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Effect of Temperature induction response on Cell viability, Cell Survivability, Malondialdehyde content and total soluble protein content of cotton (*Gossypium hirsutum* L.) genotypes

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ABSTRACT

"Temperature Induction Response" (TIR) technique was employed to investigate the effect of temperature on popular 20 cotton (*Gossypium hirsutum* L.) genotypes in a laboratory experiment conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2020-2021. Identical sized ten days old cotton seedlings were selected and subjected to inductive temperature (gradual temperature raised from 28 to 40°C) for 4 h and non-inductive temperature (46°C for 3 h, 47°C for 3 h, 48°C for 3 h and 48°C for 4 h) for specific time duration. KC3 and SVPR6 recorded highest thermotolerance among the genotypes and TSH325 and TSH357 showed moderate thermotolerance while TSH375 and TSH383 were sensitive, in terms of seedling survival, cell viability, total soluble protein and malondialdehyde compared to remaining genotypes under non-inductive temperature.

Keywords: Cotton, temperature induction response, cell viability

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the world's most important fibre economic cash crop which is essential for textile industry and is also useful for oils and livestock feed. Around 80% of world production in cotton comes from seven countries where, India is placed in third position (Zahid *et al*., 2016). Plants adapt to heat stress by inherent basal level tolerance and also acquired tolerance to severe heat stress. Mainly acquired thermo tolerance was quite rapid and it has been shown to be induced during cell acclimation, to moderately high temperature periods (Larkindale *et al*., 2005). The ability to with stand and to acclimate to supraoptimal temperature results from both prevention of heat damage and repair of heat-sensitive components. Cotton seedlings when exposed to sub lethal temperature much prior to challenge with the severe temperatures, have better growth recovery when compared to seedlings challenged directly to severe temperature stress. Though, cotton is a hardy which crop can come up in all types of climatic conditions, global warming is mainly serious emerging threat causing environmental fluctuations in most of the agricultural zones of world including cotton (Solomon, 2007). It responds to various abiotic stresses, especially, high temperature is one which can cause severe damages to cotton crop at cellular level in all growth and developmental stages ultimately limit the cotton yield (Oosterhuis, 2002). Present study is to explain the screening of cotton seedlings against thermo tolerance through the "Temperature Induction Response" technique. This method was widely used for rapid screening of cotton genotypes for high temperature tolerance (Kheir *et al*., 2012). The current screening study is mainly based on the principle of "acquired thermo tolerance" of seedlings of 20 cotton genotypes of Tamil Nadu. The accounting data of

morphological and biochemical characters of cotton seedlings were considered for the confirmation of high temperature tolerance of selected varieties.

MATERIALS AND METHODS

The laboratory screening studies through the Temperature Induction Response (TIR) technique was conducted in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2020-2021. Twenty cotton genotypes of Tamil Nadu namely, KC3, SVPR6, CO15, CO16, CO17, TSH325, TSH357, TSH358, TSH367, TSH375, TSH383, TSH387, TSH406, TSH408, TSH419, TCH1828, TCH1897, TCH1199, TCH1895 and TCH1941 were collected from the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. The uniform size of 10 days old seedlings were grown in paper cup (1:1 ratio of coir pith: vermicompost) were exposed to a gradual temperature from T1-Control, T2-46℃ for 3 h, T3-47℃ for 3 h, T4-48℃ for 3 h and T5-48℃ for 4 h. The exposed seedlings were kept for recovery under room temperature for 48 hours and analysis were done for seedling survival, cell viability (Gaff & Okong'O-Ogola, 1971), total soluble protein (Lowry *et al.*, 1950) and lipid peroxidation (MDA content) (Heath & Packer, 1968). All the treatments were maintained with 3 replications and the data were analysed under Factorial Completely Randomized Design (FCRD) by using SPSS.

