Theor Appl Genet (1999) 99: 359–367

G. Kumar · B. T. Krishnaprasad · M. Savitha
R. Gopalakrishna · K. Mukhopadhyay
G. Ramamohan · M. Udayakumar

Enhanced expression of heat-shock proteins in thermo-tolerant lines of sunflower and their progenies selected on the basis of temperature-induction response

Received: 27 August 1998 / Accepted: 28 October 1998

Abstract The major lacuna in developing stress-tolerant lines through breeding is the lack of suitable techniques for screening the segregating population. We report here the development of an efficient technique for identifying high-temperature-tolerant lines in sunflower. The rationale behind this technique is that the stress-responsive genes are expressed during sub-lethal (induction) stress, and its products impart tolerance at subsequent lethal stresses. The genetic variability in gene expression upon induction stress is responsible for the differential survival and recovery following exposure to severe lethal stress in a heterogeneous population. Optimization of induction and subsequent lethal temperature levels is a pre-requisite for developing a standardized screening protocol. The optimum induction temperature in sunflower was identified by subjecting the germinated seedlings to various sub-lethal temperatures followed by exposure to a specific lethal temperature for a fixed duration. Gradual temperature induction was found to be optimum in bringing about a maximum response in terms of the recovery growth of seedlings after exposure to a lethal temperature. Following the optimum induction treatment, seedlings were subjected to a specific high temperature for different periods to arrive at a high-stringency lethal temperature treatment. By adopting this approach an open-pollinated population of *Helianthus annuus* L. (morden) was screened for high-temperature tolerance. The seedlings which survived at the high-stringency lethal temperature following the optimum induction treatment were identified as thermo-tolerant lines. At the plant level too these identified lines showed higher tolerance as reflected by a higher membrane integrity and recovery leaf-area. The progeny population of these identified lines also exhibited higher tolerance to temperature compared to the original population, indicating the persistence of the selected trait. This tolerance was associated with a higher accumulation of heat-shock proteins (HSPs). We propose that this technique can be used as a potential tool to identify and select temperature-tolerant lines from a heterogeneous/segregating population.

Key words Thermo-tolerance · HSPs · Temperature-induction-response · Sunflower · Population variability

Communicated by H. F. Linskens

G. Kumar · B. T. Krishnaprasad · M. Savitha
R. Gopalakrishna · K. Mukhopadhyay
G. Ramamohan · M. Udayakumar (✉)
Department of Crop Physiology,
University of Agricultural Sciences,
GKVK, Bangalore 560 065, India
Fax: +91-080-3330277
E-mail: mukumar@uasblr.kar.nic.in

Introduction

High temperature is an important environmental factor significantly affecting crop productivity in arid and semi-arid regions. Temperatures higher than optimum decrease both plant growth rate and duration, which reduces the yield drastically. It has been shown in crops including wheat that for every one degree rise of mean temperature over the range of 12.2–27.5°C the yield is reduced by 4% (Howard 1924). Therefore, to sustain economic yield it is necessary to breed varieties which are tolerant to high-temperature stress. The major constraint in breeding for temperature tolerance is the lack of suitable techniques for screening both germplasm and the segregating populations.

Under natural conditions any abiotic stress is always imposed gradually. Plants therefore are exposed to a sub-lethal stress before being subjected to severe stress. Several studies have shown that plants develop the ability to withstand otherwise lethal temperatures upon exposure to sub-lethal temperatures (referred as induction stress). This phenomenon has been termed

