Pak. J. Bot., 47(6): 2085-2091, 2015.

SCREENING OF COTTON (*GOSSYPIUM HIRSUTUM* L.) GENOTYPES FOR HEAT TOLERANCE

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Abstract

Cotton yield is highly affected due to biotic (diseases and pests) and abiotic (heat, dought and salinity) Stresses. Among them, high temperature is the main environmental constraint which adversely reduces cotton yield and quality. High temperature above 36°C affects plant growth and development especially during reproductive phase. Present studies were carried out to assess the tolerance of fifty-eight newly evolved cotton genotypes to heat stresses, based on agronomic and physiological characteristics. The genotypes were screened in field conditions under two temperature regimes. The studies were conducted at experimental farm of Nuclear Institute of Agriculture, Tando Jam, Pakistan. The results showed that March sown crop experienced high temperature (i.e. > 44°C in May and June), which significantly affected crop growth and productivity. The genotypes were identified as heat-tolerant on the basis of relative cell injury percentage (RCI %), heat susceptibility index (HSI) values, boll retention and seed cotton yield (kg/ha). RCI level in cotton genotypes ranged from 39.0 to 86.0%. Out of 58, seventeen genotypes (viz.NIA-80, NIA-81, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1, NIA-Bt2, NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and check variety Sadori indicated high level of heat tolerance at both (heat-stressed and non-stressed) temperature regimes; as shown the lowest relative injury level and relatively heat resistant index (HSI<1) values. Such genotypes could be used as heat-tolerant genotypes under heat-stressed environments.

Key words: Cotton, *Gossypium hirsutum* L., High temperature tolerance, Cell Membrane-Thermostability (CMT).

Introduction

Cotton (*Gossypium hirsutum* L.) is a cash crop of Pakistan. It is highly affected by various diseases and environmental stresses like high temperature, drought and salinity. Among these all yield limiting factors, heat stress has significant effects on the growth, productivity and the quality of cotton crop. Recent research has indicated that high temperature is an important abiotic factor adversely affecting cotton yields (Oosterhuis, 2002). High temperatures (>35°C) are common throughout the cotton growing season in many regions of the world which adversely affecting growth and development of the crop and ultimately limiting the plant performance. The future cotton production is likely to occur under an increased prevalence of multiple abiotic stresses, including extreme and prolonged high temperature (Timothy.A and Michael A.Gore 2014). In Pakistan cotton is generally cultivated in warm areas (Riaz *et al*., 2013). The genotypes recommended for general cultivation in cotton growing areas, face very high temperature of about 50°C during the month of May and June, which is approximately 20°C higher than the optimum temperature required for its normal growth, thus retarding performance to higher extent. Plant growth such as shoot development, flowering and fiber quality traits are influenced largely due to high temperature (Farooq *et al*., 2015; Noshair Khan *et al.*, 2014).

The surface temperature of the planet has increased approx. 0.6°C, since the late 19th century. This rise in global temperature due to increasing concentrations of CO_2 and other greenhouse gases (e.g., methane, nitrous oxide, etc.) in the atmosphere which result from the excessive use of fossil fuels. Although cotton originated in warm climates, but it could sustain under certain level of high temperature stress. Existing and future increases in temperature during cotton crop urges the importance of taking initiatives for the evolution of germplasm with better tolerance to heat stress. Due to global warming, temperature fluctuations are faster than in the past and with increasing temperature, cotton yield have been decreased substantially. Bibi *et al.* (2008) reported that the optimum temperature for the photosynthetic carbon fixation cotton is about 33°C and photosynthesis decreased significantly at temperatures of 36°C and above. The high temperature environments (35-40°C) are frequently associated with infertility and cotton-boll retention problem and number of productive bolls, bolls retention is progressively reduced as the time per day at 40°C was increased (Reddy *et al.*, 1992b).

High temperatures also affects on Cell membrane thermo-stability (CMT). Sullivan (1972) proposed it an important criteria, which is a means to measure the amount of leakage of electrolyte from leaf discs immersed in deionized water after the exposure to heat treatment. The method has been used as a measure of thermal tolerance in various crops including rice, soybean, potato, tomato and cotton (Sing *et al*., 2007). CMT has been used in cotton as appropriate screening and selection criterion for heat tolerance and its ability to discriminate between heat tolerant and heat-sensitive genotypes (Malik *et al.*, 1999; Ashraf *et al*., 1994, Rahman *et al*., 2004, Azhar *et al.*, 2009). This technique is simpler, quicker and less expensive as compared to various other screening techniques for heat-tolerance. Potentially, it can be used with early vegetative stage leaf tissue from plants grown in field nursery environments. Keeping in view the importance of high temperature stress, the present studies were conducted to screen the newly evolved cotton germplasm and to identify the potential genotypes which possesses better tolerance to heat-stress and could produce appropriate yields in heat prone areas in the region.