Statistical Analysis

SPSS Statistics version 23.0 software (http://www.spss.com) was used for statistical analysis. The mean values of each parameter were identified and examined using analysis of variance to determine the significance for all the genotypes and treatments.

RESULTS

Cell Survivability

Higher cell survivability percentage was recorded in the genotype KC3 (64.60%) compared to remaining genotypes followed by SVPR6 (63.98%) (Table 1 and Figure 1). A lower cell survivability percentage was observed in TSH375 (40.85%) compared to remaining genotypes. Among five temperature treatments, control (T1) (73.01%) recorded significantly higher cell survivability percentage compared to remaining treatments. The temperature treatment, 48°C for 4 hrs (T5) (21.73%) showed significantly lower cell survivability percentage compared to other temperature treatments. In the genotype and treatment interaction relationship, the genotypes KC3 (90.63%) and SVPR6 (90.07%) grown under control (T1) temperature treatment showed significantly higher cell survivability percentage compared to all other genotype and treatment interactions. It was also observed that genotype TSH375 (15.90%) showed significantly lower cell survivability percentage grown under 48°C for 4 hrs (T5) treatment compared to all other combinations.

Cell Viability

Higher significant cell viability percentage was noticed in KC3 (78.51%) and SVPR6 (78.46%) among 20 genotypes (Table 2 and Figure 2). The genotypes TSH406 (77.20%) and TSH408 (77.15%) recorded higher significant cell viability percentage compared to remaining genotypes. The genotypes namely TSH375 (63.06%) and TSH383 (63.07%) recorded significantly lower cell viability percentage when compared to other genotypes. It was observed that, among all the temperature treatments control (T1) (90.18%) recorded significantly higher cell viability percentage compared to remaining treatments. Apart from control, 46°C for 3 hours (T2) (81.75%) significantly highest cell viability percentage was recorded compared to other three treatments followed by 47°C for 3 hrs (T3) (71.53%), 48°C for 3 hrs (T4) (58.94%) and 48°C for 4 hrs (T5) (53.05%). The genotype and treatment interaction relationship, observations revealed that the genotypes, KC3 (97.00%), SVPR6 (97.17%), TSH406 (96.00%) and TSH408 (96.00%) grown under control (T1) temperature treatment showed significantly higher cell viability percentage compared to all other genotype and treatment interactions. The genotypes TSH375 (46.59%) and TSH383 (46.66%) grown under 48°C for 4 hrs (T5) temperature treatment showed significantly lower cell viability percentage compared to all other combinations.

Lipid Peroxidation

The genotypes SVPR6 (0.173 nmol g^{-1}) and KC3 (0.176 nmol g^{-1}) produced significantly lower malondialdehyde content compared to all other 18 genotypes. The genotype TSH375 (0.445 nmol g^{-1}) recorded significantly higher MDA content when compared to remaining genotypes (Table 3 and Figure 3). Among five different temperature treatments, the temperature treatment control (T1) (0.166 nmol g^{-1}) revealed significantly lower malondialdehyde content compared to remaining four treatments. The temperature treatment 48°C for 4 hrs (T5) (0.399 nmol g^{-1}) registered significantly higher malondialdehyde content compared to all other temperature treatments. With respect to genotype and temperature treatment interaction, the production of malondialdehyde content was significantly lower in the genotypes SVPR6 and KC3 compared to remaining genotypes which were grown under all the five temperature treatments. Genotype TSH375 showed significantly higher MDA content compared with other genotypes grown under all five temperature treatments.

