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MOLECULAR BIOLOGY AND PHYSIOLOGY

Temperature Induction Response as a Screening Technique for Selecting High Temperature-Tolerant Cotton Lines

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ABSTRACT

Cotton is cultivated in a wide range of environments from tropical to subtropical regions. In India, approximately 70%of the total land area covered by cotton is grown under rain-fed conditions where cotton frequently experiences drought stress along with other stresses, e.g.,high temperature and high salinity. There is a need to develop a technique to screen a large number of genotypes for high temperature tolerance. In this study, a screening protocol was developed based on the principle of "acquired tolerance" in which exposure of seedlings to a sublethal level of specific stress is used to induce tolerance to a subsequent lethal level of stress. After adapting this temperature induction response (TIR) technique to cotton, several species and varieties were screened for thermotolerance. Among the tested entries, Old World cotton species showed better thermotolerance than New World cotton species. Among 36 diverse *Gossypium hirsutum* germplasm lines, significant variation in acquired thermotolerance was seen. Thermotolerant genotype *G. hirsutum* (H-28), identified by the TIR technique, demonstrated increased cell viability and protein synthesis capacity during alleviation from high temperature stress. Results suggested that TIR is a robust and powerful technique and can be used to screen breeding lines or germplasms to identify thermotolerant lines.

Global surface temperature has increased by approximately 0.6°C since the late 19th century and is projected to increase anywhere from 1.4 to 5.8°C by the end of the current century (Houghton et al.,2001) with a decrease in the diurnal temperature range (Dai et el.,2001). Temperature affects a broad spectrum of cellular components and metabolism, and temperature extremes impose stresses of variable severity that depend on the rate of temperature change, intensity, and duration. Plants overcome high temperature stress by adopting several physiological and biochemical mechanisms such as excess heat dissipation through evaporative cooling, maintenance of membrane integrity, and synthesis of heat-shock proteins (HSPs). The first mechanism is an avoidance mechanism, whereas the other two are tolerance mechanisms.

Plants adapt to high temperature stress by inherent basal level tolerance as well as acquired tolerance to severe temperature stress. Acquired thermotolerance is quite rapid and has been shown to be induced during cell acclimation to moderately high temperature periods (Hikosaka et al., 2006; Larkindale et al., 2005; Massie et al., 2003). The ability to withstand and to acclimate to supraoptimal temperatures results from both prevention of heat damage and repair of heat-sensitive components (SenthilKumar et al., 2006). Seedlings exposed to a sublethal temperature prior to challenge with severe temperature have better growth recovery than those seedlings challenged directly to severe temperature stress, and also demonstrate the ability to accumulate higher levels of low- and high-molecular-weight HSPs such as HSP18.1, HSP90, and HSP104 (Kumar et al., 1999; Srikanthbabu et al., 2002). Previous studies have shown that many of these HSPs function as molecular chaperones in maintaining homeostasis of protein folding and are thought to be responsible for the acquisition of thermotolerance (Parsell and Lindquist, 1993; Sung et al., 2003; Vierling, 1991). The number of small HSPs (sHSPs) in heat-tolerant wheat cultivars was higher than in heat-susceptible wheat cultivars. In addition, some of the sHSPs were specific to individual cultivars and detected when the temperature was raised from 37°C to 50°C (Yildiz and Terzi, 2008).A point mutation in the HSP101 gene of *Arabidopsis* abolished not only basal thermotolerance but blocked or reduced acquired thermotolerance in whole plants (Hong and Vierling, 2000). Similarly in maize, gene knockouts

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of HSP101 led to the development of plants that were found to be defective in basal and acquired thermotolerance (Nieto-Sotelo et al., 2002).

Many earlier studies have demonstrated that genetic variability for high temperature tolerance is noticed only upon induction treatment prior to severe stress (Burke, 2001; Krishnanet al., 1989; Kumar et al., 1999; Srikanthbabu et al., 2002). It is opined that the stress-signaling pathway is triggered during induction stress and induces the expression of an array of stress-responsive genes. The resulting gene products alter several physiological and biochemical processes leading to stress tolerance. (Jayaprakash et al., 1998; Kumar et al., 1999; Uma et al., 1995).

