Indian J. Plant Physiol., Vol. 12, No. 4, (N.S.) pp. 327-336 (Oct.-Dec., 2007)

HEAT SHOCK RESPONSE OF WHEAT CULTIVARS DIFFERING IN THERMOTOLERANCE

POONAM SHARMA-NATU[1]*, M. SAVITHA[2], S. BABU[3] AND M. UDAYAKUMAR[4]

[1]Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110 012
[2,3,4]Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore-560 065, India

Received on 30 Dec., 2006, Revised on 29 Dec., 2007

SUMMARY

In the present study an attempt was made to assess whether genetic variability in thermotolerance in wheat (*Triticum aestivum* L.) was associated with the differential accumulation and expression of high molecular weight (HMW) and low molecular weight (LMW) heat shock proteins (HSPs). Seedlings of wheat cvs. HD 2285 and UP 2338 (temperature tolerant) and cvs. HD 2428 and HD 2329 (temperature susceptible) were subjected to a gradual temperature induction (30^0C,1hà33^0C,1hà37^0C,2h) followed by a severe lethal temperature stress (46^0C,3h). The seedlings were allowed to recover for 68 hrs and growth during recovery was taken as a measure to quantify the relative thermotolerance of these cultivars. The temperature induced seedlings of thermotolerant cultivars showed higher recovery growth and greater ability to acquire thermotolerance as revealed by 2,3,5-triphenyl tetrazolium chloride (TTC) test. The higher recovery growth of temperature induced seedlings of tolerant cultivars was associated with the enhanced accumulation and expression of both HMW as well as LMW HSPs. However, unlike the HMW HSPs which showed constitutive expression even under non stress conditions, LMW HSPs were observed to be induced only under high temperature stress.

Key words **: Genetic variability, heat shock proteins, thermotolerance, *Triticum aestivum***

INTRODUCTION

Temperature is a major determinant of plant growth. Temperature affects both the rate of development and the duration of various developmental stages. In addition to speeding up phenological processes, high temperatures have deleterious effects generally on photosynthesis, respiration and reproduction and in particular on seedling survival (Levitt 1980). Adverse effects of high temperature on grain growth and yield of wheat have been well recognized (Mc Donald *et al.* 1983, Prakash *et al*. 2003, 2004, Sharma-Natu *et al.* 2006). High temperature stress (HTS) not only affects the phasic development of the crop but also significantly reduces the grain yield and flour quality of wheat (Slafer and Rawson 1994, Stones and Nicholas 1998, Calderini *et al*. 1999, Blum *et al*. 2001).

Breeding for high temperature tolerance has therefore been an important objective in wheat improvement programmes. A simple and realistic test to identify HTS tolerant genotypes however has been a major limitation in these programmes. Plants overcome high temperature stress by adopting several physiological and biochemical mechanisms such as excess heat dissipation through evaporative cooling, maintaining membrane integrity and synthesis of HSPs. The first mechanism is an avoidance mechanism whereas the other two mechanisms are tolerance mechanisms that are predominantly due to the altered gene expression in response to temperature stress.

*Corresponding author, E-mail: poonam_natu@yahoo.co.in

It is well documented that, plants upon exposure to high temperature synthesize a set of specific stress responsive proteins called heat shock proteins (HSPs) which are involved in altering specific biochemical processes necessary for adaptation (Iba 2002). A number of heat shock proteins have been identified and characterized based on their molecular weight. These are HSP110 (95-110kDa), HSP90 (80-90kDa), HSP70 (63-79kDa), HSP60 (53-62kDa), HSP20 (10-30kDa) and HSP8.5 (Ubiquitin) (Neumann *et al.* 1989). These HSPs function as molecular chaperones that promote the degradation of misfolded proteins, aid in refolding of denatured proteins and prevent them from aggregating and resolubilize the proteins that have already been aggregated (Vierling 1991, Parsell *et al.* 1994, Boston *et al.* 1996, Hartl 1996, Glover and Lindquist 1998, Wang *et al.* 2004). A large number of studies reveal a positive correlation between induction of HSPs with acquisition of thermal tolerance (Lin *et al.* 1984, Ristic *et al*. 1991, Nguyen *et al.* 1994, Pareek *et al.* 1995, Sumesh *et al.* 2007). Further the functional relevance of HSPs has been convincingly demonstrated by their over expression in transgenic plants (Malik *et al.* 1999, Katiyar-Agarwal *et al.* 2003), by down regulation through antisense approach (Lee and Schoffl 1996), and through knock out studies (Burke *et al*. 2000, Hong and Vierling 2000).Genetic variability in thermotolerance has been implicated to be mainly due to differential expression of temperature stress responsive (heat shock) genes (Krishnan *et al*. 1989, Joshi *et al*. 1997). These heat shock genes are predominantly expressed during the sub-lethal induction stress that would bring in required changes in the plant metabolism necessary for withstanding the subsequent severe temperature stress (Lindquist and Craig 1988). Several studies (Kumar *et al*. 1999, Burke 2001, Srikanthbabu *et al.* 2002, Senthil Kumar *et al*. 2003) have clearly shown that genetic variability for stress response could be seen only upon exposure to an induction stress before exposing to the severe stress. Based on our preliminary studies, an efficient screening technique referred as Temperature Induction Response (TIR) technique has been developed to identify thermotolerant lines. According to this technique, the seedlings are exposed to an optimum induction temperature before being subjected to severe challenging temperature and subsequently allowed to recover at room temperature. The surviving seedlings at the end of recovery period are selected as thermotolerant lines (Kumar *et al*. 1999, Srikanthbabu *et al.* 2002, Senthil Kumar *et al*. 2003). TIR is an effective technique for screening for high temperature tolerance and by following this technique we identified thermotolerant wheat cultivars. Further, an attempt was also made to understand whether genetic variability for thermotolerance in wheat cultivars and their abilities to acquire thermotolerance was associated with the differential accumulation and expression of heat shock proteins.