Total Soluble Protein

The total soluble content was found significantly higher in the genotypes KC3 (14.79 mg g^{-1}) and SVPR6 (14.30 mg g^{-1}) compared to all other genotypes (Table 4 and Figure 4). The genotypes TSH383 (8.93 mg g^{-1}), TSH358 (9.36 mg g^{-1}) and TSH375 (9.02 mg g^{-1}) had significantly lower TSP compared to remaining genotypes. Among five different temperature treatments, 48°C for 3 hrs (T4) (12.92 mg g^{-1}) recorded significantly higher TSP compared to remaining treatments followed by 48°C for 4 hrs (T4) (12.10 mg g^{-1}). The temperature treatment, control (T1) (9.92 mg g^{-1}) significantly recorded

Table 1: Effect of temperature induction response on cell survivability of 10 days old cotton seedlings

GENOTYPES	T1- CONTROL	T2 – 46°C for 3 hrs	T3 – 47°C for 3 hrs	T4 – 48°C for 3 hrs	T5 – 48°C for 4 hrs	MEAN
KC3	90.63	81.93	71.32	47.71	31.41	64.60
SVPR6	90.07	81.30	70.53	47.23	30.77	63.98
CO15	72.97	64.50	54.75	35.73	22.02	49.99
CO16	70.03	61.69	51.85	33.31	20.77	47.53
CO17	71.70	63.21	53.22	34.16	21.41	48.74
TSH325	74.90	66.29	56.45	37.18	23.07	51.58
TSH357	81.83	72.82	62.10	40.63	25.55	56.58
TSH358	65.50	56.49	47.50	29.32	17.46	43.25
TSH367	67.30	58.39	48.93	30.19	18.41	44.65
TSH375	62.67	53.51	44.82	27.35	15.90	40.85
TSH383	64.97	55.48	46.35	28.20	16.67	42.33
TSH387	65.77	56.93	47.76	29.57	17.82	43.57
TSH406	85.43	77.05	66.51	44.46	28.71	60.43
TSH408	85.67	77.19	66.63	44.31	28.41	60.44
TSH419	80.97	72.91	62.85	41.54	26.52	56.96
TCH1828	79.17	70.87	60.61	40.36	25.54	55.31
TCH1897	68.63	59.66	50.21	32.02	19.33	45.97
TCH1199	69.53	60.82	50.88	32.10	19.51	46.57
TCH1895	78.50	70.10	59.71	39.75	24.85	54.58
TCH1941	68.70	60.27	50.27	31.02	19.26	45.91
MEAN	73.01	64.34	54.52	35.07	21.73	GM=51.19
	TREATMENT		GENOTYPE		(GXT) INTERACTION	
CD	0.259		0.517		1.157	
SE (m)	0.093		0.185		0.415	
SE (d)	0.131		0.262		0.586	

Table 2: Effect of temperature induction response on cell viability of 10 days old cotton seedlings

GENOTYPES	T1- CONTROL	T2 – 46°C for 3 hrs	T3 – 47°C for 3 hrs	T4 – 48°C for 3 hrs	T5 – 48°C for 4 hrs	MEAN
KC3	97.00	91.08	79.69	65.67	59.10	78.51
SVPR6	97.16	90.94	79.58	65.57	59.01	78.45
CO15	92.00	83.77	73.30	60.40	54.36	72.76
CO16	92.00	83.40	72.98	60.13	54.12	72.53
CO17	91.00	82.39	72.09	59.40	53.46	71.67
TSH325	93.00	84.93	74.32	61.24	55.11	73.72
TSH357	95.00	86.65	75.82	62.48	56.23	75.24
TSH358	86.00	76.36	66.82	55.06	49.55	66.76
TSH367	85.00	75.93	66.43	54.74	49.27	66.27
TSH375	82.33	71.79	62.82	51.76	46.58	63.06
TSH383	82.00	71.91	62.92	51.85	46.66	63.07
TSH387	85.00	75.66	66.20	54.55	49.10	66.10
TSH406	96.00	89.37	78.20	64.44	57.99	77.20
TSH408	96.00	89.29	78.13	64.38	57.94	77.15
TSH419	94.00	87.13	76.24	62.82	56.54	75.35
TCH1828	95.00	87.50	76.56	63.09	56.78	75.78
TCH1897	90.00	81.43	71.25	58.71	52.84	70.84
TCH1199	88.00	79.49	69.55	57.31	51.58	69.18
TCH1895	94.00	86.01	75.25	62.01	55.81	74.61
TCH1941	87.00	78.40	68.60	56.53	50.87	68.28
MEAN	90.18	81.75	71.53	58.94	53.04	GM=71.83
	TREATMENT		GENOTYPE		(GXT) INTERACTION	
CD	0.464		0.928		2.072	
SE (m)	0.166		0.333		0.744	
SE (d)	0.235		0.47		1.052	