Acquired tolerance for a specific abiotic stress has been shown to give cross protection for other stresses such as salinity, chilling temperatures, and drought. Studies in finger millet and sunflower have shown that the genetic variability to withstand severe stress can be seen only in seedlings pre-exposed to subsevere concentrations of NaCl and/or polyethylene glycol prior to severe stress challenge (Uma et al., 1995 and Al- Ouda, 1999, respectively).

Cotton plants suffering from high temperature stress exhibit reductions in plant growth and development (Reddy et al., 1991, 1993, 1995) as well as reductions in pollen germination and pollen tube growth (Barrow, 1983; Kakani et al., 2005; Suy, 1979). In addition to the other approaches to avoid temperature stress, e.g., earlier planting dates, there is a vital need to develop a reliable and high-throughput screening technique to assess the genetic variability for temperature tolerance and to identify highly tolerant donor genotypes for use in plant breeding programs.

MATERIAL AND METHODS

Standardization of Optimum Induction Temperature and Challenging High Temperature for Cotton Seedlings. Induction temperature is the temperature pretreatment for a specific time duration (in hours) that is required to improve growth recovery of seedlings significantly in cultivated *Gossypium hirsutum* L. subsequently exposed to a challenging temperature. Challenging temperature is the temperature treatment for a specific time duration (in hours) required to cause more than a 90% reduction in seedling survival in noninduced seedlings of *G. hirsutum*.

Uniform seedlings of cotton (*G. hirsutum*) variety Sahana with 1 to 1.5-cm radicle length were subjected to different challenging temperatures for specific time durations (46°C for 3h, 47°C for 3h, 48°C for 3h, 48°C for 4h) without prior induction or with gradual temperature induction where the temperature was raised from 28 to 40°C over 4 h (at 0.05°C/min) before challenging them with high temperatures. These seedlings were immediately allowed to recover in an incubator at 30°C and 60% relative humidity (RH) for 48 h (Fig. 1).At the end of the recovery period, the number of seedlings that survived was recorded and percent mortality was calculated. Further, total length of surviving seedlings was recorded and percent reduction of recovery growth compared to absolute control was computed. For all experiments, three replications were maintained per treatment and each replication had 20 seedlings.

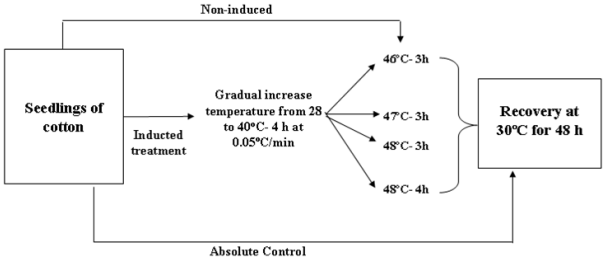

Figure1.The general protocol to study high temperature stress through the temperature induction response technique.

Temperature Stress Response in Cotton Species. Uniform seedlings from 20 genotypes belonging to four cotton species (Table 1) were adopted to a standardized induction protocol (temperature induction response, TIR) where temperature was raised from 28 to 40ºC for 4 h gradually, and then challenged by a lethal temperature of 47ºC for 3 h, then allowed to recover for 48 h at 30ºC with 60% RH. At the end of the recovery period, percent mortality and percent reduction in recovery growth was recorded.

Genetic Variability in *G. hirsutum* for Thermotolerance. Adopting the standardized induction protocol, seedlings of 36 genotypes (Table 2) belonging to *G. hirsutum* were challenged with the defined lethal temperature (47°C for 3h) and allowed to recover for 48 h at 30ºC and 60% RH. The recovery growth was measured 48 h later. By using normal Z distribution, the genotypes were classified into susceptible, moderately tolerant, and tolerant genotypes based on actual growth during recovery and percent reduction in recovery growth over absolute control.

Table 1. Cotton lines used in this study.

Cotton species	Number of lines	Common names	IC number
G.arboreum	2	A-2[z]	(1)
		A-3	(1)
G.herbaceum	9	h-1	(1)
		h-2	(1)
		h-4	(1)
		h-5	(1)
		h-8	(1)
		h-9	(1)
		h-10	(1)
		h-11	(1)
		h-12	(1)
G.barbadense	3	B-1	EC 344223
		B-2	(1)
		B-3	(1)
G.hirsutum	6	H-1	IC 357528
		H-2	IC 357536
		H-5	IC357538
		H-19	IC 357328
		H-20,	(1)
		H-25	(1)

[z] The coding of lines is by the Central Institute for Cotton Research (CICR), Nagpur, India.

