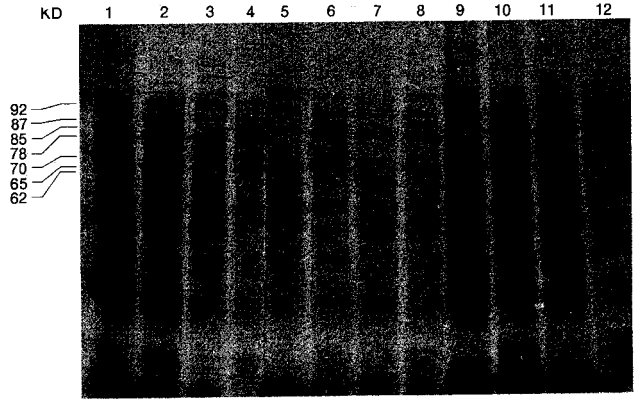

FIG. 2. Pattern of heat shock protein synthesis in sorghum seedlings. Fluorograms of SDS–polyacrylamide gel of proteins extracted from roots, embryos and whole seedlings after incubation of sorghum seedlings at 30 °C or 45 °C with ^{14}C-leucine. Samples were loaded on the basis of equal counts. Lane 1, 3, 5, 7, 9, and 11, 30 °C; lane 2, 4, 6, 8, 10, and 12, 45 °C. The genotypes used are IS 3762 (lane 1, 2, 5, 6, 9, and 10) and IS 83 (lane 3, 4, 7, 8, 11 and 12). Lane 1–4, roots; lane 5–8, embryo portion, lane 9–12 whole homogenate of seedlings. The apparent mol. wt. in kD is indicated on the left and hsps are denoted by the bars.

seen in the different parts of the seedlings. Ougham and Stoddart (1986) could also demonstrate varietal differences in some sorghum genotypes in the time at which the capacity to synthesize hsp first appeared. We have compared the hsp pattern in seedlings of IS 3762 and IS 2146 incubated at 35 °C and 45 °C and at 24 h, 48 h, and 72 h, and the fluorogram showed no significant difference in the hsp pattern (Fig. 3) at the different time periods.

The number of hsps in pearl millet seedlings at 45 °C as seen from the fluorograms (Fig. 4) was more than in sorghum (Fig. 1) and resembled other crops, such as maize (Cooper and Ho, 1983). The apparent mol. wt. of

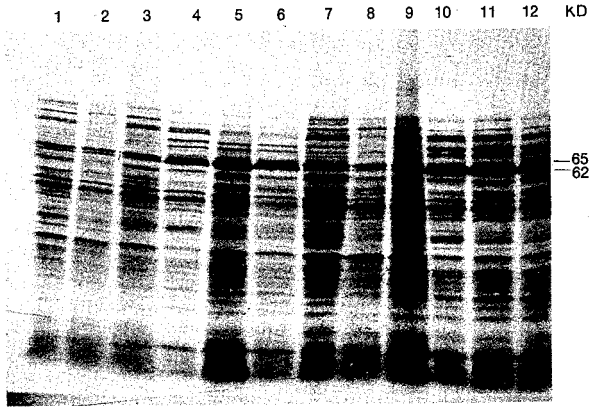

FIG. 3. Pattern of heat shock protein synthesis at different periods in sorghum seedlings. Fluorogram of SDS–polyacrylamide gels of proteins extracted from whole seedlings grown for 24 h, 48 h, and 72 h and incubated for 2 h at 35 °C or 45 °C with ^{35}S-methionine. Samples were loaded on the basis of equal protein. Lane 1, 4, 7, and 10, 24 h; lane 2, 5, 8, and 11, 48 h; lane 3, 6, 9, and 12, 72 h. Lane 1, 2, and 3 and lane 7, 8, and 9, 35 °C; lane 4, 5, and 6 and lane 10, 11, and 12, 45 °C. Lane 1–6, IS 3762, lane 7–12, IS 2146. The apparent mol. wt. in kD is indicated on the right and hsps are denoted by the bars.