lower total soluble protein compared to remaining all other temperature treatments. Genotype and temperature treatment interaction was significantly higher in the genotypes SVPR6 and KC3 compared to remaining genotypes which were grown under all five temperature treatments. Genotype TSH383 showed significantly lower TSP content compared to all other genotypes grown under all five temperature treatments.

DISCUSSION

Generally plants will overcome the stress by adopting several altered physiological, morphological and biochemical mechanisms including short-term avoidance or acclimation mechanisms to survive and produce the economically valuable yield. Under normal conditions plants will experience a

Table 3: Effect of temperature induction response on lipid peroxidation content of 10 days old cotton seedlings

GENOTYPES	T1- CONTROL	T2 – 46°C for 3 hrs	T3 – 47°C for 3 hrs	T4 – 48°C for 3 hrs	T5 – 48°C for 4 hrs	MEAN
KC3	0.112	0.146	0.179	0.213	0.230	0.176
SVPR6	0.109	0.144	0.178	0.210	0.226	0.173
CO15	0.159	0.222	0.281	0.327	0.372	0.272
CO16	0.166	0.234	0.295	0.343	0.392	0.286
CO17	0.175	0.249	0.312	0.364	0.417	0.303
TSH325	0.149	0.206	0.259	0.301	0.340	0.251
TSH357	0.153	0.212	0.268	0.312	0.352	0.260
TSH358	0.215	0.329	0.413	0.469	0.572	0.399
TSH367	0.202	0.299	0.380	0.430	0.515	0.365
TSH375	0.234	0.367	0.466	0.522	0.636	0.445
TSH383	0.227	0.352	0.443	0.501	0.615	0.428
TSH387	0.209	0.313	0.395	0.450	0.548	0.383
TSH406	0.117	0.158	0.193	0.228	0.246	0.188
TSH408	0.119	0.161	0.198	0.234	0.256	0.194
TSH419	0.127	0.173	0.214	0.251	0.280	0.209
TCH1828	0.136	0.186	0.232	0.271	0.302	0.225
TCH1897	0.183	0.263	0.329	0.383	0.443	0.320
TCH1199	0.186	0.271	0.341	0.391	0.456	0.329
TCH1895	0.141	0.194	0.243	0.282	0.317	0.235
TCH1941	0.191	0.280	0.353	0.403	0.474	0.340
MEAN	0.166	0.238	0.299	0.344	0.399	GM=0.289
	TREATMENT		GENOTYPE		(GXT) INTERACTION	
CD	0.002		0.004		0.009	
SE (m)	0.001		0.001		0.003	
SE (d)	0.001		0.002		0.005	

Table 4: Effect of temperature induction response on total soluble protein content of 10 days old cotton seedlings