MATERIALS AND METHODS

Seeds of wheat (*Triticum aestivum* L) cvs. HD 2285 and UP 2338 (temperature tolerant) and cvs. HD 2428 and HD 2329 (temperature susceptible) were obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. The pre-imbibed seeds were germinated on moist filter paper in petridishes at 23±1^{0}C. The 41h old germinated seedlings uniform in size (1.5cm) were used to evaluate the genetic variability in temperature stress response of these varieties. In all these experiments, 15 seedlings were used per replication and each treatment had three such replications.

Thermotolerance of wheat cultivars: Genetic variability for thermotolerance was assessed in four cultivars namely HD 2285 and UP 2338 (temperature tolerant) and HD 2428 and HD 2329 (temperature susceptible). The germinated seedlings (41h old) of wheat cultivars were subjected to a gradual temperature induction treatment of 30^{o}C,1h + 33^{o}C,1h + 37^{o}C,2h followed by challenge temperature (severe temperature stress) of 46^{o}C,3h in a temperature controlled incubator. The seedlings were then allowed to recover for 68h at 23±1^{o}C. At the end of recovery period, the seedlings were examined for root and shoot growth. One set of seedlings maintained at 23±1^{o}C (normal temperature) throughout the experimental period served as absolute control. The percent reduction in recovery growth over absolute control was taken as criteria to assess the genetic variability in thermotolerance in these cultivars.

The percent reduction in recovery growth over absolute control was calculated using the formula:

$$\frac{\text{(Recovery growth of absolute control seedlings)} - \text{(Recovery growth of induced seedlings)}}{\text{(Growth of seedlings in absolute control)}} \times 100$$

Recovery growth of the seedlings = Total growth of seedlings survived/Total number of seedlings taken for the experiment.

Cell viability assay: The ability of seedlings to acquire thermotolerance was assessed by examining the extent of TTC reduction (cell viability assay) by induced wheat seedlings (Kalina and Palmer 1986, Krishnan *et al*. 1989). Uniformly germinated 15 seedlings of wheat cultivars were subjected to different durations of challenge temperature (severe temperature) stress (46°C-1h, 2h, 3h) with prior induction. At the end of stress, the roots of seedlings (0.1g) were excised and transferred to 5ml of 0.1% TTC (prepared in 50mM sodium phosphate buffer, pH 7.4) and incubated for 18-20h in dark. After incubation the root segments were removed from TTC solution, rinsed with distilled water and placed in a beaker containing 5ml of methoxyethanol. The samples were boiled to total dryness and resuspended in 5ml of methoxyethanol. The colour recovered in methoxyethanol was measured at 485nm. Same quantity of seedlings maintained at 23±1°C served as absolute control. The percent TTC reduction of absolute control in induced seedlings was taken as a measure to quantify the acquired thermotolerance in contrasting wheat cultivars.

Time-lapse experiment: Seedlings (41h old) of four wheat cultivars were subjected to a gradual temperature induction treatment of 30°C,1h + 33°C,1h + 37°C,2h. After the treatment the seedlings were transferred to 23±1°C for varying durations (1h, 2h, 4h and 6h). After these time lapses the seedlings of four wheat cultivars were subjected to challenge temperature of 46°C,3h. These seedlings were allowed to recover for 68h at 23±1°C. At the end of recovery the percent reduction in recovery growth over directly induced (0h time lapse) was calculated.

Western analysis: Proteins were extracted from root tissues (1g) of induced and control seedlings by rapid homogenization in 0.1M Tris-HCl buffer (pH7.8) containing 0.02M sodium sulphite, 5mM mercaptoethanol, 5mM benzamidine, PVPP (4%) and 1mM PMSF. Protein samples (100 mg/lane) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electro blotted onto a nitrocellulose membrane according to Khyse-Anderson (1984). Blots were blocked using 4% casein in phosphate buffer saline (PBS) for 12-16h at 4°C. Later, they were probed with primary antibody (HSP104, HSP90 and HSP21) for 2h at room temperature. For detecting HSP104 and HSP90, antibodies raised in rabbits using rice HSP90 and HSP104 were employed at a dilution of 1:5000 (Pareek *et al*. 1995). For detecting HSP21 the polyclonal antibody raised against pea HSP21 was used at 1:1000 dilution (Downs *et al*. 1998). The bands in western blots were visualized after incubating with alkaline phosphatase conjugated anti-rabbit IgG for 1h at room temperature. The blots were developed by using nitrobluetetrazolium (NBT) and 5-bromo-4chloro-3indolyl-phosphate (BCIP) as a substrate (Engvall and Perlmann 1972).