acquired thermo-tolerance (Lin et al. 1984; Lindquist and Craig 1988; Hahn and Li 1990). During the induction stress, many stress-responsive genes are expressed which, in turn, trigger several physiological and biochemical processes relevant for stress tolerance. More precisely, mild heat treatment elicits a so-called heat-shock response leading to the immediate induction of a set of new proteins or the over-expression of already existing proteins known as heat-shock proteins (HSPs) and which persist over a time course at high temperature (Vierling 1991; Nguyen et al. 1992). HSPs have been known to play a role in cell protection, survival, and recovery in several species (Vierling 1991; Nguyen et al. 1992). Recently, DeRocher et al. (1991) have shown that in pea the expression of low-molecular weight HSPs, such as HSP 18.1 and HSP 17.9, were up-regulated upon a gradual increase in temperature from 30 to 42°C and had long half lives (37.7 $\pm$ 8 h) indicating their possible involvement in establishing thermo-tolerance. The cellular role of high-molecular weight HSPs has been established for chaperoning proteins (Sanchez and Lindquist 1990; Vierling 1991). For instance, HSP 104 is essential for the survival of the yeast cell at high temperature as deletion of the HSP 104 gene impaired the capacity of these cells to acquire thermo-tolerance (Sanchez and Lindquist 1990; Parsell et al. 1994). Interestingly, both soyabean and *Arabidopsis* genes have been shown to complement the thermo-tolerance defect caused by deletion of the HSP 104 gene in a mutant yeast cell (Lee et al. 1994; Schirmer et al. 1994). Molecular and electron microscope studies have shown that the probable role of yeast HSP 104 is to re-solubilise heat-inactivated proteins (Parsell et al. 1994). Next to the HSP 70 family, the HSP 90-kDa proteins are the second most predominantly expressed HSPs. These proteins appear to impart thermo-tolerance, as mutant yeast cells with an impaired capacity to make HSP 90 are incapable of growing at higher temperatures (Borkovich et al. 1989). Though these studies reveal the role of HSPs in thermo-tolerance, optimum expression of heat-shock genes is a pre-requisite which often occurs only during induction stress. On many occasions a relevant physiological trait was found not to be related to the stress tolerance in known tolerant genotypes/species, mainly because the expression of this trait was examined by directly subjecting plants to severe stress without prior optimum induction stress (Lin et al. 1984; Krishnan et al. 1989; Uma et al. 1995). Krishnan et al. (1989) have shown that genetic variability in wheat for temperature tolerance at a lethal temperature (50°C) was seen when they were subjected to prior heat-induction treatment (37°C). The studies conducted in our laboratory further strengthens the expression of genetic variability in the stress response upon induction stress (Savitha 1995; Uma et al. 1995). A relationship between stress-responsive proteins, such as LEA2 and LEA3, and stress tolerance in seedlings of finger millet and rice genotypes was seen when the seedlings were subjected to induction stress prior to their exposure to lethal stress (Jayaprakash et al. 1998).

Since the genetic variability in stress response is expressed only upon induction stress, and is accompanied by the differential accumulation of HSPs (Krishnan et al. 1989; Jorgensen et al. 1992), the intrinsically tolerant genotypes can be identified by optimizing the induction- and lethal-stress treatments. Here, we report the application of the temperature induction-responsive (TIR) technique for identifying highly thermo-tolerant lines from open-pollinated populations of sunflower.

Materials and methods

Plant material

The sunflower seeds, (*Helianthus annuus*. L.) Morden (an open-pollinated heterogeneous population), were obtained from the Project Coordinator (Sunflower), Bangalore, India. The pre-imbibed seeds were germinated on moist filter paper in Petri dishes at 30°C and 60% RH. The germinated seedlings (approximately 1.5 cm in length) and 15–20-day-old plants raised in plastic containers were used to study the genetic variability in stress response.

Identifying thermo-tolerant lines at the seedling level

Induction temperature treatments

The germinated seedlings (48 h-old) were subjected to different induction temperature treatments such as 35°C for 4 h, 40°C for 4 h, 45°C for 4 h, or to gradual induction. For gradual induction the temperature was increased from 35 to 45°C at the rate of 5°C per h and was later maintained for 2 h at 45°C.

Lethal temperature

The induced and non-induced (maintained at room temperature) seedlings were exposed to a lethal temperature of 55°C. The duration of the lethal temperature was varied from 1 to 3 h in order to achieve varying levels of severe (low-stringency lethal/high-stringency lethal) temperature stress.

Low-stringency lethal: Here the temperature treatment is severe enough to cause 50% mortality in the non-induced seedlings at the end of recovery.
High-stringency lethal: Here the temperature treatment is severe enough to cause 90–95% mortality in the induced seedlings at the end of recovery.
Recovery growth: After the lethal temperature treatment seedlings were allowed to recover by transferring them to 30°C for 72 h. At the end of the recovery period root and shoot growth and the per cent survival of seedlings were recorded.

In all these experiments 15 seedlings were used per replication and each treatment had three replications. These experiments were conducted at 60% RH.

Identification of thermo-tolerant lines

By adopting an optimum induction and a high-stringency lethal protocol, about 5000 seeds of an open-pollinated population of

sunflower were screened. The surviving seedlings (approximately 300) at the end of recovery were selected as thermo-tolerant lines and developed into plants.

Development of plants from the selected lines of the open-pollinated population

The selected lines were transferred to a plastic pot containing a red soil and sand mix (1 : 1) and established in the greenhouse for 30 days. Only 40–50% of the transplanted seedlings survived and were established as plants. Similarly, plants were also developed from the original population and were compared with the selected lines with respect to temperature tolerance.

Development of progenies from the selected lines

The selected lines were allowed to open-pollinate among themselves and the resulting seeds were bulked and termed the progeny population.

Stress response of selected lines at the plant level

Leaf-area recovery

The 30 day-old plants of selected lines and the original population were subjected to a lethal temperature (50°C for 1 h), with or without prior optimum induction, and later were allowed to recover in the greenhouse for 6 days. Leaf-area recovery was recorded at the end of the recovery period.