(1) Selected stabilized lines maintained At CICR, Nagpur, India.

Cell Viability of the Seedlings During Recovery From High Temperature Stress. As an additional measure to quantify genotypic variability, in the context of survival after exposure to challenging temperature, the level of cell viability in the seedling tissue was assessed using the method developed by Gaff and Okong'o-Ogola (1971).Seedlings of a *Gossypium* species classified as tolerant (*G.hirsutum*, H-28) and as susceptible (*G. barbadense* L., B-4) were subjected to high temperature stress as explained above in the TIR protocol. Subsequently, seedlings were allowed to recover at 30°C with 60% RH. After 3 h of recovery, 20 seedlings were incubated in Evan's blue solution (0.1 %) for 2 h and then washed in distilled water (DW) to remove the excess dye. The dye from the seedlings was extracted in absolute alcohol maintained at 50°C for 15 min and the absorbance was measured at 600nm using a UV-visible spectrophotometer (UV-2450, Shimadzu

Table 2. Cotton lines belonging to *G.hirsutum* species used in this study.

Serial #	Common name	IC number
1	H-1[z]	IC 357528
2	H-2	IC 357536
3	H-3	IC 357524
4	H-4	IC 357529
5	H-5	IC 357538
6	H-6	IC 357510
7	H-7	(1)
8	H-8	(1)
9	H-9	(1)
10	H-10	(1)
11	H-11	IC 358915
12	H-14	(1)
13	H-15	(1)
14	H-17	(1)
15	H-18	(1)
16	H-19	IC 357328
17	H-20	(1)
18	H-21	(1)
19	H-24	(1)
20	H-25	(1)
21	H-28	(1)
22	H-29	(1)
23	H- 30	(1)
24	H-31	(1)
25	H- 32	(1)
26	H-33	(1)
27	H-35	(1)
28	H-37	(1)
29	H-38	(1)
30	H-39	(1)
31	H-40	(1)
32	CNH-21-I	(2)
33	CNH-32	(2)
34	DTS-22	(2)
35	DTS-380	(2)
36	LRA-5166	(2)

[z] The coding of lines is by the Central Institute for Cotton Research (CICR), Nagpur, India.

(1) Selected stabilized lines maintained At CICR, Nagpur, India

(2) Developed lines at CICR, Nagpur.

Corp., Kyoto, Japan). Percent cell survival was computed (Gaff and Okong'o-Ogola, 1971; Taylor and West, 1980) as

$$(\frac{T}{C}\times 100)$$

where, T: absorbance at 600 nm of dye extracted from induced or noninduced seedlings, C: absorbance at 600 nm of dye extracted from absolute control seedlings.

Protein Synthetic Capacity of the Seedlings After Exposure to High Temperature Stress. Cotton seedlings of high-temperature-tolerant (H-28) and susceptible (B-4) genotypes were subjected to high temperature stress as explained in the TIR protocol. At the end of the challenging temperature treatment, 20 seedlings were incubated in a solution containing 2 ml Tris-HCl (50mM, pH7) buffer with 10 µl of radioactive ^{35}S methionine (specific activity 5000 mC_i/ml) for 4 h. Thereafter, seedlings were washed three times in DW followed by phosphate-buffered saline (PBS). The total soluble protein in seedlings was extracted in 10 mM PBS (pH 7.5 NaCl 0.13 M, KCl, KH_2PO_4 1.5 mM, Na_2HPO_4 7.8 mM, Tween-20 0.1%). The homogenate was centrifuged at 10,000 rpm for 10 min at 4ºC and the volume of supernatant was recorded (volume of extract). Debris was washed two times and used for quantification of the total ^{35}S methionine uptake. The protein in supernatant was precipitated with 1/10 volume of 10% of trichloroacetic acid (TCA). The supernatant was kept aside for radiolabel quantification. The pellet was washed three times with 80% acetone to remove the traces of TCA. The total soluble protein was estimated by Bradford (1976). The radiolabel was quantified in debris, supernatant, and protein by using Liquid Scintillation CounterWallac–1049 (Perkin Elmer Wallac, Inc., Waltham, MA) as:

$$S^{35}\text{ incorporation into protein (\%)} = \frac{\text{Radioactivity (CPM) in protein fraction}}{\text{Radioactivity (CPM) taken up by seedling}}\times 100$$

where, CPM: count per min.

