Screening of Cotton Genotypes for Heat Tolerance

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Abstract

Cotton (*Gossypium hirsutum* L.) is being grown in both the tropical and temperate regions of the world and is greatly influenced by climatic factors. Cotton requires more than 180 days with temperature above 15°C and excessive temperature (above 36°C) affects its growth, development and reproduction significantly. The present investigation was carried out with 40 genotypes of cotton, collected from different parts of the country, at Agricultural College Farm, Bapatla to elicit information on heat tolerance using cellular membrane thermostability and enzyme viability. The mean relative electrical conductivity values showed gradual increase from 32.06 at 25°C to 84.15 at 50°C indicating higher temperatures had direct effect on the leakage of fluids from the cells and higher levels of cell injury. The dehydrogenase enzyme viability was reduced with the increase in the temperature from 30°C and 50°C. The genotypes L788, RAH 912 and Pee Dee 0113 showed high levels of heat tolerance in both the experiments at 50°C and these genotypes can be exploited in the breeding programmes for heat tolerance. Genotypic differences for cellular membrane thermostability and enzyme viability were observed. The genotypic and phenotypic coefficients of variation were moderate while heritability coupled with high genetic advance as percentage of mean was high for these characters indicating the operation of additive gene action in controlling these traits. This study also indicated the importance of these parameters as easy screening tools for heat tolerance in cotton.

1. Introduction

High temperature causes irreversible damage to plant function and development. The magnitude of heat stress effect rapidly increases as temperature increases above a threshold level. The predicted global warming further magnifies the significance of high temperature mediated effects on plant productivity. High temperature affects membrane-linked processes due to alteration in membrane fluidity and permeability (Abdelbagi et al., 1999 and Sangwan et al., 2002). Enzyme function is also sensitive to changes in temperature. Heat- induced alteration in enzyme activity can lead to imbalance in metabolic pathways or heat can cause complete enzyme inactivation due to protein denaturation (Vierling, 1991).

Cotton production, in both the tropical and temperate regions of the world, is greatly limited by climatic factors. Cotton has an optimal thermal kinetic window of 23 to 32°C in which metabolic activity is most efficient. High temperatures (>35°C) throughout the growing season are commonplace among the cotton production areas and exceed the thermal kinetic window for which metabolic activity is most efficient in cotton plants, thereby limiting the growth and development of the crop and hence yield and fibre quality.

In the last century, carbon dioxide concentration (CO_2) has risen rapidly from about 350 µmol mol^{-1} in 1980 to 378 µmol mol^{-1} at present. At the current rate of gas emissions and population increase, it is predicted that (CO_2) will double by end of this century. These changes in CO_2 and other greenhouse gases are predicted to increase surface mean temperature in the range of 1.4-5.8ºC, in addition, studies showed that future climate will have more frequent short episodes of high temperature. Preferential selection for heat tolerant cultivars may delay the onset of heat stress in the plant throughout the season, thereby minimizing yield loss whilst maintaining fibre quality in a hot year (Singh et al., 2007).

Different physiological mechanisms may contribute to heat tolerance in any crop-improvement programme. Therefore it is imperative to use cost efficient, reproducible and reliable techniques to screen available germplasm for various ecophysiological, morphological and reproductive traits to assist their utilization in crop breeding programmes. Several

methods in both field and controlled environment facilities are commonly being used for screening heat tolerance. Field studies are more advantageous than controlled environment as they represent the true nature of farmer's and breeder's field conditions. However, the major limitation is the lack of control of the environment, which makes the screening procedure difficult.

The conventional screening of large germplasm has been very resource intensive and time consuming because the success of any breeding programme depends on the effective evaluation and utilization of available and suitable germplasm. Laboratory based assays are generated for identification of cultivar specific heat tolerance under field conditions. The effectiveness of concurrent measurements of membrane integrity, enzyme viability, gas exchange and chlorophyll fluorescence for field grown leaf material to resolve genotypic difference in heat tolerance may be very helpful in identifying the desirable genotypes for higher temperatures (Singh et al., 2007). Keeping this in view a systematic effort was made to elucidate information on the existence of heat tolerance in Indian upland cotton genotypes.

2. Materials and Methods

The experiment was conducted during *kharif* 2011-12 in randomized block design with 40 germplasm lines obtained from all over India with three replications following spacing of 120×60 cm at Agricultural College Farm, Bapatla, Andhra Pradesh. Youngest fully expanded leaves from five plants replication^{-1} genotype^{-1} were taken at peak flowering period for estimating cellular membrane thermostability and enzyme viability.