Membrane integrity

The membrane integrity in the leaves of the treated plants (30 day-old) was analyzed 16 h after temperature treatment by following the Leopold et al. (1981) method. Leaf punches were taken from the top third leaf and incubated in 10 ml of de-ionised water on a shaker for 3 h. At the end of 3 h, initial absorbance (D) was measured at 273 nm. Following this the leaf punches were boiled over a hot water bath for 30 min and the final D was recorded. The percent leakage was computed as follows:

$$\text{percent leakage} = \frac{\text{Initial } D}{\text{Final } D} \times 100.$$

Western blots

Proteins were isolated from induced and non-induced seedlings and leaf tissues by rapid homogenization in 0.1 M tris-HCl buffer (pH 7.8). Protein samples (100 μg/lane) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto a nitrocellulose membrane according to Kyhse-Andersen (1984). Blots were blocked using 4% casein in PBS for 12 h at 4°C. Later, they were probed with primary antibody for 2 h at room temperature. For detecting HSP 90 and HSP 104, the antibodies raised in rabbit against rice HSP 90 and HSP 104 were employed at a 1 : 2500 dilution (Ashwani Pareek et al. 1995); HSP 18.1 was detected by using an antibody raised in rabbit against a fusion protein containing a C-terminal conserved region of pea HSP 18.1 at a 1 : 250 dilution (DeRocher et al. 1991). The bands in Western blots were visualized after incubating with alkaline phosphatase-conjugated-goat anti-rabbit IgG for 1 h at room temperature and developed by using nitro blue tetrazolium and 5- bromo-4-chloro-3-indolyl-phosphate as a substrate (Engvall and Perlmann 1972).

Frequency analysis

The germinated seedlings ($n = 15$) of the original and progeny populations were subjected to the lethal temperature with or without prior gradual temperature-induction. The recovery growth of the seedlings were grouped into ten size classes. The level of significance of the frequency distribution in induced and non-induced seedlings of the two populations was calculated as described by Siegel (1956).

Results

Induction response

Seedlings exposed to the induction temperature prior to the lethal temperature exhibited a higher growth recovery compared to the non-induced seedlings. Further, the recovery growth varied significantly across different induction temperatures (Figs. 1, 2). The maximum recovery growth was obtained following gradual temperature-induction treatment.

One of the possible mechanisms for the enhanced recovery growth of induced seedlings is by the synthesis of heat-shock proteins which impart thermo-tolerance (Lindquist and Craige 1988; Hahn and Li 1990; Vierling 1991; O'Connel 1994). We examined the

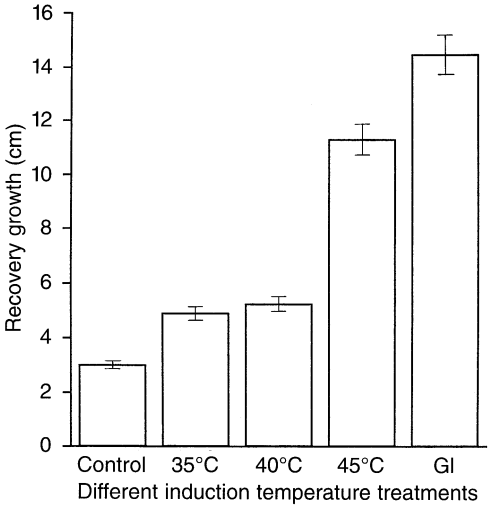

Fig. 1 Recovery growth of sunflower seedlings exposed to high-temperature stress following different induction treatments. Germinated sunflower seedlings were exposed to different induction temperatures including 35°C, 4 h; 40°C, 4 h; 45°C, 4 h separately, and a gradual induction and (GI) of 35°C, 1 h → 40°C, 1 h → 45°C, 2 h. A non-induced control was maintained at 30°C. Later, they were transferred to a lethal temperature of 55°C, 1 h, following which the seedlings were recovered at 30°C for 72 h. At the end of recovery the root and shoot growth was recorded. Three independent experiments were conducted and the data were averaged. *Bars* represent the standard error of means at the 5% level