An ANOVA was performed for each variable in this experiment; subsequently ANOVA was used to determine whether there were differences among cotton lines. Cotton line means were separated by use of critical differenceat $P\leq 0.05$ obtained by using MSTAT-C software (Anonymous, 1998). The standard error of the mean is the standard deviation of the sample mean estimate of a population mean.

RESULTS

Standardization of Optimum TIRProtocol in Cotton-Challenging Temperature. In *G.hirsutum* var. Sahana, as the challenging temperature increased, seedling survival and recovery growth decreased in both induced and noninduced seedlings. Noninduced seedlings exposed to the challenging temperature showed high percent mortality as well as marked reduction in recovery growth compared to seedlings exposed to gradually increasing temperature (induction treatment) prior to challenging with high temperatures (Fig. 2).This is clear evidence of an induction response in cotton. Noninduced seedlings challenged at 47ºC for 3 h showed a high percent reduction in recovery growth (99.6%), whereas the reduction in recovery growth in induced seedlings challenged at 47ºC for 3 h was approximately 50%. Consequently, a treatment consisting of 47ºC for 3 h was selected as the challenging temperature stress, and a standard protocol for TIR was established. This involved subjecting seedlings to a gradual temperature increase from 28 to 40ºC over 4 h (induction treatment), immediately followed by challenging at 47ºC for 3 h. Subsequently, seedlings are allowed to recover at room temperature (30ºC and 60% RH) for 48 h.

Temperature Stress Response in Cotton Species. Adopting the standardized protocol developed, genotypes of four cultivated cotton species were examined for high temperature tolerance. At the end of recovery, species differed significantly ($P<0.01$) in seedling mortality and recovery growth. Similarly, for interactions between species and treatment in percent mortality and percent reduction in recovery growth a significant ($P< 0.01$) difference was observed. The results revealed that prior induction temperature treatments identified significant variability among entries belonging to four cotton species in both seedling mortality and recovery growth after challenging with high temperature ranging from 18.3 to 60% (Table 3). On the other hand, no significant genotypic differences exist in seedling mortality amongst the cotton entries. However, a large variation in seedling survival among species was noticed only in induced treatment. A similar trend in species variation in recovery growth of seedlings was noticed in induced seedlings compared to noninduced seedlings (Table 3).

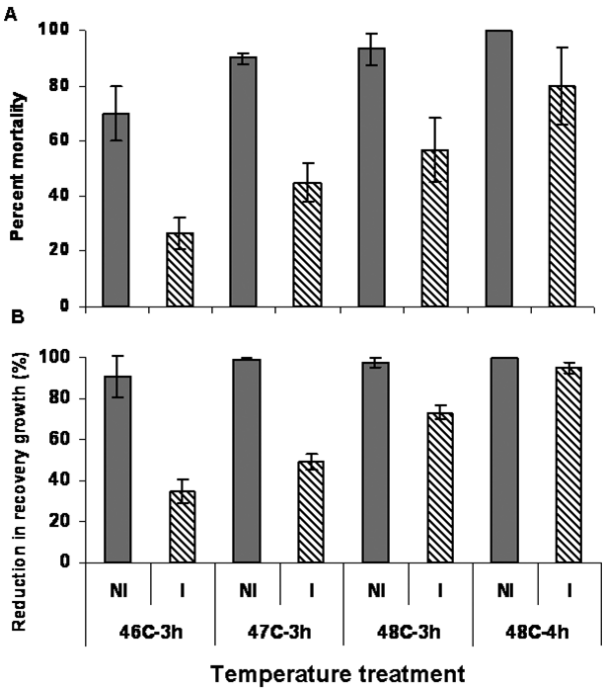

Figure 2. Induction response of cotton seedlings (*G. hirsutum* var. Sahana) to high temperature stress. Seedlings (36 h old) of cotton were induced with a gradual temperature increase (temperature increased from 28 to 40°C over 4 h) and challenged with high temperature (diagonal-lined columns). A subset of seedlings were directly challenged with severe temperatures (46ºC for3 h, 47ºC for 3 h, 48ºC for 3h, and 48ºC for 4 h) (gray columns), and allowed to recover at 30ºC and 60% RH for 48 h. At the end of recovery period percent mortality (A) and percent reduction in recovery growth of the seedlings (B) was computed. Data shown are an average of three replicates, each replicate had 20 seedlings and error bars represent SD. I: induced, NI: noninduced.