Northern analysis: Total RNA was isolated from the roots (1.5g-2.0g) of induced and control seedlings following the modified sucrose-SDS method (Datta *et al*.1989). RNA samples (10mg/lane) were separated on formaldehyde denaturing agarose gel (0.8%), transferred to a hybond N^+ membrane (Sambrook *et al*. 1989), UV-crosslinked and hybridized with cDNA probes of LMW and HMW *hsps*. Appropriate cDNA inserts (40-50ng) were labeled using ^{32}PdATP using Gibco-BRL random labeling module. Unincorporated nucleotides present in the reaction were removed by passing through a G-50 Sephadex column. Membranes were prehybridized and hybridized (with probe) in Church-Gilbert buffer (0.5M sodium phosphate buffer, pH7.2 containing 1mM EDTA and 7% SDS) at 55°C for 16-20h. Membranes were washed in 4xSSC, 0.1%SDS at 55°C for 30min, 2xSSC, 0.1%SDS at 55°C for 30min and again at 2xSSC without SDS at room temperature. After the washes membranes were exposed to Kodak X-ray film following standard procedures (Sambrook *et al*. 1989).

RESULTS

Thermotolerance of wheat cultivars: The induction response of four wheat cultivars, differing in thermotolerance to challenging temperature (46°C, 3h) was examined. The data indicated that temperature

induced seedlings of tolerant wheat cultivars UP2338 (T1) and HD 2285 (T2) showed 37% and 42% percent reduction in recovery growth over absolute control, respectively when exposed to 46^{0}C for 3h. On the other hand, susceptible cultivar HD2428 (S1) showed 65% reduction and HD2329 (S2) 68% reduction in growth over absolute control (Fig.1). It suggests that upon induction the thermotolerant cultivars performed better than their sensitive counterparts.

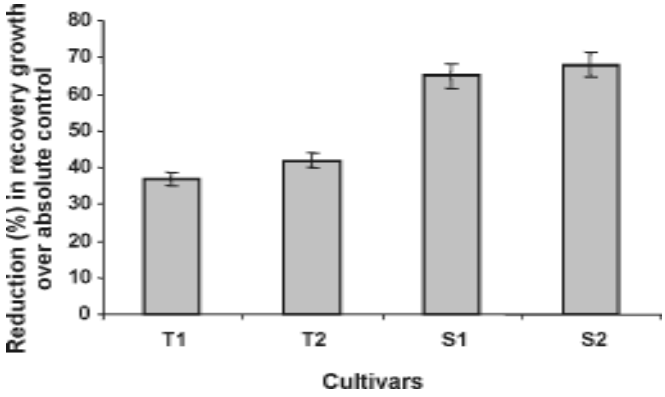

Fig. 1. Induction response of wheat cultivars differing in thermotolerance (T1: UP2338; T2: HD2285; S1: HD2428; S2:HD2329)

Cell viability assay: The ability of seedlings upon induction to acquire thermotolerance was assessed by cell viability assay in the induced seedlings of contrasting wheat cultivars. At all levels of severe temperature stress (46^{0}C-1h, 2h, 3h) the thermotolerant cultivars showed relatively higher percent of cell survival compared to the susceptible cultivars. Even at severe stress (46^{0}C, 3h) the percent TTC reduction was almost 55-60% of the control plants in thermotolerant cultivars (Fig. 2).

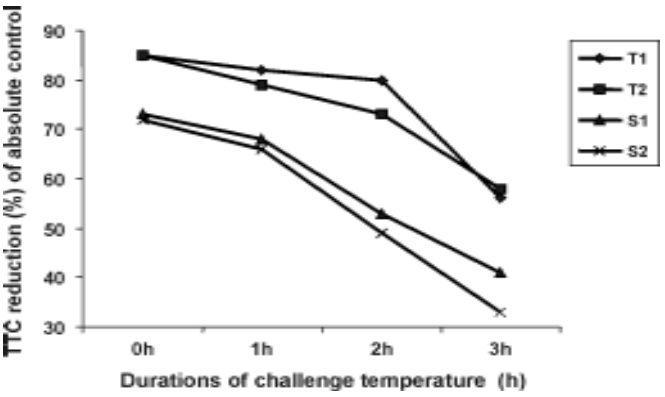

Fig. 2. Maintenance of cell viability in the temperature induced seedlings of wheat cultivars at different durations of challenge temperature (T1: UP2338; T2: HD2285; S1: HD2428; S2:HD2329)

Time-lapse experiment: The induction response of induced seedlings may possibly decrease when transferred to ambient temperature before subjecting to challenge temperature (Kumar *et al*. 1999). This phenomenon was examined by exposing the induced seedlings to different levels of time lapse (1h, 2h, 4h and 6h) before exposing to severe temperature stress (46^{0}C, 3h). The thermotolerant cultivars showed less percent reduction in recovery growth compared to susceptible cultivars at all the different durations of time lapse (Fig. 3). When the time lapse given was 6h between the induction treatment and exposure to challenge temperature, the susceptible cultivars did not survive where as the thermotolerant cultivars showed maximum reduction in recovery growth but still survived.