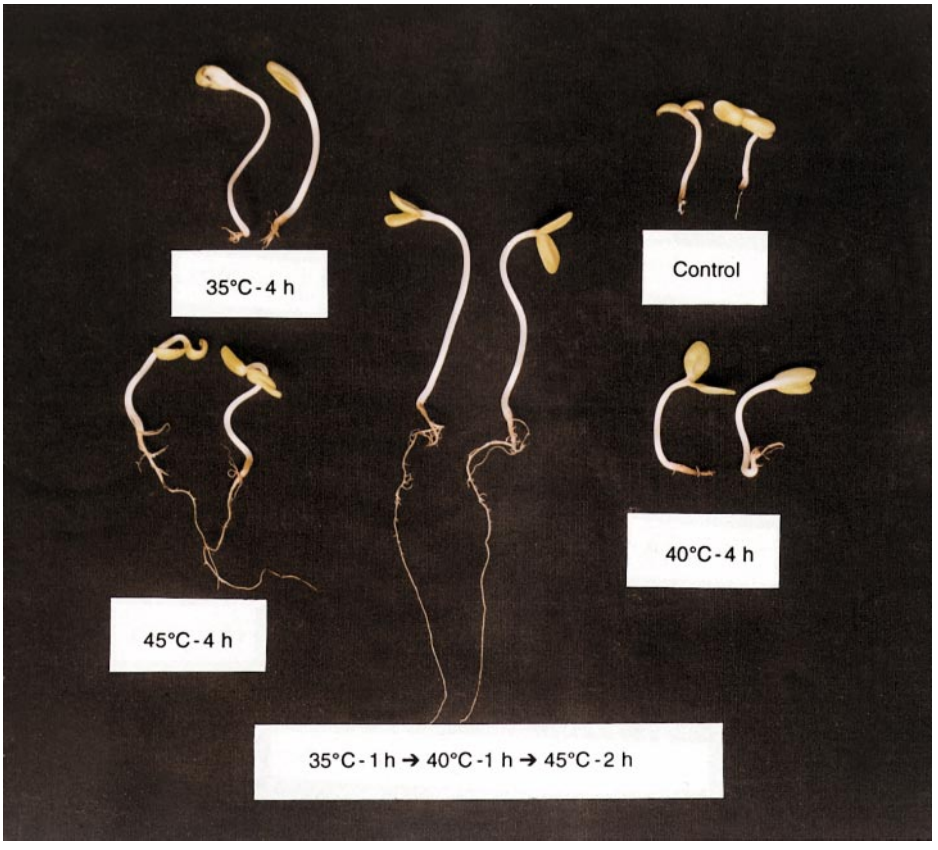

Fig. 2 Response of sunflower seedlings to different induction temperature treatments. Germinated sunflower seedlings were exposed for 1 h of lethal temperature stress, 55°C, following different temperature-induction treatments. The photograph was taken after 72 h of recovery (for details refer to Fig. 1)

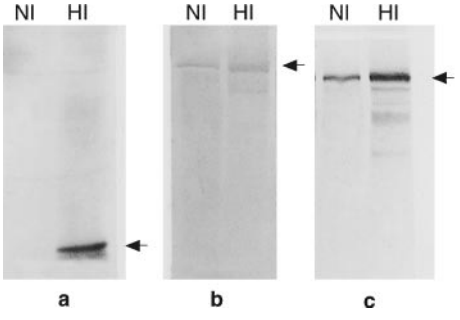

Fig. 3 A Western blot showing the enhanced expression of HSP 18.1 (**a**), HSP 90 (**b**) and HSP 104 (**c**) in temperature-induced seedlings. Protein was extracted at the end of induction from both heat-induced (*HI*) and non-induced (*NI*) germinated seedlings of sunflower and separated by SDS-PAGE (100 μg/lane) and subjected to immunoblot analysis using the indicated antisera

expression of HSP 18.1, HSP 90 and HSP 104 following temperature induction. There was enhanced synthesis of all these HSPs upon temperature induction (Fig. 3) and this result suitably complemented the recovery growth response of the induced seedlings.

Selection of thermo-tolerant lines from an open-pollinated population of sunflower

The original population used for this study was highly heterogeneous. In order to identify thermo-tolerant lines, the seedlings were initially subjected to gradual temperature induction, followed by a lethal temperature for different durations. With an increase in the duration of the lethal temperature, the percent survival of seedlings was markedly reduced (Figs. 4, 5). Lethal stress at which only 5–10% of the seedlings survived was considered as high-stringency lethal (explained in Materials and methods). The surviving seedlings at high-stringency lethal stress were selected as thermo-tolerant lines and subsequently developed into plants.

Evaluation of selected lines at the plant level to validate the screening done at the seedling level

The 30 day-old plants of selected lines and the original population were subjected to a high-temperature stress of 50°C for 1 h 30 min following gradual temperature induction. The level of stress tolerance was assessed by monitoring the leaf-area recovery and the maintenance of membrane integrity. The selected population exhibited a higher leaf-area recovery and also a higher membrane integrity under temperature stress (Table 1, Fig. 6). These results thus confirm that the selected lines are intrinsically tolerant and, therefore, also validates the initial screening done at the seedling level. Further, to test the heritability of the identified trait