Screening of *G.hirsutum* Genotypes for Thermotolerance Using TIR Technique. Acquired thermotolerance among 36 diverse genotypes of *G. hirsutum* was assessed. Significant genotypic variation in seedling survival and recovery growth were expressed after induction treatment (Table 4, Fig. 3). Genetic variation in seedling mortality during recovery from challenging temperature ranged significantly ($P< 0.01$) from 50 to 100% in noninduced seedlings and 10 to 93.3% in induced seedlings (Table 4). Genetic variation in seedling growth during recovery from challenging temperature showed a similar trend, in which seedling growth was distinctly higher in induced seedlings. There was no correlation in recovery growth between induced and noninduced seedlings (Fig. 4), suggesting that levels of basal tolerance and acquired tolerance to high temperature varies between genotypes.

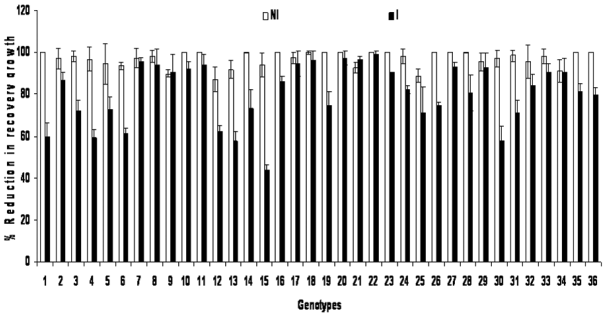

Figure 3. Genetic variability in intrinsic and acquired thermotolerance in cotton (*G.hirsutum*) genotypes screened by TIR technique. Data shown are averages of three replicates, each replicate had 20 seedlings and error bars represent SD. I: induced, NI: noninduced. Numbers 1 to 36 are the 36 *G.hirsutum* genotypes screened for high temperature tolerance using TIR technique.

Table 3. Variation in thermotolerance of different cotton species in terms of percent mortality and percent reduction in growth[z].

	n	Seedling Mortality (%)		Reduction in recovery growth (%)	
		Induced	Noninduced	Induced	Noninduced
G. arboreum	2	18.30±6.7	93.35±6.7	68.27±4.2	99.14±0.9
G.herbaceum	9	27.78±6.6	84.07±4.8	53.28±6.9	95.17±2.2
G.barbadense	3	60.00±10.3	81.11±10.6	90.30±7.1	98.44±1.56
G. hirsutum	6	48.88±3.6	88.32±4.3	82.89±3.0	97.17±1.4
LSD @ 5 %		13.62		11.02	

[z] Germinated seedlings from four cultivated species (*G.arboreum, G.herbaceum, G. hirsutum* and *G.barbadense*) were exposed to challenging temperature of 47ºC for 3 h with or without prior induction treatment. Subsequently, seedlings were allowed to recover for 48 h at 30ºC and 60% RH. At the end of recovery period the mortality and recovery growth of seedling was recorded. A set of seedlings maintained at 30ºC as absolute control. Data shown was average of three replicates, each replicate had 20 seedlings. LSD is less significant difference at 5% between species. n: number of genotypes per species. Means are followed by standard errors of the means.

Table 4. Genetic variability in thermotolerance among36 *G. hirsutum* genotypes[z].

	Seedling Mortality (%)		Reduction in recovery growth (%)	
	Induced	Noninduced	Induced	Noninduced
Mean	**49.77±4.2**	**88.61±2.2**	**80.54±2.4**	**96.94±0.6**
Min	**10.00**	**50.00**	**43.59**	**87.22**
Max	**93.30**	**100.00**	**96.56**	**100.00**
LSD @ 5 %	**24.63**		**16.20**	

[z] The induction response of seedlings was assessed among 36 genotypes of *G. hirsutum* as described in Table 1. Data shown is average of three replicates, each replicate had20 seedlings. LSD is less significant difference at 5% between genotypes. Means are followed by standard errors of the means.