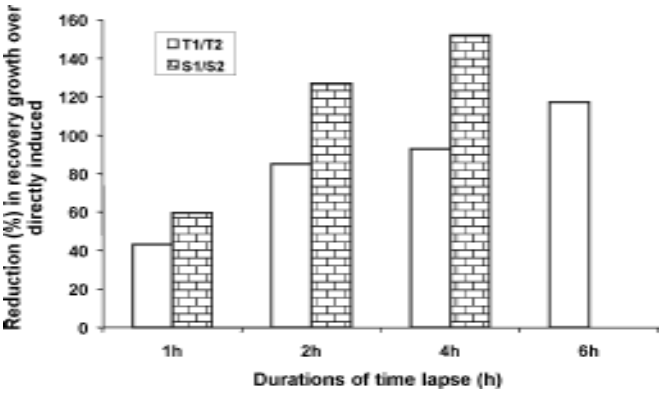

Fig. 3. Response of wheat seedlings subjected to different durations of time lapse after induction (T1: UP2338; T2: HD2285; S1: HD2428; S2:HD2329)

Expression studies: Variation in thermotolerance among different wheat cultivars could be due to differential expression of temperature stress responsive (heat shock) genes. Since optimum expression is seen only upon induction, the variability in thermotolerance was examined through the accumulation and expression of heat shock proteins (HSPs) in the induced seedlings of these four wheat cultivars (Fig. 4&5) The results indicated that thermotolerant cultivars showed enhanced expression of *hsp*70 and LMW*hsp* (18-20 Kda)

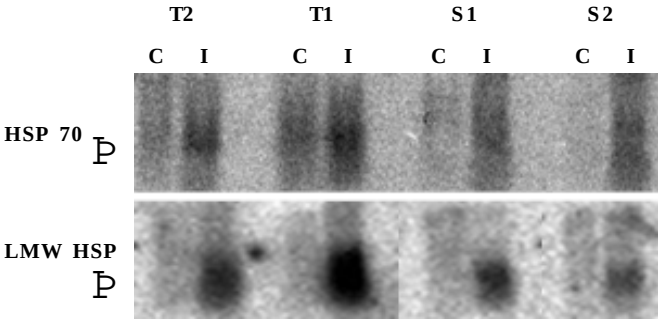

Fig. 4. Northern blots showing the expression levels of heat shock protein transcripts in the induced (I) and control (C) seedlings of thermotolerant and susceptible cultivars (T1: UP2338; T2: HD2285; S1: HD2428; S2:HD2329)

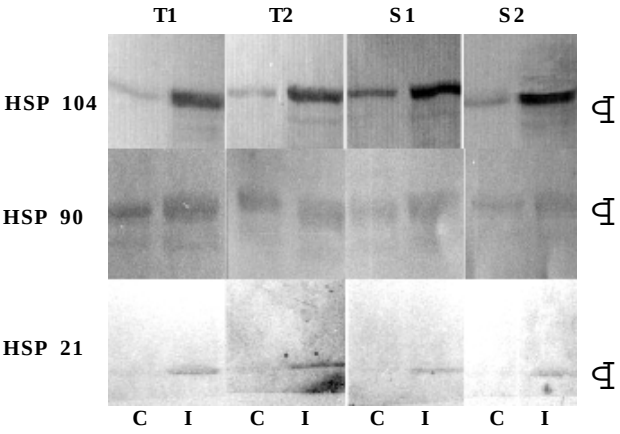

Fig. 5. Western blots showing the accumulation levels of heat shock proteins in the induced (I) and control (C) seedlings of thermotolerant and susceptible cultivars (T1: UP2338; T2: HD2285; S1: HD2428; S2:HD2329)

compared to the susceptible cultivars. However, constitutive expression of *hsp*70 was observed even in the control seedlings of tolerant cultivars whereas in case of susceptible cultivars it was inducible (Fig. 4) On the contrary, LMW*hsp* expression was highly inducible in all the four cultivars, level of expression being substantially high in case of tolerant cultivars than susceptible cultivars (Fig. 4). There was also higher accumulation of HSP104, HSP90 and HSP21 in the induced seedlings of thermotolerant cultivars compared to susceptible cultivars (Fig. 5). These results suggest a positive correlation between level of thermotolerance and accumulation and expression of HSPs in the wheat cultivars.