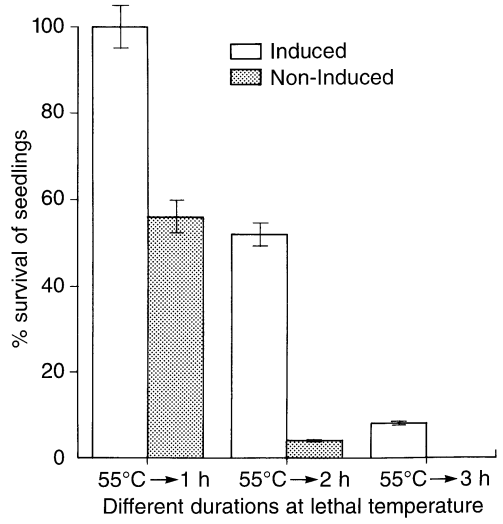

Fig. 4 Percent survival of seedlings exposed to different lethal-temperature durations following a gradual temperature induction. Germinated sunflower seedlings were exposed to different durations of a lethal temperature (55°C, 1 h or 55°C, 2 h or 55°C, 3 h) with or without prior gradual temperature induction. After 72 h of recovery the percent survival of the seedlings was recorded. The data presented here is the average of three experiments and the *bar* represents the mean standard error at the 5% level

(thermo-tolerance) the progenies of the selected lines were assessed for high-temperature tolerance.

Evaluation of the progeny population of selected lines for temperature tolerance

The germinated seedlings of the progeny population were subjected to high-temperature stress with or without prior gradual temperature induction. The induced progeny population of the selected lines showed a higher growth recovery than the induced original population (Fig. 7). However, in the non-induced treatment there was no significant difference in the recovery growth of the two populations. Further, the higher recovery growth of the induced progeny population was associated with enhanced synthesis of HSP 104 (Fig. 8).

Variability in the recovery growth of the seedlings of the original population and the progenies of the selected lines

Since the population used for this study was heterogeneous, a considerable variation in the recovery growth of induced seedlings was observed. The extent of variability was measured using the frequency distribution of the recovery growth of the induced and non-induced seedlings of both the original and the progeny populations (Fig. 9). The non-induced seedlings did not show any variation in recovery growth, i.e. a large number of seedlings grouped into the same class interval (Fig. 9. a,c, CV = 23.07 and 26.75% respectively). But, when the induced seedlings were exposed to high-temperature stress, they exhibited considerable variation in recovery growth, i.e. the seedlings were distributed in different class intervals (Fig. 9.b. CV = 54.41%, D-max between induced and non-induced = 0.733, $P < 0.01$). More interestingly, when the induced seedlings of progenies of the selected lines were subjected to high-temperature stress they exhibited a remarkably higher variation in recovery growth, i.e. the seedlings were distributed in different class intervals (Fig. 9.d., CV = 52.87%, D-max between induced and non-induced = 0.933, $P < 0.01$). Therefore, this suggests that genetic variability for stress tolerance is seen only upon optimum induction stress.

Discussion

Our results clearly demonstrate that the induced seedlings exhibited a higher recovery growth compared to the non-induced and also accumulated higher levels of a few low- and high-molecular weight HSPs, such as HSP 18.1, HSP 90 and HSP 104. The synthesis and localization of some of these HSPs trigger several important physiological and biochemical parameters (Chen et al. 1990), including the maintenance of membrane stability (Kader et al. 1991) and chaperoning of the proteins (Sanchez and Lindquist 1990; Vierling and Nguyen 1992). These changes facilitate the maintenance of cellular function under stress. However, the expression of stress-induced genes and the subsequent changes in physiological processes occur predominantly during induction stress. Therefore, only the seedlings which were pre-exposed to the optimum temperature-induction stress exhibited better recovery growth. More importantly, though the induced and non-induced seedlings are not different genetically, the induced seedlings showed higher tolerance by the enhanced expression of stress-responsive genes.

The thermo-tolerant lines were identified from the open-pollinated (heterogeneous) sunflower population by varying the duration of the lethal temperature of the induced seedlings (Fig. 4). The plants which developed from these identified lines were also found to be tolerant to high temperature, and so validate the initial screening done at the seedling level. In a similar study Blumenthal et al. (1990) have shown that the coleoptiles of field-resistant wheat cultivars exposed to a lethal temperature upon heat shock exhibited better growth compared to a field-susceptible cultivar. This indicates that screening for temperature tolerance at the seedling level is valid. Further, the performance of the selected lines under temperature stress was superior to that of the original population. This was principally because

5

Fig. 5 Effect of different durations of lethal temperature on seedling survival. The percent survival of seedlings in induced (*Cy.I*) and non-induced (*C*) conditions of different durations in lethal temperature, such as 55°C-1 h (*a*), 55°C-2 h (*b*), 55°C-3 h (*c*) was recorded and the photographs were taken 72 h after the stress treatment

6

Fig. 6 Variation in the thermotolerance of original and selected lines at the plant level. The 30-day old plants, developed from original population (*CI*) and selected lines of the original population (*SCI*) were subjected to a lethal temperature of 50°C for 1 h following gradual temperature induction. The photograph was taken after 6 days of recovery

Table 1 Higher membrane stability and leaf area recovery of selected lines under high-temperature stress.

