Research Article

Estimation of Relative Cell Injury in Response to Heat Stress in *Gossypium hirsutum* L.

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Abstract | Pakistan's main source of fiber is cotton. Both living (biotic) and non-living (abiotic) stimuli, including high temperatures, have a deleterious impact on its formation. The goal of the current study was to investigate the genetic foundation of upland cotton's heat tolerance. On the basis of CMT%, canopy temperature, seed cotton production, node number of first fruiting branch, days to first effective boll, heat index (HI), and heat tolerance index (HTI), fifty upland cotton genotypes were screened against high temperatures. Based on the traits utilized for screening, there were fifty genotypes with significant variation. Five heat-sensitive genotypes (Ali akbar-703, Ali akbar-708, IR-1524, Tarzan-1, CIM-598) and seven heat-tolerant genotypes (CIM-602, Cyto-178, MNH-1020, FH-142, MNH-1026, MNH-886 and IUB-222) were chosen. These selected heat tolerant genotypes can be utilized further in breeding program

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Introduction

The world's hot, semi-arid regions are where cotton is found. Extreme temperatures have an impact on this crop's growth, development, and reproduction for over one hundred eighty (180) days between temperatures of 15°C to 36°C (Baloch *et al*., 2000). Although heat and high temperatures negatively impact cotton output and fiber quality, biotic (diseases and pests) and abiotic (warmth, dry spell, and salty) stressors also have a significant impact on cotton yield (Abro *et al*., 2015). After industrialization's mechanical transition in the 1940s, a developing and abnormal weather change drift started, and it has continued without end ever since. The increase in temperature around the turn of the century was about 0.7 °C. According to Rasul *et al*. (2011), this growth has outpaced the first decade of the twenty-first century by about 1 °C. The unburned carbon dioxide, nitrogen dioxide, methane, and hydrocarbons emitted from vehicle exhaust are the main contributors to an increase in Earth's temperature. Since 1980 till 2006, the amount of carbon dioxide has increased from 350 mol^{-1} to around 378 mol^{-1}, and by the end of the twenty-first century, it is expected to have doubled. It is projected that methane, carbon dioxide, and nitrogen dioxide will increase mean temperature. Thylakoid membrane leaking was the primary cause of this decreased rate (Bibi *et al*., 2008). According to Ashraf *et al*. (1994), the cotton belt of Pakistan frequently experiences summer temperatures of up to 50 °C, however cotton seeds do not germinate at that temperature. As a result, rising high temperature stress is becoming a concern, particularly for cotton crops that are grown throughout the hot season in hot regions of the world. Different methods have been created by plants to cope with extreme temperature stress. Plant reproducers face a challenge due to the complex genetic makeup of heat resistance, which is further strengthened by the occurrence of wide levels of genic and epistatic connections (Cossani and Reynolds, 2012). The physiological processes, biochemical reactions, and morphological indications of the cotton plant are drastically altered under high temperature stress. These changes have an impact on plant growth and development, which significantly reduces cotton seed output and, ultimately, economic yield. By creating genotypes that are tolerant of high temperatures using traditional and molecular methods, these adverse effects can be reduced (Wahid *et al*., 2007). The type of crop and stage of plant growth that are exposed to the heat stress episode affect the severity of potential damages. It is unclear, though, whether the detrimental effects of high temperature stress occurring at various phases of growth are cumulative (Wollenweber *et al*., 2003).

Species and cultivars respond differently to heat stress depending on their developmental phases. However, high temperature stress has a major impact on growth phases that are almost vegetative and reproductive. For instance, high daytime temperatures during the vegetative growth stage harm the leaf's gaseous exchange system. A brief period of heat stress during the reproduction stage is what causes floral buds and flowers to shed. There is a wide range of tolerance and sensitivity among plant species (Guilioni *et al*., 1997; Young *et al*., 2004). Due to the greater sensitivity of pollen grains to high temperatures than ovules, high temperatures result in less fertilization. Compared to other vegetative tissues, pollen grains and tubes demand more energy (Burke *et al*., 2004). High temperatures impede carbohydrate generation and dispersion for growing sinks, which ultimately results in less of it being produced (Liu *et al*., 2006). Additionally, this process raises dark respiration and photorespiration while decreasing photosynthetic capacity. According to Snider *et al*. (2009), heat stress also lowers the pollen grains carbohydrate content and the amount of ATP in the stigmatic tissues. In many agricultural species, this mechanism results in fewer blooms per plant, fewer fruits per plant, and eventually a poorer yield per plant (Peet *et al*., 1998; Sato *et al*., 2006). However, some physiological and morphological characteristics allowed scientists to detect variations in response to high temperatures. For instance, higher CMT% suggests greater tolerance and stability in cotton output under heat stress circumstances, while lower canopy temperature shows stronger resistance to high temperatures.