Materials and Methods

To evolved cotton genotypes were evaluated in field conditions under two temperature regimes using varying sowing dates (15th March and 15th May) at experimental farm of Nuclear Institute of Agriculture (NIA), Tando Jam, Pakistan during year 2013-14. In the first study sowing was conducted on 15th March (heat stress regime 1) as to expose material to high temperature stress during flowering and boll setting stage; and second sowing was conducted on 15th May (non-heat stress regime 2 to expose the material to normal temperature during flowering and boll setting stage. Both studies were carried out with three replicates in randomize manner using randomize complete block design (RCBD). Each genotype was sown in four rows, 75 cm apart in plot size 6.1m x 3.0m. Distance between plants within rows was 30cm. All agronomic practices were done at proper time. The field observations were recorded from five plants, selected randomly from each genotype per replicate. Data on seed cotton yield/plant (kg/ha), bolls retention and Staple length (mm) were recorded at each picking.

Physiological trait i.e., relative cell injury percentage (RCI %) was measured for analysis of the CMT youngest fully expanded leaves 20-22 days age main stem in the peak flowering period. Meteorological data on daily minimum and maximum temperature (^{o}C) and precipitation was measured during entire crop season.

After completing the required data of each character, the analysis of the variance of each characteristic was performed according to Gomez & Gomez (1984). The samples from cotton genotypes were tested for CMT conducted through the method proposed by Sullivan (1972) method. The combination of temperature and duration of 50^{o}C for 60 minutes was used as done by (Rahman *et al*., 2004). The lowest values of RCI% are the indicators of less damage to cell membrane and vice versa. Therefore, the lower RCI% was interpreted as greater thermal stability of the cell membrane (CMT).

Formula: $RCI\% = 1 - [\{1 - (T1/T2)\} / \{1 - (C1/C2)\}] \times 100$

when
T1= EC of sap in 50°C before autoclaving
T2= EC of sap in 50°C after autoclaving
C1= EC of sap in 25°C before autoclaving
C2 = EC of sap in 25°C after autoclaving

The heat susceptibility index (HSI) was used as a measure of heat tolerance in terms of minimization of the reduction in yield caused by unfavorable versus favorable environments. HSI was calculated for each genotype according to Fisher and Maurer (1978).

Heat susceptibility index (HSI) = $(1\text{-}Y/Yp)/(1\text{-}X/Xp)$

where
Yh= seed cotton yield of genotye in a stress environment
Yp= seed cotton yield of genotye in a stress-free environment
X = Mean Yh of all genotypes
Xp = Mean Yp of all genotypes

Meterological data and physio-chemical analysis of experimental site: Meteorological data recorded during cotton crop season (2013-14), indicated high temperature from the month of April to third week of July (i.e. 37-44^{o}C). The precipitation (rainfall) was also inadequate only occur during the time of picking in the fourth week of July and third week of August (Fig. 1). The studies have shown that the cotton grow well around 32°C and the temperatures above 35°C had negative and drastic effects on the yield and quality. The optimum and ideal temperature for cotton is ranging from 20 to 30°C (Reddy *et al*, 1991 and Bibi *et al*., 2008). However, screening under high temperature through early sowing provided a good opportunity to screen the material to various heat stresses and to select high yielding advance breeding material with better tolerance to heat (Table 1).

Statistical analysis: Data recorded on various traits were analyzed statistically using analysis of variance (ANOVA) according to Gomez and Gomez (1984).

Table 1. Physico-chemical properties of soil (before sowing) at experimental site.