Cellular membrane thermostability was measured following the method proposed by Sullivan (1972). The vials were incubated in a controlled temperature water bath and at various temperatures (specific for each experiment) for 1 h at a specified incubation temperature (t=35^{o}, 40^{o} and 50^{o}C). Samples incubated at high water bath temperatures (>25^{o}C) were left to cool to 25^{o}C. Initial electrical conductivity (IECt), a measure of membrane leakage, was determined using EC bridge. Discs were then autoclaved at 121^{o}C and 103 k Pa for 15 minutes then cooled to 25^{o}C. Final electrical conductivity (FECt) of the solution was measured with the calibrated conductivity meter. Relative electrical conductivity (RECt) was then determined by using the formula RECt (%)=(IECt÷FECt)×100**.** Relative cellular injury (RCIt) was also determined as per Rahman et al. (2004). Lower values of RCI% indicates less damage to cellular membranes and *vice versa*. Hence, lower RCI% was interpreted as higher cellular membrane thermostability (CMT) due to heat tolerance and higher RCI% as lower cellular membrane thermostability.

In enzyme viability assay, enzyme extraction was done following the method proposed by Mahadevan and Sridhar (1982). Triphenyl tetrazolium chloride (TTC) dye was included in the reaction mixture and the rate of reaction was measured according to the degree of reduction of the dye. Colorimetric method first introduced by Kun and Abood (1949) was used to know the reduction of the dye.

The data was statistically analyzed to estimate phenotypic and genotypic coefficients of variation (PCV and GCV) using the formula proposed by Burton (1952). Heritability in broad sense was estimated as per Lush (1940) while genetic advance was estimated as per the formula proposed by Lush (1940) and Johnson et al. (1955).

3. Results and Discussion

In the present study cellular membrane thermostability and enzyme viability were used to know the heat tolerance of the cotton genotypes.

3.1. Cellular membrane thermostability assay

Cellular membrane thermostability (CMT) was measured indirectly through measuring relative cell injury (RCI%), which reflects the relative electrical conductivity (REC) i.e., the relative amount of electrolytes leaked out because of damage to cell membranes caused by heat shock. Relative cell injury% is an indicator of cellular membrane thermostability.

In the present study, cellular membrane thermostability was studied by using REC values. REC values were recorded at controlled temperature (25^{o}C), 35^{o}C, 40^{o}C and 50^{o}C to know the effect of higher temperatures on the cell membrane. The REC values of 40 genotypes at four different temperatures are presented in Table 1. REC values recorded variation i.e., 10.71 (L 788) to 64.71 (P 403) at controlled temperature (25^{o}C) to 56.00 (RAH 912) to 100.00 (L 763 and L 765) at 50^{o}C which represents the effect of higher temperature on cell membrane and electrolytes release from the cells. The genotypes P 403 (64.71), L 761 (63.64), NDLH 1939 (55.00) and L 766 (53.33) recorded maximum REC values where as the genotypes L 788 (10.71), KGL 54620 (11.90) and JK-276-4 (18.18) recorded the lowest values at controlled temperatures (25^{o}C).

The genotypes P 403, NA 1584, NDLH 1938, L 763 and L 766 recorded higher REC values at while the genotypes, L 788 (14.29) and RAH 912 (16.00) showed the lowest values at 35^{o}C indicating their levels of tolerance to normal cotton growing temperature. The genotypes, L 763 (93.33), L 761 (90.91) and NDLH 1939 (90.00) recorded REC values more than 90 at 40^{o}C indicating their susceptibility to higher temperature. Whereas the genotypes L 788 (32.14), KGL 54620 (35.71) and RAH 912 (36.00) recorded lowest REC values at 40^{o}C revealing their high levels of heat tolerance and their usefulness

Table 1: REC values at control (25°C), 35°C, 40°C and 50°C in 40 genotypes of cotton (*Gossypium hirsutum* L.)