GENOTYPES	T1- CONTROL	T2 – 46°C for 3 hrs	T3 – 47°C for 3 hrs	T4 – 48°C for 3 hrs	T5–48°C for 4 hrs	MEAN
KC3	12.45	13.74	15.26	16.79	15.72	14.79
SVPR6	12.08	13.30	14.73	16.21	15.18	14.30
CO15	10.04	10.88	11.88	13.07	12.24	11.62
CO16	9.89	10.69	11.71	12.88	12.06	11.45
CO17	10.00	10.80	11.83	13.01	12.18	11.56
TSH325	10.49	11.40	12.51	13.76	12.89	12.21
TSH357	10.32	11.22	12.28	13.50	12.64	11.99
TSH358	8.24	8.73	9.52	10.48	9.81	9.36
TSH367	9.00	9.57	10.47	11.52	10.79	10.27
TSH375	8.00	8.43	9.16	10.07	9.43	9.02
TSH383	7.91	8.29	9.09	10.00	9.36	8.93
TSH387	8.60	9.17	10.03	11.03	10.33	9.84
TSH406	11.94	13.13	14.58	16.04	15.02	14.14
TSH408	11.65	12.76	14.10	15.51	14.52	13.71
TSH419	11.62	12.71	14.02	15.43	14.44	13.64
TCH1828	11.32	12.36	13.61	14.98	14.02	13.26
TCH1897	9.53	10.27	11.24	12.37	11.58	11.00
TCH1199	9.48	10.19	11.15	12.26	11.48	10.91
TCH1895	11.01	12.00	13.18	14.50	13.57	12.85
TCH1941	9.43	10.10	11.05	12.16	11.38	10.82
MEAN	9.91	10.71	11.75	12.92	12.10	GM=11.78
	TREATMENT		GENOTYPE		(GXT) INTERACTION	
CD	0.321		0.642		1.435	
SE (m)	0.115		0.230		0.514	
SE (d)	0.163		0.325		0.727	

gradual increase in stress over a period of time which gradually progression results in the exposure of plants to mild stress before plants undergo severe stress. When plants were exposed to induction stress, acquired tolerance is induced, which is referred to as increased tolerance by plants to lethal stress. Acquired tolerance is like ubiquitous and it is been demonstrated in several species like Arabidopsis mutants (Burke *et al*., 2000; Flahaut *et al*., 1996; Hong & Vierling, 2000; Larkindale *et al*., 2005; SenthilKumar *et al*., 2006; Vierling, 1991). Plants upon exposure to acclimation of temperature stress, many heat shock proteins, other stress response genes and some transcription factors are up-regulated (Kumar *et al*., 1999; SenthilKumar *et al*., 2003; Srikanthbabu *et al*., 2002; Uma *et al*., 1995; Visioli *et al*., 1997; Woolf & Lay- Yee, 1997).