Characteristics	Value	
	0-15 cm	15-30 cm
ECe (dS m^{-1})	1.33	1.29
pH	8.0	8.1
Olsen's P (μg^{g-1})	8.9	7.76
Organic matter (%)	0.88	0.79
Kjeldhal's N (%)	0.057	0.049
Exchangeable K (μg^{g-1})	195	188
Textural class	Clay loam	Clay loam

Results and Discussion

Physiological and morphological traits: The mean square for different traits from analysis of variance presented in Table 2 showed that the genotypes and sowing dates are significantly different among each other. Relative cell injury percentage (RCI %) of 58 genotypes is presented in Fig. 2. Cell injury is a relative indicator of cell or tissue tolerance to heat. Low RCI reflects high cell membrane thermo stability (CMT) and high RCI low CMT. The CMT may be useful in the screening of heat tolerant cotton and heat sensitive. In this study, the thermo-stability of the cell membrane was studied by using RCI% values were recorded at controlled temperature 50°C to determine the effect of higher temperatures on the cell membrane. The mean values for RCI % of genotypes ranged from 39 to 86%. Relative cell injury level recorded was below 70% in twenty two genotypes; while it ranged from 60 to 70% in other twenty lines and 50-60% in eight genotypes. However, eight genotypes showed the relative level of cell damage less than 50%. The better performance genotypes having comparatively low relative level of cellular damage including NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and Sadori had the lowest relative injury level. While, among cotton advanced lines NIA-80, NIA-82, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1 and NIA-Bt2 had the lowest relative injury level (high cell membrane thermo-stability) at 50^{o}C with high level of tolerance to higher temperature. Our results suggested that these genotypes had the ability to maintain the integrity of the cell membrane and the high temperature structure. Therefore, these genotypes can be considered for heat tolerance. Heat tolerant genotypes obviously incurred less RCI % under heat stressed and non-stressed environments and therefore possessed greater CMT than heat-susceptible cultivars. NIA-Okra, NIA-86, NIA-HM-1, NIA-M32, NIA-M2, NIA-HM1 NIA-H67 CIM-496 and CRIS-121 had the highest relative injury level (low cell membrane thermo stability) indicating that high temperatures had direct effect on the electrolytes leakage and damage to the cells. Out of 58 entries, 17 genotypes showed stability to CMT; hence more tolerant to heat stress. Exposure to high temperature prior to CMT test, therefore, produced better discrimination between heat-tolerant and heat-susceptible genotypes. Our results are in agreement with earlier findings of to Azhar *et al.* (2009), Azeem *et al*. (2008), Rahman *et al.* (2004) Wang (1988), who used relative cell injury percentage to measure heat tolerance in cotton, and reported that heat-tolerant accessions were more stable performance and produced more seed cotton with improved fiber quality accessions heat intolerant under high temperature stress, the membrane structure is altered, increased permeability, electrolyte leakage increases, and eventually, the cell dies. Therefore exposure to high temperatures before the CMT test produces a better distinction between tolerant cultivars susceptible to heat and above criteria were used to solve heat tolerant and sensitive cotton accessions for further breeding program.

Wide variation in seed cotton yield existed due to high temperature stress among the tested cultivars of cotton. Seed cotton yield ranged between 2215 to 4115 kg ha-^{1} at under heat stress and non-stress regimes. Among them eighteen genotypes performed well in both temperature regimes and showed heat tolerance. Among cotton cultivars NIA-80, NIA-82, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1, NIA-Bt2, NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and Sadori had the highest seed cotton yield. The low seed cotton yields were obtained NIA-Okra, NIA-86, NIA-HM-1, NIA-M32, NIA-M2, NIA-HM1 NIA-H67, CIM-496 and CRIS-121 respectively under heat stress and non-stress regimes (Fig. 3). These techniques for screening is accordance to research of Emine Kardemir *et al*., 2012, who evaluated cotton genotypes for heat tolerance based on agronomic and physiological characteristics in field conditions where the temperature exceeded 40°C in July and August. Genotypes showed statistically significant at 0.01 levels of probability differences in seed cotton yield.

Heat susceptibility index: Heat susceptibility index (HSI) provides a measure of stress tolerance based on minimization of yield loss under stress as compared to optimum conditions, rather than on yield level under stress pre-se. Clarke *et al.* (1984), Bruckner & Frohberg (1987) and Fisher & Wood (1979) concluded that heat susceptibility index could be used to estimate stress injury because it accounted for variation in yield potential and stress intensity. Low stress susceptibility (HSI<1) is synonymous with higher stress tolerance. Results in Fig. 4, indicated that the values of heat susceptibility index ranged from 0.32 to 10.85% for genotypes NIA-80, NIA-82, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1, NIA-Bt2, NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and Sadori which gave the lowest value for heat susceptibility index. The superior genotypes for heat tolerance gave the least values of heat susceptibility index (HSI < 1) and high yield under heat stress.