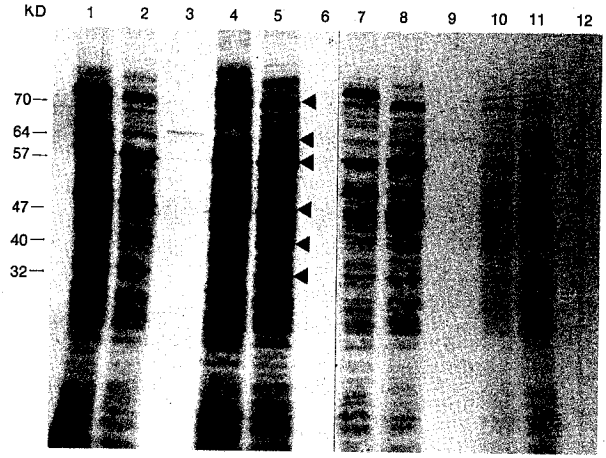

FIG. 4. Pattern of heat shock protein synthesis in pearl millet seedlings. Fluorogram of SDS–polyacrylamide slab gel of proteins extracted from homogenates of whole seedlings after incubation for 2 h at 35 °C, 45 °C, or 50 °C with ^{35}S-methionine. Samples were loaded on the basis of equal protein. Lane 1, 4, 7 and 10, 35 °C; lane 2, 5, 8 and 11, 45 °C and lane 3, 6, 9, and 12, 50 °C. Lane 1–3, IP 3120; lane 4–6, IP 3374, lane 7–9, IP 14000 and lane 10–12, IP 14085. The apparent mol. wt. of hsps in kD are denoted on the left by the bars and the hsps of one genotype are indicated by the arrows.

these hsps ranged from 30 kD to 70 kD. Some varietal differences in the production of hsps were also seen at 45 °C among the contrasting pearl millet genotypes (lanes 2 and 5 versus lanes 8 and 11) which seems to be more in pearl millet than in sorghum genotypes. Since pearl millet is considered as more heat tolerant and generally grown in hotter areas than sorghum, hsps were looked for at 50 °C as well. At 50 °C most of the normal protein synthesis was prevented but a faint hsp band of 65 kD was seen in all the genotypes to varying degrees (better visible in IP 3120, lane 3 and IP 14000, lane 9). The significance of this residual hsp synthesis which was neither present at 35 °C nor at 45 °C is not clear. Thermal adaptation is an important aspect in temperature tolerance, and a pre-exposure of seedlings to a higher temperature like 40 °C can afford protection against otherwise lethal temperatures of 50 °C, as shown in the case of soybean (Altschuler and Mascarenhas, 1982; Key *et al.*, 1985).

The heat shock response is a function of temperature and is tissue specific (Altschuler and Mascarenhas, 1982; Cooper *et al.*, 1984) which would imply the existence of a controlled system in the cell that is very sensitive and adaptive to temperature. The important question about hsps is whether or not they confer any thermotolerance on the plants in which they are synthesized on exposure to a higher temperature. Burke, Hatfield, Klein, and Mullet (1985) have shown the accumulation of hsps in substantial amounts in the leaves of dryland cotton and suggested that dryland crops might synthesize hsps to overcome the elevated canopy temperatures. In the seedling establishment of sorghum and pearl millet, the soil temperature is a critical factor and the genotypes selected in our present study have shown some contrasting differences. Though we observe some genotypic differences in the pattern of hsp production in both sorghum and pearl millet, the real advantage of the better establishing genotypes over poor ones in terms of the number or quantity of hsps produced are not very obvious in our present study. It appears that production of hsps may be a general response to temperature in the crop species studied and both quantitative and qualitative differences in hsp production in several genotypes need to be compared and better understood in order to relate the presence of hsps with the thermotolerance of the various genotypes.

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