The 30 day-old plants, developed from the original population and the selected lines of (for details see Materials and methods) the original population were subjected to a lethal temperature of 50°C for 1.5 h following an optimum gradual temperature induction. Later, recovered for 6 days. During recovery (16 h after recovery) the leaf punches were collected from these plants for membrane-integrity assessment. The leaf-area recovery was recorded 6 days after the stress treatment

Treatment	Recovery leaf area (cm^2)	Membrane integrity (% leakage)
Selected induced	223	29.56
Selected non-induced	Nil	32.65
Original induced	124	31.62
Original non-induced	Nil	44.08
LSD at 5%	10.29	0.40

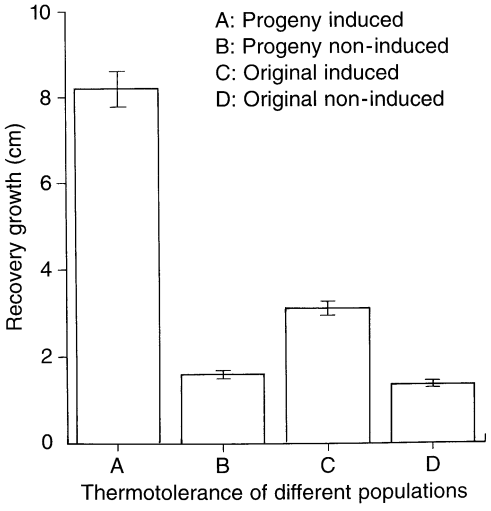

Fig. 7 Differential thermo-tolerance of the original, and progeny populations of the selected lines at the seedling level. Germinated seedlings of the original, and progenies of the selected, lines were subjected to the lethal temperature with or without prior gradual temperature induction. The seedlings were recovered at 30°C for 72 h and the root and shoot growth was recorded. This experiment was repeated three times and the average data is presented. *Bars* represent the standard error of the means at the 5% level

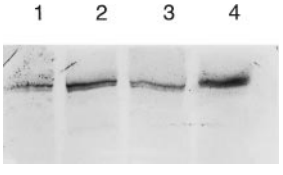

Fig. 8 Enhanced expression of HSP 104 in the progenies of selected lines. *Lanes 1 and 3* Non-induced original (*1*) and progeny of selected lines (*3*). *Lanes 2 and 4* Induced original (*2*) and progeny of selected lines (*4*). Proteins extracted at the end of induction from the top-third leaves of 30 day-old plants of the original and progeny population was separated by SDS-PAGE (100 µg/lane) and subjected to immunoblot analysis using HSP 104 antisera

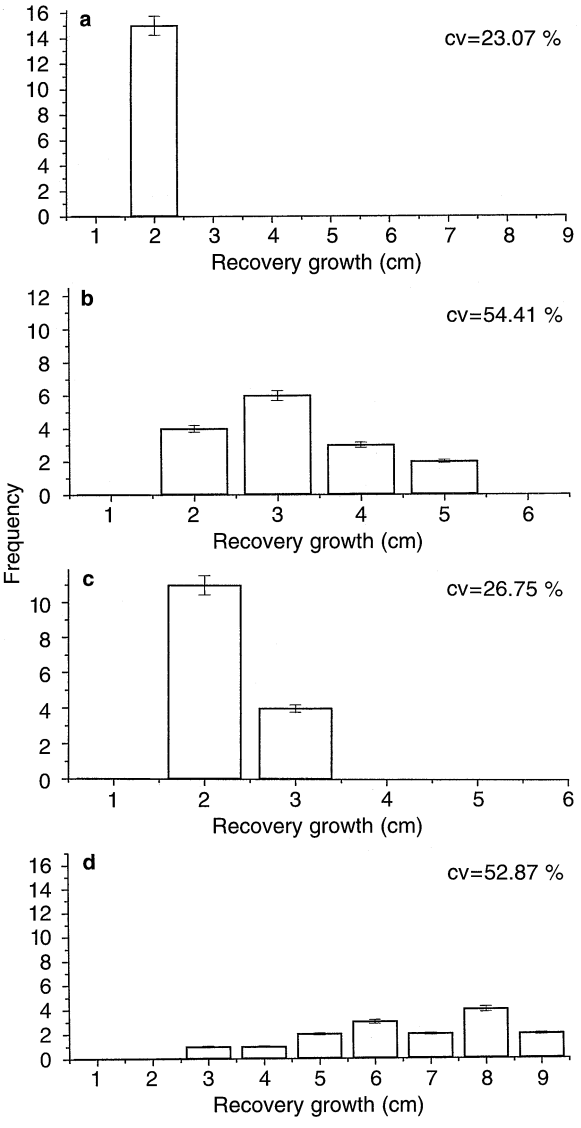

Fig. 9a–d Frequency distribution of the recovery growth of original and progeny populations. **a** non-induced original population, **b** induced original population, **c** non-induced progeny population and **d** induced progeny population. The Kolmogorov-Smirnov test indicated a significant difference in the frequency distribution in the induced seedlings of the original (D-max between induced and non-induced: 0.733, $P < 0.01$) and the progeny (D-max between induced and non-induced: 0.933, $P < 0.01$) populations. But in the non-induced it was non-significant (D-max between non-induced original and non-induced progeny population: 0.222, NS)

they were selected by imposing a high selection pressure upon optimum induction. These selected lines showed better membrane integrity under stress, a trait which broadly reflects intrinsic tolerance (Mckersie and Leshem 1994).