Sl. No.	Genotype	REC (25°C)	REC (30°C)	REC (40°C)	REC (50°C)
1	GSHV 97/291	21.05	42.11	57.89	73.68
2	GJHV 338	38.89	55.56	77.78	88.89
3	GBHV 164	39.13	47.83	78.26	82.61
4	G.ageti	38.10	57.14	66.67	90.48
5	G.cot 12	25.00	41.67	62.50	95.83
6	HAG 812	41.67	58.33	83.33	95.83
7	HS 271	26.67	40.00	73.33	86.67
8	HLS 72	31.25	50.00	81.25	87.50
9	H 492	38.89	50.00	72.22	88.89
10	JK-5	29.41	41.18	58.82	76.47
11	JK 276-4	18.18	31.82	54.55	86.36
12	JK 276-10-5	27.27	40.91	72.73	95.45
13	KDCKAD	31.58	52.63	78.95	89.47
14	KH 121	21.43	32.14	53.57	82.14
15	NDLH 1938	45.45	63.64	81.82	90.91
16	KGL 54620	11.90	21.43	35.71	80.95
17	NISC 40	46.67	53.33	80.00	86.67
18	NA 1584	47.06	64.71	82.35	88.24
19	NDLH 1939	55.00	70.00	90.00	90.00
20	NA 1290 BP	45.45	54.55	81.82	86.36
21	Pee Dee 0113	30.77	34.62	50.00	57.69
22	P 403	64.71	70.59	76.47	82.35
23	RS 419	31.25	50.00	75.00	75.00
24	RAH 902	22.58	29.03	45.16	83.87
25	RS 2557	22.22	38.89	66.67	72.22
26	RAH 912	16.00	16.00	36.00	56.00
27	RAH 216	33.33	53.33	73.33	73.33
28	TCH 1716	15.79	31.58	68.42	89.47
29	TSH 9974	24.14	31.03	68.97	89.66
30	VIKRAM	15.63	25.00	40.63	62.50
31	Narasimha	33.33	53.33	80.00	93.33
32	L 761	63.64	72.73	90.91	90.91
33	L 766	53.33	60.00	80.00	86.67
34	L 763	40.00	60.00	93.33	100.00
35	L 769	22.73	36.36	72.73	77.27
36	L 765	25.00	40.00	85.00	100.00
37	L 770	21.21	27.27	57.58	93.94
38	L 603	25.00	40.00	75.00	95.00
39	L 604	31.03	37.93	62.07	82.76
40	L 788	10.71	14.29	32.14	60.71
Mean		32.04	44.77	68.82	84.15
CD (p=0.05)		1.34	1.08	1.73	1.61

in breeding programmes.

At 50°C temperature, the genotypes L 763 and L 765 recorded REC values 100.00 indicating their very survival at higher temperatures is in doubt. While the genotypes L 603 (95.00), JK-276-10-5 (95.45), G.cot (95.83) and HAG 812 (95.83) recorded more than 95% of REC values indicating their inability to tolerate high temperature. The genotypes, RAH 912 (56.00), Pee Dee 0113 (57.69), L 788 (60.71) and VIKRAM (62.50) showed the lowest REC values at 50°C indicating their higher levels of tolerance to increase in temperature and their utilization in the heat tolerance breeding programmes. These genotypes may have high levels of buffering capacity to tolerate high temperature by adjusting the cell membrane permeability and injury to the cell membrane.

The mean values of REC at four different temperatures are presented in Figure 1. This clearly indicated the gradual increase of REC from 32.06 at 25°C to 84.15 at 50°C. The higher temperatures had direct effect on the leakage of fluids from the cells which in turn showed higher REC values (Rahman et al., 2004; Bibi et al., 2006; Senthil Kumar and Udayakumar, 2004; Singh et al., 2007 and Cottee et al., 2010).

The calculated relative cell injury% (RCI%) values were ranged between 31.66 (Pee Dee 0113) and 100 (L 763 and L 765) with a mean of 78.27 (Table 2). The RCI% values showed that the cell injury levels were very high in the genotypes like L 763 and L 765 indicating higher temperatures had direct effect on the electrolytes leakage and damage to the cells. The genotypes, Pee Dee 0113 (31.66), RAH 912 (44.27), VIKRAM (54.69) and L 788 (56.99) recorded low levels of cell injury at 50°C indicating their high levels of tolerance to higher temperature and these genotypes had the ability to maintain the cell membrane integrity and structure at high temperature. Hence, these genotypes may be considered for heat tolerance

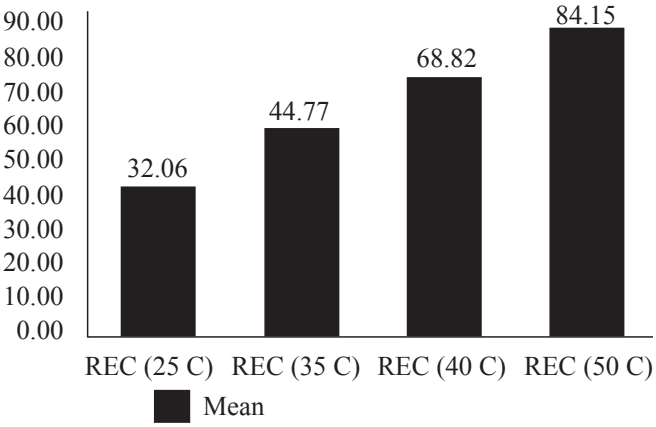

Figure 1: Representation of mean REC values at control (25°C), 35°C, 40°C and 50°C in 40 genotypes of cotton (*Gossypium hirsutum* L.)