Combining ability effects enabling the researchers to better understand the general and specific patterns of how various genotypes interact with one another to produce enticing results (Braden *et al*., 2003). High values of SCA effects revealed the role of non-additive dominant genes in proposing hybrid breeding programs for crop development, while significant values of GCA effects demonstrated the role played by additive genes in advising early choices for the improvement of the cotton crop. Due to the effective application of hybrid (heterosis) breeding in corn crops, the construct can be used to evaluate F1 predominance over superior parents in cotton and other species as well. The degree of dominance, the hereditary separation between selected parental genotypes, and the genetic differences that predominate among parents are all factors in manipulating heterotic effects. Considering the significance of genetic.

Materials and Methods

Screening of cotton germplasm for heat tolerance

For testing against high temperature stress, 50 upland cotton genotypes were gathered from the Cotton Research Institute (CRI) in Multan. The experiment was carried out in the field in 2021 using two sowing dates: mid-June for normal temperature at peak flowering and sowing in April for maximum temperature at peak flowering. The split plot arrangement was used, along with a randomized complete block design (RCBD) with two replications. By building a tunnel out of polythene sheets, bamboos, and ropes and leaving it open at night for 15 days while the plant was 50% flowering, the temperature was regulated and maintained. Cellular membrane thermostability (CMT), canopy temperature, days to first effective boll, and node number of first fruiting branch were used to choose the available germplasm.

Cell membrane thermostability

Five plants of each genotype were selected for heat treatment at 50% blooming, under both normal and heat-stressed conditions. For the same sample size, leaves were cut into equal-sized circles and submerged in distilled water in falcon tubes at 250°C for 24 hours. Before autoclaving, EC meters were used to measure T1 (EC of sap (treatment)) and C1 (EC of sap (control)). The electrolyte was then released from these falcon tubes by placing them in a water bath at 500C for an hour. The EC of sap in these falcon tubes was measured as T2 (EC of sap (treatment) after autoclaving) and C2 (EC of sap (control) after autoclaving) after being allowed to cool at room temperature. The following formula was used to determine Relative Cellular Injury%:

$$RCI\% = (1-[\{1-(T_1/T_2)\}/\{1-(C_1/C_2)\}]) \times 100$$

Where; T_1= EC of sap (treatment) before autoclaving; T_2= EC of sap (treatment) after autoclaving; C_1= EC of sap (control) before autoclaving; C_2= EC of sap (control) after autoclaving

CMT% is reciprocal term of RCI% showing same effects so both can be used antagonistically for thermo tolerance. RCI% and CMT% are related by the formula given below:

$$CMT\% = 100 - RCI\%$$

Therefore, formula for CMT% was modified and used in this study as below:

$$CMT\% = [\{1-(T_1/T_2)\}/\{1-(C_1/C_2)\}] \times 100$$

Canopy temperature

Canopy temperature of five guarded plants is an important criterion to differentiate genotypes of *Gossypium hirsutum* L. Therefore, in the present investigation canopy temperature of five guarded plants were measured with the help of Infrared thermometer (IRT) at 11:00 am until 04:00 pm.

Days to first effective boll

From five selected plants of each genotype days to first effective (splitted) boll were counted and then arithmetic mean was taken.

Node number of first fruiting branch

Node number of first fruiting branch was counted from each selected plant in such a way that node cotyledon was counted as zero node.

Results and Discussion

Seed cotton yield

The yield of seed cotton is the ultimate outcome of all biotic and abiotic stressors. Picking began when the dew drops on five chosen plants had dried up at maturity. Two pickings of the chosen plants were done. A separate paper bag was used to gather the yield from a single plant. It was measured how much seed cotton each plant produced on average.

Twelve genotypes were chosen based on the performance of the germplasm measured by cell membrane thermostability, canopy temperature, days to the first effective boll, and node number of the first fruiting branch. Of these genotypes, seven (CIM-602, Cyto-178, MNH-1020, FH-142, MNH-1026, MNH-886 and IUB-222) were high temperature tolerant, while five (Ali Akbar-703, Ali Akbar-708, IR-1524, Tarzan-1, CIM-598) showed less yield.

Table 1: *Mean square values for canopy temperature, node number of first fruiting branch, days to first effective boll and seed cotton yield RCBD.*

Sov.	d.f.	CT	NFFB	DFEB	SCY
Rep	1	4.86	0.03604	0.16	34.7
Trt	1	1638.52**	0.02703	74.82**	20134.7**
Error a (rep*trt)	1	0.02	0.04324	0.75	0.7
Genotype	49	7.41 **	5.40760*	2715.16**	1426.1**
Trt*Genotype	49	4.31**	0.03546*	4.31**	38.0**
Error b	98	0.48	0.03002	0.31	6.2
Total	199				

Cell membrane thermostability

CMT% mean values for fifty genotypes range from 35.97 (CIM-598) to 71.38 (MNH-1020). The genotypes with the greatest values were taken as tolerant parents for breeding, while the genotypes with the lowest values were thought to be prone to heat stress. According to Table 1, CIM-598 had the lowest values (35.97), followed by IR-1524 (36.07), Ali Akbar-708 (36.55), AA-703 (38.69), and Tarzan-1 (39.84). While tolerant values ranged as MNH-1020 (71.38), Cyto-178 (69.14), CIM-602 (65.68), and MNH-1026 (65.02) had the highest values (Table 2). Cell membrane thermostability percentage (CMT%)