The progenies of the selected lines also exhibited greater tolerance to high temperature compared to the original population, and this trait was associated with a higher accumulation of HSP 104. The importance of HSP 104 in acquisition of thermo-tolerance has been

proved in systems such as yeast (Parsell et al. 1994; Sanchez and Lindquist 1990), soyabean and arabidopsis (Lee et al. 1994; Schirmer et al. 1994). This suggests that the population selected based on the temperature induction response at high stringency lethal are highly thermo-tolerant and this trait is heritable. Recently, Joshi et al. (1997) have shown the genetic linkage between the acquired thermo-tolerance trait and the differential expression of a unique member of the HSP 26 gene family in wheat, and this further strengthens our findings.

In this study, when an open pollinated population was subjected to high temperature stress upon an optimum induction, considerable variation in recovery response was seen (Fig. 4). Such a variation is in fact, a pre-requisite for identifying highly tolerant lines at high stringency lethal stress. The lines thus selected and their progenies showed higher thermo-tolerance. Enhanced expression of the HSPs could be one of the mechanisms attributed to high temperature tolerance, as it was shown in cotton (Susan and O'Connel 1989), maize (Ristic et al. 1991; Jorgenson et al. 1992), sorghum (Howarth 1989; Ougham and Stoddart 1986), wheat (Krishnan et al. 1989; Blumenthal et al. 1990, Vierling and Nguyen 1992) and sugar cane (Moisyadi and Harrington 1989).

Our results conclusively showed that by developing optimum temperature induction stress protocol and arriving at high stringency lethal stress; thermo-tolerant lines can be identified. Further, the thermo-tolerant trait was positively associated with enhanced accumulation of HSP 104 which therefore, can be used as a biological marker to confirm the identified tolerant genotype. This technique is amenable for screening large number of seedlings and since it is non-destructive, the identified tolerant lines can be developed into subsequent progenies. Therefore, this will be a novel approach to screen a segregating population.

Acknowledgements We thank Dr. Elizabeth Vierling, Department of Molecular and Cellular Biology and Biochemistry University of Arizona, Tucson, Arizona, 85721, USA, for providing pea HSP 18.1 antibodies, and Dr. Anil Grover, Department of Molecular Biology, University of Delhi South Campus, New Delhi-110021, India, for providing HSP 90 and HSP 104 antibodies. Ganesh Kumar thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, for a Senior Research Fellowship Award.

References

Ashwani Pareek, Sneh Lata Singla, Anil Grover (1995) Immunological evidence for accumulation of two high-molecular-weight (104 and 90 kDa) HSPs in response to different stresses in rice and in response to high-temperature stress in diverse plant genera. Plant Mol Biol 29 : 291–301

Borkovich KA, Farrelly FW, Finkelstein DB, Taulien J, Lindquist S (1989) HSP 82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol Cell Biol 9 : 3919–3930

Blumenthal C, Bekes F, Wrigely CW, Borlow EWR (1990) The acquisition and maintaince of thermo-tolerance in Australian wheats. Aust J Plant Physiol 17 : 37–47

Chen Q, Lauzon LM, DeRocher AE, Vierling E (1990) Accumulation, stability, and localization of a major chloroplast heat-shock protein. J Cell Biol 110 : 1873–1883

DeRocher AE, Kenneth WH, Lisa ML, Vierling E (1991) Expression of conserved family of cytoplasmic low-molecular-weight heat-shock proteins during heat stress and recovery. Plant Physiol 96 : 1038–1047

Engvall E, Perlmann PJ (1972) Enzyme-linked immunosorbent assay (ELIAS), III. Quantification of specific antibodies by enzyme-labelled anti-immunogloubulin in antigen-coated tubes. J Immunol 109 : 129–135

Hahn GM, Li GC (1990) Thermo-tolerance, thermo-resistance and thermo-sensitization. In: Morimoto RI, Tissieres A, Georgopoulos C (eds) Stress proteins in biology and medicine. Cold Spring Harbor Press, Cold Spring Harbor, New York, pp 79–100

Howard A (1924) Crop production in India: a critical survey of its problems, Oxford University Press, UK