Table 2: RCI% values at 50°C in 40 genotypes of cotton (*Gossypium hirsutum* L.)

Sl. No.	Genotype	RCI (%)
1	GSHV 97/291	64.91
2	GJHV 338	85.19
3	GBHV 164	77.87
4	G.ageti	87.85
5	G.cot 12	93.75
6	HAG 812	94.02
7	HS 271	80.74
8	HLS 72	80.56
9	H 492	82.41
10	JK-5	70.59
11	JK 276-4	79.55
12	JK 276-10-5	93.94
13	KDCKAD	86.73
14	KH 121	75.45
15	NDLH 1938	88.07
16	KGL 54620	72.29
17	NISC 40	79.49
18	NA 1584	83.53
19	NDLH 1939	84.76
20	NA 1290 BP	81.49
21	Pee Dee 0113	31.66
22	P 403	73.91
23	RS 419	66.67
24	RAH 902	76.81
25	RS 2557	65.28
26	RAH 912	44.27
27	RAH 216	63.08
28	TCH 1716	86.84
29	TSH 9974	86.64
30	VIKRAM	54.69
31	Narasimha	90.77
32	L 761	84.55
33	L 766	76.97
34	L 763	100.00
35	L 769	70.17
36	L 765	100.00
37	L 770	90.68
38	L 603	94.19
39	L 604	73.63
40	L 788	56.91
Mean		78.27
CD (p=0.05)		1.64

breeding programmes in cotton.

3.2. Enzyme viability assay

High temperature stress can alter the conformation, composition and permeability of the thylakoid membrane in the chloroplasts. Cyclic phosphorylation is unable to compensate for the leakiness of the lipid membrane resulting in disruption of electron transport between PSI and PSII thereby reducing energy availability. This may further reduce or alter enzymatic activity and limit net photosynthesis at temperatures above 35°C. The physiological viability of a large number of plant samples at a particular point of time could be assessed by assessing the dehydrogenase activity in mitochondrial respiratory electron transport chains. The tetrazolium viability test is a simple assay that may be used to determine the physiological viability of a large number of plant samples at a particular point of time. Heat tolerant plants are better able to reduce 2,3,5-triphenyltetrazolium salts to an insoluble red formazan compound by accepting electrons from the electron transport chain *via* the dehydrogenase pathway, this reduction can be correlated to the level of enzyme viability. The dehydrogenase enzyme viability of the genotypes was studied by taking the absorbance values of the extracted enzyme of the genotypes at 30°C and 50°C. The absorbance values showed inverse relationship with high temperature i.e., the dehydrogenase activity reduces with increase in temperature. The genotypic variation in absorbance values with increase in temperature are presented in Table 3 and the results are discussed here under.

The genotypes Pee Dee 0113 (0.34), NDLH 1939 (0.32), L 766 (0.31), L 788 (0.31) and RAH 912 (0.29) recorded highest absorbance values, while the genotypes L 769 (0.12), L763 (0.14), JK-276-4 (0.15) and GBHV 164 (0.15) recorded lowest absorbance values at 30°C. This indicates the genotypes

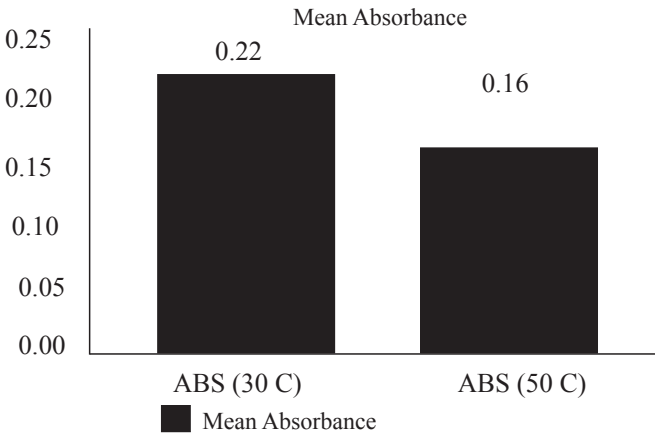

Figure 2: Representation of mean absorbance values at 30°C and at 50°C in 40 genotypes of cotton (*Gossypium hirsutum* L.)