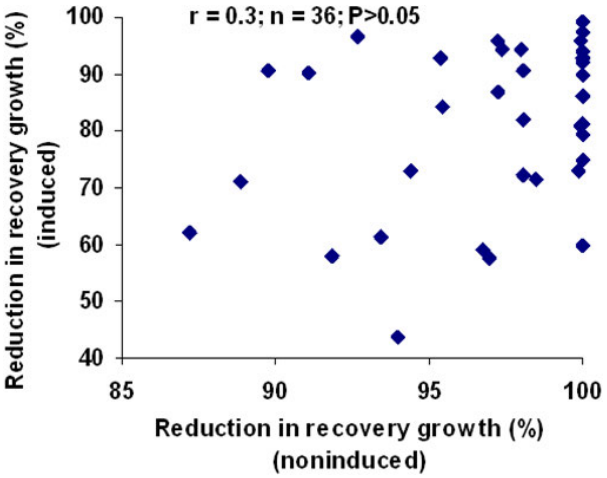

Figure 4. Relationship between basal and acquired thermotolerance across *G. hirsutum* genotypes. Seedling recovery growth was assessed for a set of seedlings directly exposed to 47°C for 3 h (basal level thermotolerance, noninduced) and with prior exposure to induction temperature stress (acquired thermotolerance).

Assessment of Physiological Response Under High Temperature Stress in Selected Cotton Genotypes. The screened genotypes were classified (based on absolute growth after recovery period and percent reduction in recovery growth) into three different categories: tolerant, moderately tolerant, and susceptible (Fig.5). One genotype showing tolerance, *G. hirsutum* (H-28), and one susceptible genotype, *G. barbadense* (B-4),were selected to assess the stress response based on cell viability and protein synthesis after exposure to challenging temperature stress. Both H-28 and B-4 had similar basal thermotolerance, however they differed in acquired thermotolerance (Fig. 6A). Percent cell viability as estimated by Evan's blue method was low (34.41%) in B-4, whereas it was significantly higher (87.01%) in H-28. However, no significant difference between these genotypes was observed under the noninduced treatment Fig. 6B). In addition, whether differences between tolerant and susceptible genotypes were due to differences in protection of protein synthesis machinery under stress was evaluated through the incorporation of ^{35}S methionine into protein. ^{35}S methionine incorporation to protein fraction was less in both induced and noninduced seedlings of susceptible genotype (B-4). In contrast, the tolerant genotype (H-28) maintained higher ^{35}S methionine incorporation into the protein fraction both under noninduced and induced treatments. In addition, ^{35}S methionine incorporation into the protein fraction showed a significant increase in induced seedlings exposed to the challenging temperature (Fig. 6C).

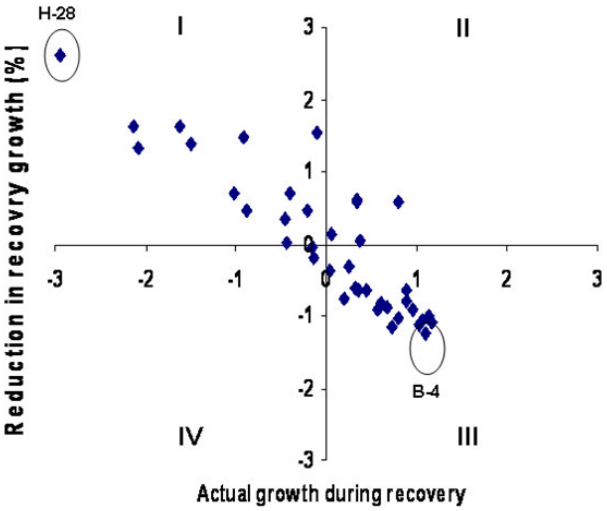

Figure 5. Normal Z-distribution of New World cotton genotypes based on absolute growth after recovery period and percent reduction in recovery growth over control. Quadrant I: heat tolerant genotypes, Quadrant II and IV: moderately heat tolerant genotypes, and Quadrant III: susceptible genotypes. H-28 and B-4 are the contrasting (circled) tolerant and susceptible genotypes belonging to *G. hirsutum* and *G. barbadense*, respectively.