Table 2: *Mean performance of fifty upland cotton genotypes for heat index (HI), heat tolerance index (HTI) and cell membrane thermostability (CMT%).*

S. No.	Genotypes	HI	HTI	CMT
1	CIM-600	106.05	87.64	60.92
2	Lalazar	122.27	72.28	50.04
3	FH-312	119.21	65.27	51.06
4	MNH-988	124.59	65.97	52.31
5	FH-113	110.11	87.29	63.40
6	MNH-992	117.94	65.59	53.36
7	BH-185	118.91	62.34	48.32
8	CIM-602	110.13	86.45	65.68
9	RH-647	128.26	70.94	42.11
10	SLH-8	121.53	63.11	52.26
11	BS-52	121.31	55.88	43.26
12	FH-114	112.18	87.59	64.29
13	IR-1524	130.43	48.69	36.07
14	AGC-999	115.78	67.03	52.35
15	NS-131	120.64	72.42	48.22
16	Tarzan-1	132.17	39.70	39.84
17	Rajat	123.55	61.58	45.04
18	ARK-2	116.89	71.05	51.41
19	CIM-598	130.11	32.48	35.97
20	ARK-5	116.17	59.52	53.18
21	HRV/O/P2	121.92	74.23	45.89
22	G-67/P3	134.66	67.37	48.70
23	FH-35	116.78	73.81	52.62
24	FH-215	121.10	76.60	55.83
25	TARZAN-1	116.15	61.25	52.05
26	NIAB-820	124.55	79.48	46.63
27	MNH-1026	113.06	82.35	65.02
28	IR-NIBGE-7	125.85	67.90	39.75
29	IR-NIBGE-6	123.45	64.80	46.14
30	AA-802	136.65	34.47	38.69
31	A-555	121.02	68.82	46.81
32	AA-703	116.42	67.01	50.71
33	KEHKASHAN	122.19	69.76	45.36
34	PB-896	122.34	70.15	59.80
35	MNH-1020	110.24	86.39	71.38
36	CRS-2007	116.70	74.08	58.19
37	S-3	124.60	65.42	48.80
38	AA-708	130.82	48.37	36.55
39	IR-3701	120.49	71.88	48.22
40	FH-118	123.97	69.49	48.59
41	FH-214	116.94	74.99	58.32
42	MNH-886	120.73	66.09	55.84
43	VH-15	116.69	74.91	58.57
44	CRS-456	123.89	60.92	44.30
45	FH-142	128.49	59.42	42.81
46	IUB-63	122.22	67.95	49.34
47	IUB-75	117.24	74.86	56.48
48	IUB-013	116.01	72.40	55.05
49	AGC-2	117.48	67.50	52.72
50	Cyto-178	111.16	86.93	69.14

is regarded as a trustworthy and quick method of avoiding thermotolerance. Both CMT% and RCI%, which are antagonistic terms, exhibit cell membrane leakage in response to high temperature stress, which lowers the rate of photosynthetic activity and, eventually, lowers yield.

Canopy temperature

Cotton's thermo-tolerance was measured using the canopy temperature. Under typical conditions, the average canopy temperature for fifty genotypes ranges from 26.59 (MNH-886) to 32.38 (Cyto-178). The genotypes with lower values were chosen as tolerant, whilst those with higher values were taken as sensitive under stressful conditions for crossing. The lowest value of canopy temperature demonstrated thermos-tolerance. MNH-1026 (28.04), MNH-1020 (27.89), FH-142 (28.05), MNH-886 (26.59), and IUB-222 (28.06) had the lowest results. Under typical circumstances, Cyto-178 (32.38) had higher values, followed by AA-703 (32.21) and AA-708 (32.16). The mean values under stress ranged from 32.47 (Cyto-178) to 40.89 (AA-708).

Conclusions and Recommendations

Heat stress is the one of the major crop growths limiting factor. Water stress at reproductive stage of cotton results in shedding of squares, flowers and ultimately reduced no of bolls and boll weight. Fiber quality characters are also badly affected by drought imposition. Hence there is dire need to develop drought tolerant cotton varieties that can perform well even under limited moisture condition. Farmers are advised to choose drought tolerant varieties for cultivation so that they can get maximum yield output from limited water resources.

Novelty Statement

This research study will help in selection of desirable parents of breeding programme development of heat tolerant genotypes in cotton.

Author's Contribution

Javed Iqbal: Research idea and write-up
Mamoona Hanif: Data Analysis
Nadia Hussain Ahmad: Write-up support
Amna Bibi: Helped in Data collection
Zaib-Un-Nisa: Result interpretation

Sadia Kanwal: Helped in proofreading and editing

Conflict of interest

The authors have declared no conflict of interest.

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