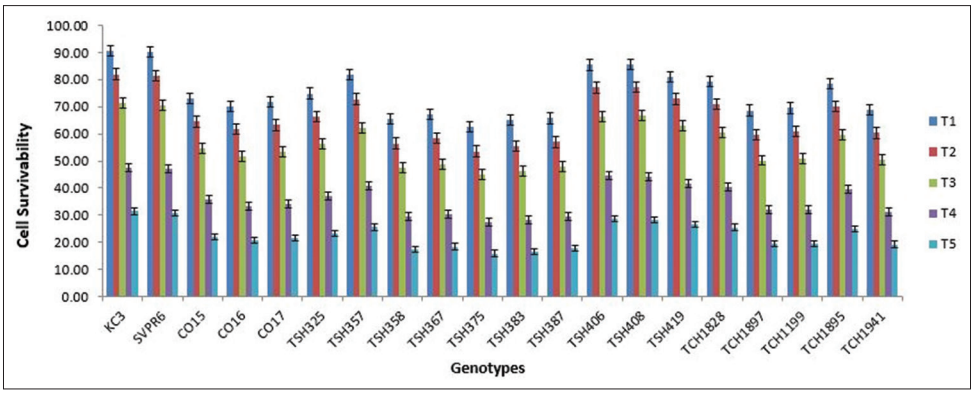

Figure 1: Graphical representation of cell survivability percentage

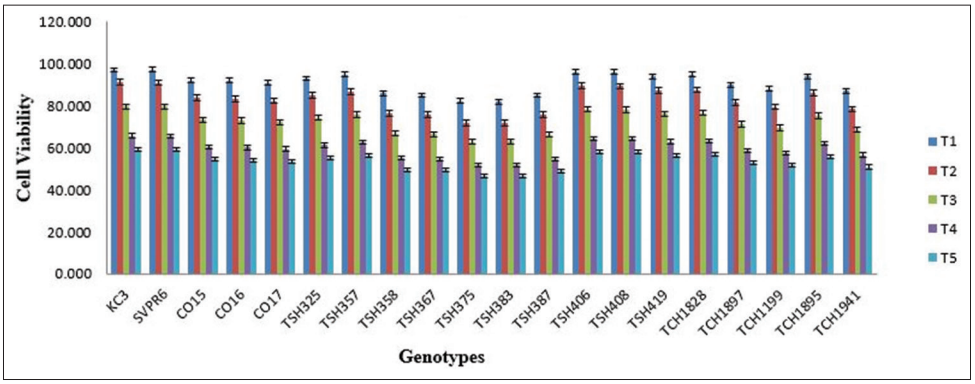

Figure 2: Graphical representation of cell viability percentage

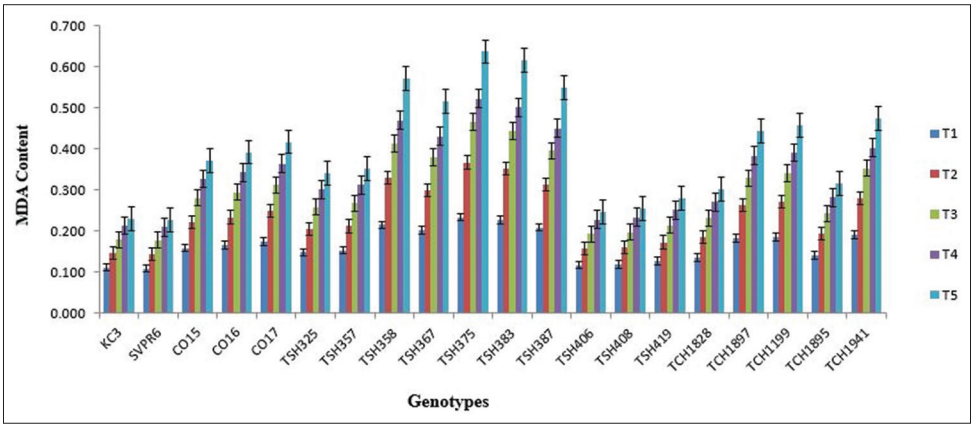

Figure 3: Graphical representation of MDA content

The threshold temperature for tolerance capacity will differ among the different species. In sunflower 49°C for 2 hrs is a severe temperature stress (SenthilKumar *et al*., 2006), whereas it is 52°C in groundnut (Lokesh *et al*., 2004) and in peas also (Srikanthbabu *et al*., 2002). Similarly the induction stress required for optimum expression of stress response genes also varies among the different species.

Some of the researchers observed that high recovery growth of induced seedlings is only because of altered metabolism in response to acclimation seen in some crops like sunflower (Kumar *et al*., 1999; Senthil Kumar *et al*., 2006), sorghum, pearl millet (Howarth *et al*., 1997), beans (Keeler *et al*., 2000), wheat (Burke, 1994), and groundnut (Srikanthbabu *et al*., 2002). Several stress-adaptive mechanisms are enhanced signifying that coordinated expressions of several temperature stress-responsive genes that occur upon the induction. Physiological and biochemical processes as mentioned by Chen *et al*., (1990) including maintenance of membrane stability (Berry & Bojorkman, 1980; Grover *et al*., 2000) and sustaining the macromolecules without getting destroyed (Sanchez & Lindquist, 1990; Vierling & Nguyen, 1992) were showed to occur in response to induction stress treatment.