DISCUSSION

Adaptation to temperature stress is a consequence of expression of specific stress responsive genes, which brings about altered metabolism for acclimation (Iba K 2002). These temperature stress responsive (heat shock) genes are expressed during the early stress periods. Plants develop the ability to withstand otherwise lethal temperature stress on prior exposure to sub-lethal induction stress. This phenomenon is common for all stresses and has been termed as acquired tolerance. It is during this induction phase, several physiological and biochemical changes take place that impart tolerance to subsequent lethal stress (Vierling 1991). In the present study also genetic variability for thermotolerance in wheat cultivars could be seen only after exposure to gradual temperature induction treatment prior to severe lethal stress. The induced seedlings of thermotolerant cultivars (UP2338 and HD2285) showed significantly higher recovery growth compared to susceptible cultivars (HD2428 and HD2329). Cultivar UP2338 (T1) showed the least reduction in recovery growth over absolute control where as HD2329 (S2) exhibited maximum reduction in recovery growth (Fig. 1). Blumenthal *et al.* (1990) also reported that the coleoptiles of field grown, temperature resistant wheat cultivar exposed to a high temperature exhibited better growth compared to susceptible cultivar. Under field grown conditions the seedlings are always exposed to a gradual increase in temperature but not directly to higher temperature thereby suggesting that genetic variability for thermotolerance can be observed only after exposure to an induction temperature. Several other studies (Lin *et al* 1984, Krishnan *et al.* 1989, Kumar *et al.* 1999, Srikanthbabu *et al.* 2002, Senthil-Kumar *et al.* 2003) have revealed enhanced thermotolerance of plants following exposure to a sub-lethal induction stress.

The ability of seedlings to adapt to high temperature stress following prior induction treatment was assessed using a cell viability assay that involved the extent of TTC reduction by induced seedlings. TTC reduction by seedlings is an indicator of mitochondrial functioning which in turn reflects the cell viability (Kalina and Palmer 1986). Our studies revealed that induced seedlings of tolerant cultivars (UP2338 and HD2285) showed

significantly higher cell viability at all the temperature stress treatments (46^0C-1h, 2h, 3h) compared to the susceptible cultivars (Fig. 2). Even at severe stress level (46^0C, 3h) the induced seedlings of tolerant cultivars were able to maintain considerably higher cell viability than the induced seedlings of susceptible cultivars. A positive correlation between cell viability and acquisition of thermotolerance has also been reported earlier (Chen *et al*. 1982, Krishnan *et al.* 1989, Porter *et al*. 1994).

One of the mechanisms for the acquired thermotolerance upon exposure to induction treatment is the upregulation of unique temperature responsive (heat shock) proteins. These upregulated proteins enhance the expression of specific stress proteins that in turn impart tolerance to the severe lethal stress (Vierling 1991). In the present study after the induction stress the seedlings were shifted to normal temperature for varying durations (1h to 6h). After the time gap the seedlings were exposed to severe lethal stress and allowed to recover. The thermotolerant cultivars showed better growth compared to the susceptible cultivars at all the different durations of time lapse (Fig. 3). However the induction response declined with increase in time lapse after induction. Interestingly, in tolerant cultivars the induction response was still seen even after a considerable time lapse (6h) compared to susceptible cultivars This could be because the thermotolerant cultivars might be able to retain HSPs for longer time even when the stress was alleviated and hence perform better under severe lethal stress. The same may not be true in case of susceptible cultivars.

The enhanced recovery growth of the induced seedlings of thermotolerant cultivars and their ability to acquire thermotolerance is due to the synthesis of heat shock proteins (HSPs), which impart thermotolerance (Nagao *et al*. 1990, Vierling 1991). The HSPs are known to be synthesized in crop plants exposed to heat stress in both field and controlled environments (Harrington *et al*. 1994). These proteins are known to function as molecular chaperones that aid to stabilize the cellular structures in response to elevated temperatures or function to repair the damage caused by high temperature stress, thereby playing an important role in cell protection, survival and recovery (Parsell and Lindquist 1993, Vishwanathan and Khanna-Chopra 1996). A large number of studies reveal a positive correlation between induction of HSPs with acquisition of thermal tolerance (Ristic *et al.* 1991, Howarth and Skot 1994, Yeh *et al*. 1994, Lee and Schoffl 1996, Hong and Vierling 2000). The enhanced expression and accumulation of these HMW and LMW HSPs in response to high temperature stress has been observed in rice (Pareek *et al*. 1995), sunflower (Kumar *et al*. 1999, Senthil Kumar *et al*. 2003) and garden peas (Srikanthbabu *et al.* 2002).