Howarth CJ (1989) Heat-shock proteins in *Sorghum bicolor* and *Pennisetum americannum*. Plant Cell Environ 12 : 471–477

Jayaprakash TL, Ramamohan G, KrishnaPrasad BT, Ganesh Kumar, Prasad TG, Udaya Kumar M (1998) Genotypic variation in differential expression of LEA2 and LEA3 genes and proteins in finger millet and rice seedling systems. Ann Bot 82 : 513–522

Jorgensen JA, Weng J, Ho T-HD, Nguyen HT (1992) Genotypic-specific heat-shock proteins in two maize inbreds. Plant Cell Rep 11 : 576–580

Joshi CP, Klueva NY, Morrow KJ, Nguyen HT (1997) Expression of a unique plastid-localized heat-shock protein is genetically linked to acquired thermo-tolerance in wheat. Theor Appl Genet 95 : 834–841

Kader JC, Ostergaard J, Vergnolle C, Renard M (1991) Bifunctional lipid transfer fatty acid-binding proteins in plants. In: Quinn PJ, Harwood JL (eds) Plant lipid biochemistry: structure and utilization. Portland Press, London, pp 212–214

Krishnan M, Nguyen HT, Burke JJ (1989) Heat-shock protein synthesis and thermal tolerance in wheat. Plant Physiol 90 : 140–145

Kyshe-Andersen J (1984) Electroblotting of multiple gels: a simple apparatus without buffer for rapid transfer of proteins from polyacrylamide to nitrocellulose. J Biochem Biophys Methods 10 : 203–209

Lee YRJ, Nagao RT, Key JL (1994) A soybean 101-kDa heat-shock protein complements a yeast HSP 104 deletion mutant in acquiring thermo-tolerance. Plant Cell 6 : 1899–1909

Leopold AC, Musgrave ME, Williams KM, (1981) Solute leakage resulting from leaf desiccation. Plant Physiol 68 : 1222–1225

Lin CY, Roberts JK, Key JL (1984) Acquisition of thermo-tolerance in soybean seedlings: synthesis and accumulation of heat-shock proteins and their cellular localisation. Plant Physiol 74 : 152–160

Lindquist SL, Craig E (1988) The heat-shock proteins. Annu Rev Genet 22 : 631–637

Mckersie DB, Leshem YY (1994) Heat stress. In: Stress and stress coping in cultivated plants, chapter 8. Kluwer Academic Publishers, Netherlands, pp 181–193

Moisyadi S, Harrington Hm (1989) Characterization of heat-shock response in cultured sugarcane cells. Plant Physiol 90 : 1156–1162

Nguyen HT, Hendershot KL, Joshi CP (1992) Molecular genetics for stress breeding: heat-shock proteins In: Buxton DR et al. (eds). Crop Science Society of America, Maryland. Int Crop Sci 1 : 541–547

O'Connell MA (1994) Heat-shock proteins and thermo-tolerance. In: Basra AS (ed) Stress-induced gene expression in plants. Harwood Academic Publishers, Switzerland, pp 163–184

Ougham Hj, Stoddart JL (1986) Synthesis of heat-shock protein and acquisition of thermo-tolerance in high-temperature-tolerant and high-temperature-susceptible lines of sorghum. Plant Sci 44 : 163–167

Parsell DA, Sanchez Y, Stitzel JD, Lindquist S (1994) Protein disaggregation mediated by heat-shock protein HSP 104. Nature 372 : 475–478

Ristic Z, Gifford DJ, Cass DD, (1991) Heat-shock proteins in two lines of *Zea mays* L. that differ in drought and heat resistance. Plant Physiol 97 : 1430–1434

Sanchez Y, Lindquist S (1990) HSP 104 is required for induced thermo-tolerance. Science 248 : 1112–1115

Savitha M (1995) Genetic variability in recovery growth and synthesis of stress proteins to temperature stress in groundnut. MSc thesis, University of Agricultural Science, Bangalore, India

Schirmer EC, Lindquist S, Vierling E (1994) An *Arabidopsis* heat-shock protein complements a thermo-tolerance defect in yeast. Plant Cell 6 : 1899–1909

Siegel S (1956) Non-parametric statistics for the behavioural sciences. McGraw Hill Kagakusha Ltd., New Delhi, India

Susan EF, O'Connell MA (1989) Heat-shock protein expression in thermo-tolerant and thermo-sensitive lines of cotton. Plant Cell Rep 8 : 37–40

Uma S, Prasad TG, Udayakumar M (1995) Genetic variability in recovery growth and synthesis of stress proteins in response to polyethylene glycol and salt stress in Finger millet. Ann Bot 76 : 3–49

Vierling E (1991) The role of heat-shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 42 : 579–620

Vierling E, Nguyen HT (1992) Heat-shock-protein gene expression in diploid wheat genotypes differing in thermal tolerance. Crop Sci 32 : 370–377