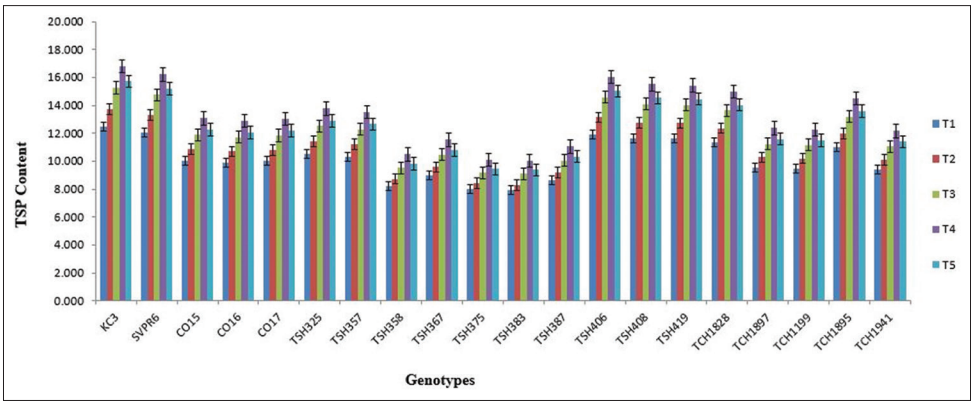

Figure 4: Graphical representation of TSP content

Therefore, by keeping all these points into consideration a study about temperature response across the genotypes of different species, optimum induction and challenging temperatures were to be standardized. In the present investigation development of a Temperature induction response protocol for cotton genotypes was accomplished. In this protocol, seedlings were initially exposed inductive temperature (gradual temperature raised from 28 to 40℃) for 4 h and non-inductive temperature (46℃ for 3 h, 47℃ for 3 h, 48℃ for 3 h and 48℃ for 4 h) for specific time duration. Further, the recovery growth period at the end of 48 hrs 30°C temperature was assessed.

In the present investigation despite the exposure of different cotton genotypes to optimum induction temperature, lethal temperature and the recovery growth differed among the different genotypes. Variation in the stress adaptive mechanisms in between the genotypes might be the reason for observed differences for thermo tolerance. Previously it was reported that induction stress mainly alters the gene expression and brings greater adaptation to temperature stress and genetic variability in thermo tolerance is seen upon induction stress.

When the temperature is increasing the membrane damage will be more and it will disrupt the leaf membranes, then the proteins will shuffle in order to change the positions and thereby will be a less chance for its survival when it get damaged. Percentage of seedlings survivability was declined when the plants are subjected to continuous stress period. Similarly, this trend was shown in some crops like cotton (Kheir *et al*., 2012), rice (Vijayalakshmi *et al*., 2015), maize (Dar *et al*., 2016) and chickpea (Raghavendra *et al*., 2017).

It showed that the cell viability and high temperature was indirectly correlated with each other. With increase in gradual temperature it showed decreasing the viable cell counts. Similarly, Kheir *et al.* (2012) reported that the cell viability percentage of cotton estimated by Evan's blue method which mainly had higher viable cell count (87.01%) in heat tolerant genotype as that of heat susceptible (34.41%).

High temperature stress that mainly impairs the mitochondrial functions thereby resulting in the induction of oxidative damage which leads mainly that manifests in lipid peroxidation, detected by malondialdehyde content (Larkindale & Knight 2002; Vacca *et al*., 2004). Heat stress also causes increased membrane damage due to lipid peroxidation (Amirjani, 2012). Malondialdehyde content will be more in the susceptible genotypes when compared to the tolerant genotypes because the damage which will be more in case of susceptible genotypes as they undergo more lipid peroxidation whereas tolerant genotypes will be able to overcome the effect by decreasing the effect of ROS production. Similarly, Vijayalakshmi *et al.* (2015) observed that lipid peroxidation (MDA content) was lower in inductive temperature when compared to non-inductive temperature in rice.

Increase in total soluble protein content with the occurrence of high temperature might be a reason for restructuring and accumulation of protein fractions. Similarly Ashraf *et al.* (1994) reported in sorghum and barley where the majority of heat shock proteins were associated with the soluble fractions only.