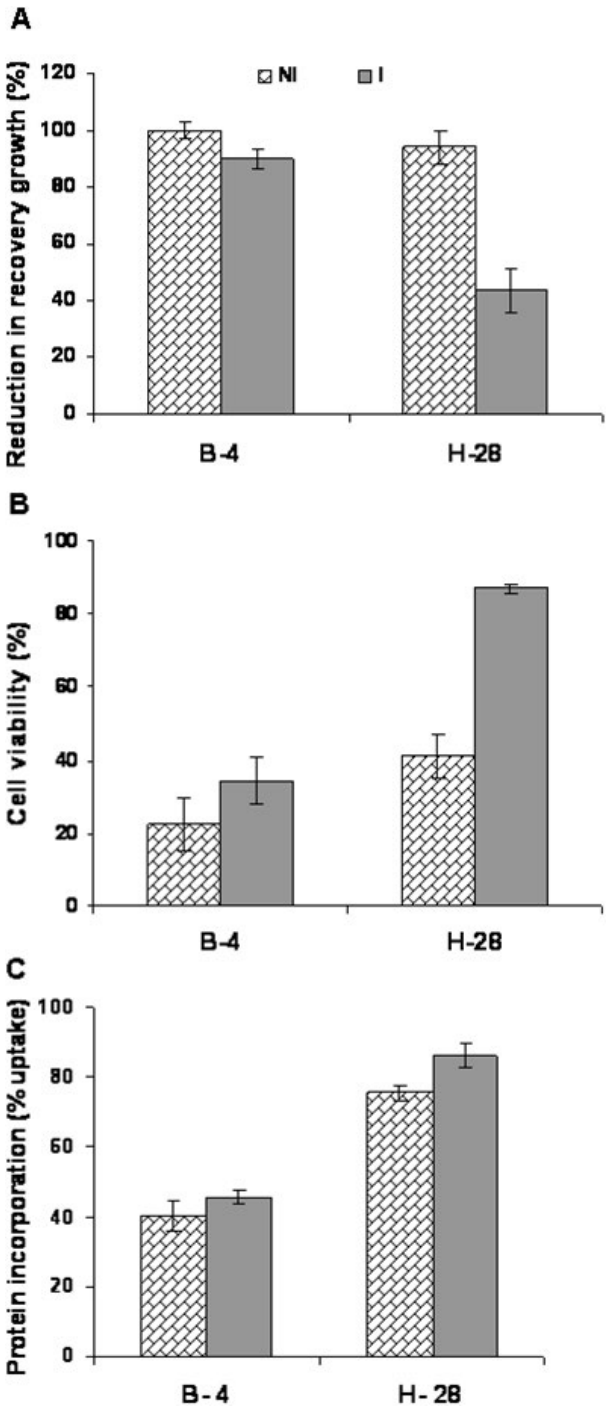

Figure 6. Stress response of heat tolerant (H-28) and heat susceptible (B-4) cotton genotypes as measured by (A)percent reduction in recovery growth, (B) cell viability, and (C) protein synthesis. The recovery growth of induced and noninduced seedlings was assessed at the end of recovery period. The cell viability and protein synthesis data were assessed at the end of stress treatment. Data shown are an average of three replicates, each replicate had 20 seedlings. Error bars represent SD. I: Induction treatment, NI: Noninduced treatment.

DISCUSSION

Plants overcome stress by adopting several physiological and biochemical mechanisms including morphological or short-term avoidance or acclimation mechanisms. Under natural conditions plants experience a gradual increase in stress over a period of time. This gradual progression results in the exposure of plants to milder stress before plants experience severe stress. Increased tolerance to otherwise lethal stress in plants exposed to induction stress is referred to as acquired tolerance. Acquired tolerance is ubiquitous and has been demonstrated in several species (Burke et al., 2000; Flahaut et al., 1996; Hong and Vierling, 2000; Larkindale et al., 2005; Massie et al., 2003; SenthilKumar et al., 2006; Vierling, 1991). Upon exposure to acclimation temperature stress, many of HSPs and other stress-response genes are up-regulated (Kumar et al., 1999; SenthilKumar et al., 2003; Srikanthbabu et al., 2002; Uma et al., 1995; Visioli et al., 1997; Woolf and Lay-Yee, 1997).The observed higher recovery growth of induced seedlings is mainly because of altered metabolism in response to acclimation as seen in sunflower (Kumar et al., 1999; SenthilKumar et al., 2006), sorghum, pearl millet (Howarth et al., 1997), beans (Keeler et al., 2000), wheat (Burke, 1994, 1998), and groundnut (Srikanthbabu et al., 2002). Several stress-adaptive mechanisms are triggered signifying that coordinated expression of several temperature stress-responsive genes occurs upon induction. Several physiological and biochemical processes (Chen et al., 1990) including maintenance of membrane stability (Berry and Bojorkman, 1980; Grover et al., 2000; Kader et al., 1991) and protection to macromolecules (Sanchez and Lindquist, 1990; Vierling and Nguyen, 1992) were shown to occur in response to induction stress treatment.