In the present investigation enhanced expression of *hsp*70 and LMW*hsp* (Fig. 4) and greater accumulation of HSP104, HSP90 and HSP21 (Fig.5) in the induced seedlings of thermotolerant wheat cultivars compared to susceptible cultivars was observed. A high constitutive accumulation of HSP90 and HSP 104 and expression of *hsp*70 transcripts was seen in the control seedlings of thermotolerant wheat cultivars (Fig. 4 &5). It has been reported that constitutive expression of low levels of HMW HSPs is an added advantage to the plants, conferring resistance to both high and low temperature stress (Lee *et al.* 1995, Prandl *et al*. 1998, Queitsch *et al*. 2000). Queitsch *et al*. (2000) has reported that transgenic *Arabidopsis* plants constitutively expressing HSP101 (at 22^oC) tolerated sudden shifts to extreme temperatures than vector controls (expressing HSP101 only at 38^oC).Thermotolerance for grain growth in wheat has been reported to be associated with higher activity of soluble starch synthase (SSS) at elevated temperature and higher level of HSP 100 (Sumesh *et al.* 2007). It is not known whether heat induced HSPs are functionally different from developmentally controlled HSPs that accumulate in the absence of heat stress (Maestri *et al*. 2002).Nevertheless, HMW HSPs have been shown to play a definite role in thermotolerance (Pareek *et al*. 1995, Katiyar-Agarwal *et al.* 2003). Transgenic rice lines overexpressing *A. thaliana* HSP 101 (*Athsp* 101) showed significantly better growth performance in the recovery phase following stress (Katiyar-Agarwal *et al*. 2003). Schroda *et al*. (1999) reported that a chloroplast targeted heat shock protein 70 (HSP 70) contributes to photoprotection and repair of photosystem II during and after heat stress. HSP 90 is also suspected to function as chaperone, although there is little evidence related to temperature stress in plants (Iba *et al*. 2002).

Unlike the HMW HSPs (HSP70, HSP90 and HSP104), the constitutive accumulation and expression of LMWHSPs (Fig.4 & 5) was not observed in the control seedlings of wheat cultivars and these were expressed only when induced. Further, tolerant cultivars showed significantly higher levels of induction of these LMW HSPs compared to susceptible cultivars. The results suggests a positive correlation between level of thermotolerance and accumulation and expression of these LMW HSPs among the wheat cultivars in response to induction. LMW HSPs are very efficient at binding denatured proteins and prevent irreversible protein aggregation and insolubilization, thereby increasing the stress resistance of cells (Basha *et al.* 2004).The pea HSP 18.1 works to prevent the aggregation of proteins denatured by heat and reactivate them (Lee *et al*. 1997). The role of LMW HSPs in acquisition of thermotolerance has been reported by several workers (Joshi *et al*. 1997, Heckathorn *et al*. 1998, Malik *et al*. 1999). The functional significance of a small methionine rich chloroplastic HSP in photosynthetic (PS II) thermotolerance has been demonstrated by Heckathorn *et al*. (1998). Joshi *et al*.(1997) reported that acquired thermotolerance in wheat seedlings is linked with 26 Kda plastid localized heat shock protein. Studies by Malik *et al*. (1999) indicated that thermotolerance can be altered (increased/decreased) by modifying the expression of HSP 17.7 in carrot.

The above results indicated that significant genetic variability in thermotolerance of wheat cultivars could be seen only upon exposure to an optimum induction temperature prior to severe temperature stress. Induced seedlings of temperature tolerant cultivars showed significantly higher recovery response compared to the susceptible cultivars, suggesting that this technique can be adapted to screen wheat germplasm and segregating populations. Further, the differential thermotolerance of wheat cultivars was found to be related with accumulation and expression of both HMW and LMW HSPs. Unlike the HMW HSPs which showed constitutive expression even under non stress conditions, LMW HSPs were found to be induced only under high temperature stress. The results suggest that LMW HSPs can be used as biological markers for identifying high temperature tolerant cultivars under severe heat stress conditions.

ACKNOWLEDGEMENTS

Poonam Natu thanks Dr. M. C. Ghildiyal, Principal Scientist, Division of Plant Physiology, IARI, New Delhi for the support and encouragement.

REFERENCES

Basha, E., Lee, G.J., Demeler, B. and Vierling, E. (2004). Chaperone activity of cytosolic small heat shock proteins from wheat. *Eur. J. Biochem.* **271:** 1426-1436.

Blum, A., Kluera, N. and Nguyen, H.T. (2001). Wheat cellular thermotolerance is related to yield under heat stress. *Euphytica* **117:** 117-123.

Blumenthal, C, Bekes, F., Wrigely, C.W. and Borlow, E.W.R. (1990). The acquisition and maintenance of thermotolerance in Australian wheats. *Aust. J. Plant Physiol.* **17:** 37-47.

Boston, R.S., Viitanen, P.V. and Vierling, E. (1996). Molecular chaperones and protein folding in plants. *Plant Mol. Biol.* **32:** 191-222.

Burke (2001). Identification of genetic diversity and mutations in higher plant acquired thermotolerance. *Physiol. Plant.* **112:** 167-170.

Burke, J.J., Mahony, P.J.O. and Oliver, M.J. (2000). Isolation of *Arabidopsis* mutants lacking components of acquired thermotolerance. *Plant Physiol.* **123:** 575-587.

Calderini, D.F., Abeledo, L.G., Savin, R. and Slafer, G.A. (1999). Final grain weight in wheat as affected by short periods of high temperature during pre and post anthesis under field conditions. *Aust. J. Plant Physiol.* **26:** 453-458.

Chen, H.H., Shen, Z.Y. and Li, P.H. (1982). Adaptability of crop plants to high temperature stress. *Crop Sci*. **22:** 719-725.