CONCLUSION

This study mainly concludes that, the screening of 10 days old cotton seedling through the temperature induction response (TIR) expressed that the seedling survival, cell viability, TSP and MDA content was higher in KC3, SVPR6 and moderate in TSH325, TSH357 and least in TSH375, TSH383. Further it was inferred that KC3, SVPR6 varieties had shown high thermo tolerance capacity when compared to all other genotypes when they are subjected to high thermo tolerance. Finally due to induction of high temperature by TIR protocol the plants which are subjected to severe stress condition will be affected by damage caused to the thylakoid membrane which leads to damage of ATP synthesis due to loss in proton motive force, so the cell may loss its function even though some of the enzymes which will work on optimum temperature also. Some of the tolerant genotypes which showed positive values maintain their resistant mechanism by decreasing the ROS production by increasing the antioxidant enzymes capacity.

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SUPPLEMENTARY MATERIALS

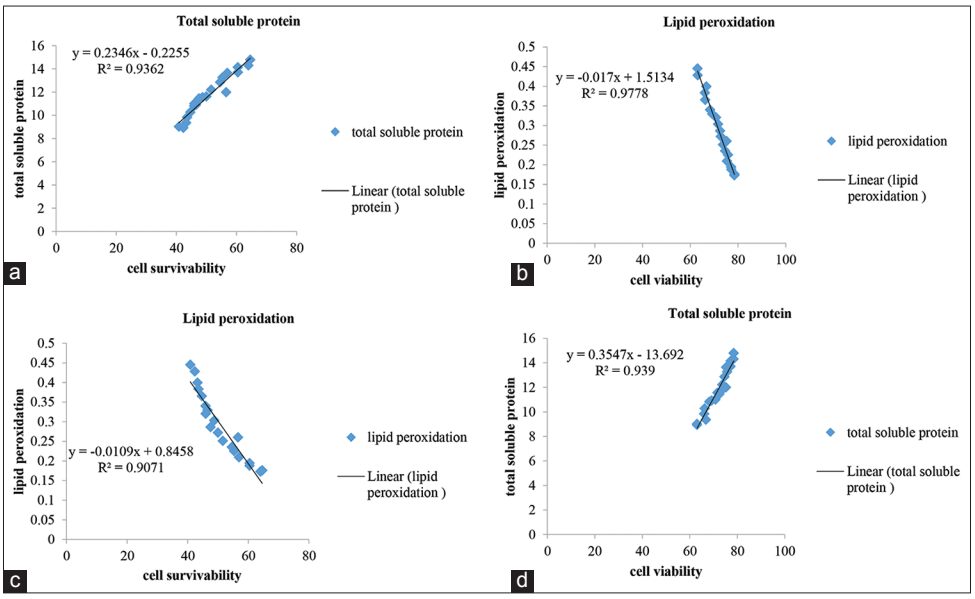

Figure S1: Regression for genotypes. All the genotypes and treatments are correlated with each other and regression graphs are represented above.

Figure S2: Heat map analysis. Heat map for following 20 genotypes which are distributed based on their values for each representing colours for each parameter and they are representing as >50 dark green, < 50 light green, decimals in red and above 10 orange colour.

Figure S3: a) 10 days Cotton seedlings in cups, b and c) Induction of stress in TIR, d) Lab analysis for all treatments.

Table S1: Correlation of genotypes

	Cell survivability	Cell viability	Lipid peroxidation	Total soluble protein
Cell survivability	1.000			
Cell viability	0.947	1.000		
Lipid peroxidation	-0.952	-0.989	1.000	
Total soluble protein	0.968	0.969	-0.987	1.000

Table S2: Correlation of treatments

	Cell survivability	Cell viability	Lipid peroxidation	Total soluble protein
Cell survivability	1.000			
Cell viability	0.989	1.000		
Lipid peroxidation	-0.973	-0.989	1.000	
Total soluble protein	-0.853	-0.915	0.891	1.000