Table 3: Absorbance values at 30°C and 50°C in 40 genotypes of cotton (*Gossypium hirsutum* L.)

Sl. No.	Genotype	Absorbance (30°C) (µg)	Absorbance (50°C) (µg)
1	GSHV 97/291	0.17	0.12
2	GJHV 338	0.18	0.13
3	GBHV 164	0.15	0.09
4	G.ageti	0.28	0.21
5	G.cot 12	0.25	0.19
6	HAG 812	0.18	0.12
7	HS 271	0.20	0.14
8	HLS 72	0.25	0.12
9	H 492	0.18	0.12
10	JK-5	0.25	0.20
11	JK 276-4	0.15	0.12
12	JK 276-10-5	0.16	0.13
13	KDCKAD	0.19	0.12
14	KH 121	0.18	0.13
15	NDLH 1938	0.23	0.16
16	KGL 54620	0.21	0.16
17	NISC 40	0.25	0.16
18	NA 1584	0.24	0.16
19	NDLH 1939	0.32	0.26
20	NA 1290 BP	0.22	0.17
21	Pee Dee 0113	0.34	0.30
22	P 403	0.27	0.20
23	RS 419	0.17	0.10
24	RAH 902	0.21	0.15
25	RS 2557	0.22	0.19
26	RAH 912	0.29	0.26
27	RAH 216	0.21	0.16
28	TCH 1716	0.22	0.16
29	TSH 9974	0.16	0.10
30	VIKRAM	0.28	0.23
31	Narasimha	0.30	0.27
32	L 761	0.13	0.08
33	L 766	0.31	0.24
34	L 763	0.14	0.09
35	L 769	0.12	0.10
36	L 765	0.16	0.12
37	L 770	0.29	0.22
38	L 603	0.19	0.15
39	L 604	0.19	0.12
40	L 788	0.31	0.28
Mean		0.22	0.16
CD (p=0.05)		0.03	0.02

Pee Dee 0113, NDLH 1939, L 766 and L 788 had higher levels of dehydrogenase activity at 30°C. The low levels of dehydrogenase activity at 30°C in the genotypes L 769, L763, JK-276-4 and GBHV 164 indicates their low levels of dehydrogenase enzyme activity in mitochondrial energy production system of these genotypes.

At 50°C, the genotypes Pee Dee 0113 (0.30), L 788 (0.28), Narasimha (0.27), RAH 912 (0.26) and NDLH 1939 (0.26) showed higher levels of absorbance indicating the maximal performance of dehydrogenase activity even with high temperature of 50°C. So these genotypes can be exploited for heat tolerance breeding programmes. The mean absorbance values decreased from 0.22 at 30°C to 0.16 at 50°C which indicates the reduction in dehydrogenase activity with increase in temperature (Figure 2).

3.3. Genetic parameters for RCI and enzyme viability

The analysis of variance showed significant differences for RCI% and enzyme viability among the genotypes at 50°C (Table 3). The estimates of PCV and GCV (18.45 and 18.40) for RCI% were moderate. High heritability (99.51) and high genetic advance as percentage of mean (37.82) were also observed for this trait indicating the operation of additive gene action (Table 4). The PCV and GCV estimates of enzyme viability (35.91 and 35.22) were high indicating the genetic basis of variability in these genotypes for this parameter.

High heritability (96.21) and high genetic advance as percentage of mean (71.18) were also observed for this trait indicating the operation of additive gene action (Table 4). The

Table 4: Genetic variability, heritability (broad sense) and genetic advance as percentage of mean (GAM) for seed cotton yield and yield components in cotton (*Gossypium hirsutum* L.)

Parameter	Relative cell injury % (RCI%)	Enzyme viability (Absorbance)(µg)
PCV	18.45	35.91
GCV	18.40	35.22
Heritability	99.51	96.21
GAM	37.82	71.18

experiments of cellular membrane thermostability and enzyme viability clearly indicated the existence of variation in the genotypes for thermotolerance.

The genotypes Pee Dee 0113, RAH 912 and L788 showed high levels of tolerance in both the experiments at 50°C and these genotypes can be used as sources for heat tolerance genes in the breeding programmes.

4. Conclusion

Thus, the genotypes, Pee Dee 0113, RAH 912 and L788

showed high levels of tolerance in both the experiments at 50°C and their usefulness as source for heat tolerance genes in the breeding programmes. This study also highlighted the use of cellular membrane thermostability and enzyme viability as large germplasm screening tools for heat tolerance in cotton.

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