Datta, K., Schmidt, A. and Marcus, A. (1989). Characterization of two soybean repetitive proline rich proteins and a cognate cDNA from germinated axes. *The Plant Cell* **1:** 945- 952.

Downs, C.A., Heckathorn, S.A., Bryan, K.J. and Coleman, S.J. (1998). The methionine-rich low-molecular weight heat shock protein: An evolutionary conservation and accumulation in relation to thermotolerance. *Amer. J. Bot.* **85:** 175-183.

Engvall, E. and Perlmann, P.J. (1972). Enzyme linked immunosorbent assay (ELISA) III Quantification of specific antibodies by enzyme labelled anti-immunoglobulin in antigen-coated tubes. *J. Immunol*. **109:** 129-135.

Glover, J.R. and Lindquist, S. (1998). HSP 104, HSP 70 and HSP 40 A novel chaperone system that rescues previously aggregated proteins. *Cell* **94:** 73-82.

Harrington, H.M., Dash, S., Dharmasiri, N. and Dharmasiri, S. (1994). Heat shock proteins: a search for functions. *Aust. J. Plant Physiol*. **21:** 843-855.

Hartl, F.U. (1996). Molecular chaperones in cellular protein folding. *Nature* **381:** 571-580.

Heckathorn, S.A., Downs, C.A., Sharkey,T.D. and Coleman, J.S. (1998). The small methionine rich chloroplast heat shock protein protects photosystem II electron transport during heat stress. *Plant Physiol*. **116:** 439-444.

Hong, S.W. and Vierling, E. (2000). Mutants of *Arabidopsis thaliana* defective in the acquisition of tolerance to high temperatures stress. *Proc. Natl. Acad. Sci*. **97:** 4392-4397.

Howarth, C.J. and Skot, K.P. (1994). Detailed characterization of heat shock protein synthesis and induced thermotolerance in seedlings of *Sorghum bicolor* L. *J. Exp. Bot.* **45:** 1353-1363.

Iba, K. (2002). Acclimation response to temperature stress in higher plants: Approaches of gene engineering for temperature tolerance. *Annu. Rev. Plant Biol*. **53:** 225-245.

Joshi, C.P., Kluge, M., Morton, R.K. and Nguyen, H.T. (1997). Expression of a unique plastid- localized heat shock protein is genetically linked to acquired thermotolerance in wheat. *Theor. Appl. Genet*. **95:** 834-841.

Kalina, M. and Palmer, J.M. (1986). The reduction of tetrazolium salts by mitochondria. *Histochem*. **14:** 366-374.

Katiyar-Agarwal, S.K., Agarwal, M. and Grover, A. (2003). Heat-tolerant basmati rice engineered by over expression of *hsp 101*. *Plant Mol. Biol.* **51:** 677-686.

Khyse-Anderson, 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.

Krishnan, M., Nguyen, H.T. and Burke, J.J. (1989). Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol*. **90:** 140-145.

Kumar,G., Krishnaprasad, B.T., Savitha, M., Gopalkrishna, R., Mukhopadhyay, K., Ramamohan, G. and Udayakumar, M.(1999). Enhanced expression of heat shock proteins in thermotolerant lines of sunflower and their progenies selected on the basis of temperature induction response. *Theor. Appl. Genet*. **99:** 359-367.

Lee, G.J., Roseman, A.M., Saibil, H.R. and Vierling, E. (1997). A small heat shock protein stably binds heat denatured model substrates and can maintain a substrate in a folding competent state. *EMBO J.* **16:** 659-671.

Lee, J.H., Hubel, A. and Schoffl, F. (1995). Derepression of the activity of genetically engineered heat shock factor causes constitutive synthesis of heat shock proteins and increased thermotolerance in transgenic *Arabidopsis*. *Plant J*. **8:** 603-612.

Lee, J.H. and Schoffl, F. (1996). An HSP 70 antisense gene affects the expression of HSP 70/HSC 70, the regulation of HSF and the acquisition of thermotolerance in transgenic *Arabidopsis thaliana*. *Mol .Genl. Genet*. **252:** 11-19.

Levitt, J. (1980). Responses of Plants to Environmental Stresses, Chilling, Freezing and High Temperature Stress. Academic Press, New York

Lin, C.Y., Roberts, J.K. and Key, J.L. (1984). Acquisition of thermotolerance in soybean seedlings synthesis and accumulation of heat shock proteins and their cellular localization. *Plant Physiol*. **74:** 152-160.

Lindquist ,S. and Craig, E.A. (1988). The heat shock proteins. *Annu. Rev. Genet.* **22:** 631-677.

Maestri, E., Klueva, N., Perrotta, C., Gulli, M., Nguyen, H.T. and Marmiroli, N. (2002). Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Mol. Biol*. **48:** 667-681.

Malik, M.K., Slovin, J.P., Hwang, C.H. and Zimmerman, J.L. (1999). Modified expression of a carrot small heat shock protein gene HSP 17.7 results in increased or decreased thermotolerance. *Plant J*. **20:** 1-11.

Mc Donald, G.K., Sutton, B.G. and Ellison, F.W. (1983). The effects of time of sowing on the grain yield of irrigated wheat in the Namoi valley, New South Wales. *Aust. J. Agri. Res*. **34:** 229-240.