Further, the threshold temperature for tolerance differs among species. For instance, 49ºC for 2 h is a severe temperature stress for sunflower (SenthilKumar et al., 2006), whereas it is much higher (52ºC) in groundnut (Lokesh et al., 2004; Srikanthbabu et al., 2002). Similarly, the induction stress required for optimum expression of stress-response genes also varies among species. Therefore, to study temperature response across the genotypes of a species, optimum induction and challenging temperatures have to be standardized. In this study we developed a TIR

protocol for cotton. In this protocol, seedlings were initially exposed to a gradual temperature increase from 28 to 40ºC over 4 h (induction temperature treatment) followed by a challenging temperature treatment of47ºC for 3 h. Subsequently, the recovery growth at the end of 48 hat 30ºC temperature was assessed (Fig. 2).

As expected, differences in stress response were noticed among cotton entries belonging to different species. Induced seedlings of both *G. arboretum* L. and *G. herbaceum* L. species showed a low percent mortality and high recovery growth after high temperature stress (challenging stress) compared with *G. hirsutum* and *G. barbadense* seedlings. On the other hand, variability in basal thermotolerance was narrow (Table 3). This may explain why diploid cotton is generally cultivated in marginal drought-prone environments in Asia due to their inherent ability to withstand abiotic stress (Kulkarni et al., 2009).Many other studies have shown that genetic variability is only seen upon stress acclimation treatment prior to severe stress (Burke, 2001; Jayprakash et al., 1998; Krishnan et al., 1989; Kumar et al., 1999; Srikanthbabu et al., 2002; Uma et al., 1995)

The usefulness of TIR to identify genotypic variation in high temperature tolerance was shown in 36 cotton (*G. hirsutum*) lines. Significant genetic variability was seen in acquired thermotolerance (Table 2). A number of earlier studies showed that thermotolerant genotypes selected based on TIR technique have better leaf area and membrane integrity, and thus better recovery growth after exposure to heat stress at the whole-plant level. This indicates that the thermotolerant genotypes selected based on TIR technique at the seedling level are intrinsically tolerant at the plant level (Al-Ouda, 1999; Kumar et al., 1999; Senthilkumar et al., 2003; Srikanthbabu et al., 2002).Quantitative indicators of the potential capacity of a genotype to tolerate high temperature stress are its ability to maintain cellular membrane integrityand protein synthetic capacity immediately after exposure to challenging temperature stress. In this study, induced seedlings of the thermotolerant *G. hirsutum* genotype H-28 showed signifcantly higher recovery growth, cellular membrane integrity, and protein synthesis than the susceptible *G. barbadense* genotype B-4. However, differences between these genotypes under noninduced conditions were not marked. In similar studies, Howarth et al. (1997) and Kumar et al. (1999) demonstrated higher protein synthesis during recovery from severe stress only upon early induction stress. These results suggest that the TIR technique is a robust and powerful technique to identify genetic variability in high temperature tolerance in cotton within a short time period and is suitable for screening a large number of genotypes.

In summary, breeding for heat tolerance is often complicated by the lack of an efficient and easily implemented screening technique and inadequate information on the availability of genetic variability. Here we demonstrate that the TIR technique is a robust method for screening cotton seedlings for heat tolerance. Using this technique it was demonstrated that there is sufficient genetic variability present among cotton lines for high temperature tolerance. Lines selected as tolerant to high temperature should be useful in breeding programs seeking to overcome this yield limitation.

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