Nagao, R.T., Kimpel, J.A. and Key, J.L. (1990). Molecular and cellular biology of heat shock response. *Adv. Genet*. **28:** 235-274.

Neumann, D., Nover, L., Parthier, B., Reiger, R., Scharf, K.D., Wollgelhn, R. and Zur Neiden, U. (1989). Heat shock and other stress response systems of plants. *Biol. Zentralbl*. **108:** 1-155.

Nguyen, H.T., Joshi, C.P., Klueva, N., Weng, J., Hendershot, K.L. and Blum, A. (1994). Heat shock response and expression of heat shock proteins in wheat under diurnal heat stress and field conditions. *Aust. J.Plant Physiol*. **21:** 857-867.

Pareek, A., Singla, S.L. and Grover, A. (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:** 293-301.

Parsell, D.A., Kowal, A.S., Singer, M.A. and Lindquist, S. (1994). Protein disaggregation mediated by heat shock protein HSP 104. *Nature* **372:** 475-478.

Parsell, D.A. and Lindquist, S. (1993). The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genet*. **27:** 437-496.

Porter, D.R., Nguyen, H.T. and Burke, J.J. (1994). Quantifying acquired thermal tolerance in winter wheat. *Crop Sci*. **34:** 1686-1689.

Prakash, P., Sharma-Natu, P. and Ghildiyal, M.C. (2003). High temperature effect on starch synthase activity in relation to grain growth in wheat cultivars. *Indian J. Plant Physiol.* (Spl. issue) **8:** 390-398.

Prakash, P., Sharma-Natu, P. and Ghildiyal, M.C. (2004). Effect of different temperature on starch synthase activity in excised grain of wheat cultivars. *Indian J. Exp. Biol*. **42:** 227-230.

Prandl, R., Hinderhofer, K., Eggers-Schumacher, G. and Schoffl, F. (1998). HSF 3, a new heat shock factor from *Arabidopsis thaliana*, derepresses the heat shock response and confers thermotolerance when overexpressed in transgenic plants. *Mol. Gen. Genet*. **258:** 269-278.

Queitsch, C., Hong, S.W., Vierling, E. and Lindquist. S. (2000). Heat shock protein 101 plays a crucial role in thermotolerance in *Arabidopsis. The Plant Cell* **12:** 479-492.

Ristic, Z., Gifford, D.J. and Cars, D.D. (1991). Heat shock proteins in two lines of *Zea mays* L. that differs in drought and heat resistance. *Plant Physiol*. **97:** 1430-1434.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning – A Laboratory Manual. Cold Spring Harbor Laboratory Press ,New York.

Schroda, M., Vallon, D., Wollmann , F.A. and Beck, C.F. (1999). A chloroplast targeted heat shock protein 70 (HSP 70) contributes to photo protection and repair of photosystem II during and after photoinhibition. *Plant Cell* **11:** 1165-1178.

Senthil-Kumar, M., Srikanthbabu, V., Mohan Raju, B., Ganeshkumar, Shivaprakash, N. and Udayakumar, M. (2003). Screening of inbred lines to develop thermotolerant sunflower hybrid through temperature induction response (TIR) technique: A novel approach by exploiting residual variability. *J. Exp. Bot*. **54:** 2569-2578.

Sharma-Natu, P., Sumesh, K.V., Lohot, V.D. and Ghildiyal, M.C. (2006). High temperature effect on grain growth in wheat cultivars: An evaluation of responses. *Indian J. Plant Physiol*. **11:** 239-245.

Slafer, G.A. and Rawson, H.M. (1994). Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. *Aust. J. Plant Physiol*. **21:** 393-426.

Srikanthbabu, V., Ganeshkumar, Krishnaprasad, B.T., Gopalakrishna, R., Savitha, M. and Udayakumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *J. Plant Physiol*. **159:** 535-545.

Stones, P.J. and Nicholas, M.E. (1998). The effect of duration of heat stress during grain filling on two wheat varieties differing in heat tolerance: grain growth and fractional protein accumulation. *Aust. J. Plant Physiol*. **26:** 453-458.

Sumesh, K.V., Sharma-Natu, P. and Ghildiyal, M.C. (2007). Starch synthase activity and heat shock protein in relation to thermal tolerance of developing wheat grains. *Biol. Plant.* (in press).

Vierling, E. (1991). The roles of heat shock protein in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **42:** 579-620.

Vishwanathan, C. and Khanna-Chopra, R. (1996). Heat shock proteins- Role in thermotolerance of crop plants. *Curr. Sci*. **71:** 275-284.

Wang, W., Vinocur, B., Shoseyov, O. and Altman, A. (2004). Role of plant heat shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci*. **9:** 244-252.

Yeh, K.W., Jinn, T.L., Yeh, C.H., Chen, Y.M. and Lin, C.Y. (1994). Plant low molecular mass heat shock proteins: Their relationship to the acquisition of thermotolerance in plants. *Biotechnol. Appl. Biochem*. **19:** 41-49.