Less shedding of flowers/bolls at high temperature stress is also considered as important criteria for the selection of heat tolerant genotypes in cotton. The mean values regarding boll retention revealed that both the planting dates (early and normal) had affected differently. Only eighteen genotypes retained maximum boll in both sowing dates (heat stress regimes and non-stress regime). The genotype NIA-80, NIA-81, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1, NIA-Bt2, NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and Sadori showed maximum boll retention under both sowing conditions which indicate less effect of terminal heat stress in these advance genotypes (Fig. 5). While, in remaining genotypes comparatively higher shedding of boll was observed as a result of high temperature stress (early sowing treatment). It has been reported that unexpected periodic episodes of extreme heat stress at the peak time of flowering cotton may cause low fruit set due to pollen infertility, which results in lower boll and fiber yield (Zhi Liu *et al.*, 2006; Reddy *et al.*, 1996). The results with respect to quality character (staple length) were non-significant. However, staple length increase for all the genotypes ranges from 27-30 mm as a results of normal sowing dates (non-heat stress regime) as compare to early sowing (heat stress regime). Among the 58 genotypes only 17 showed less reduction in staple length in both regimes because high temperature can be attributed to a decrease in fiber length and high temperatures throughout the filling phase of the bolls and the development of the fiber may reduce the quality of the fiber (Fig. 6). Reduction in staple length due to heat stress also reported in earlier studies (Rahman, 2006; Pettigrew 2008; Roussopoulos *et al.*, 1998), who stated that high temperature can be attributed to a decrease in fiber length.

The genotypes NIA-80, NIA-81, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1 ,NIA-Bt2, NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and Sadori were sorted as tolerant and genotypes NIA-85, NIA-86, NIA-H329, NIA-H335, NIA-148, NIA-32, NIA-H35, NIA-M2, NIA-M320, NIA-Bt-3, NIA-Okra, CRIS-121 and CIM-496 were regarded as sensitive to heat (Table 3).

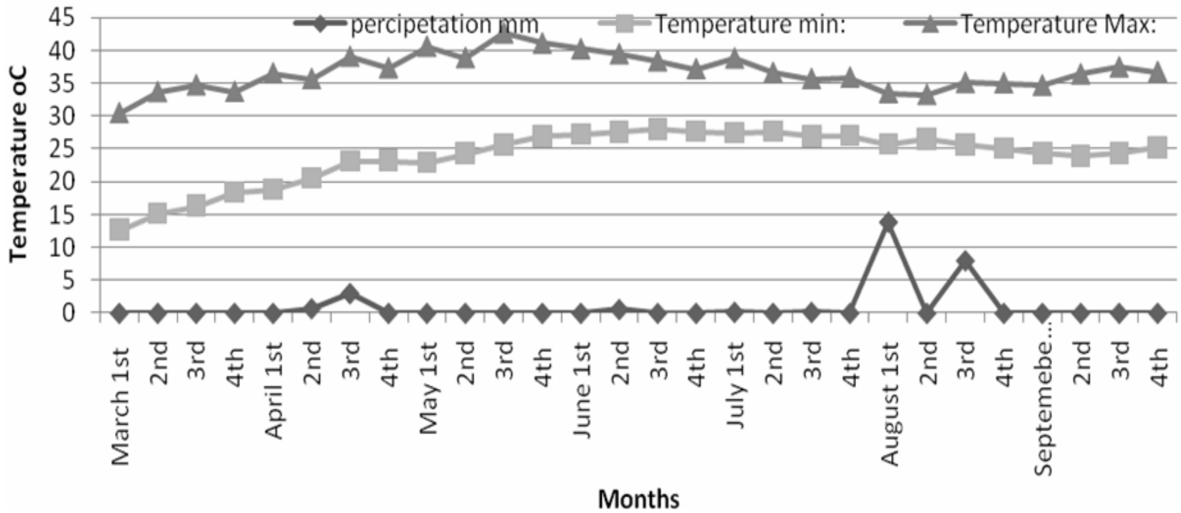

Fig. 1. Meteorological data on daily minimum and maximum temperature (^{o}C) and precipitation.

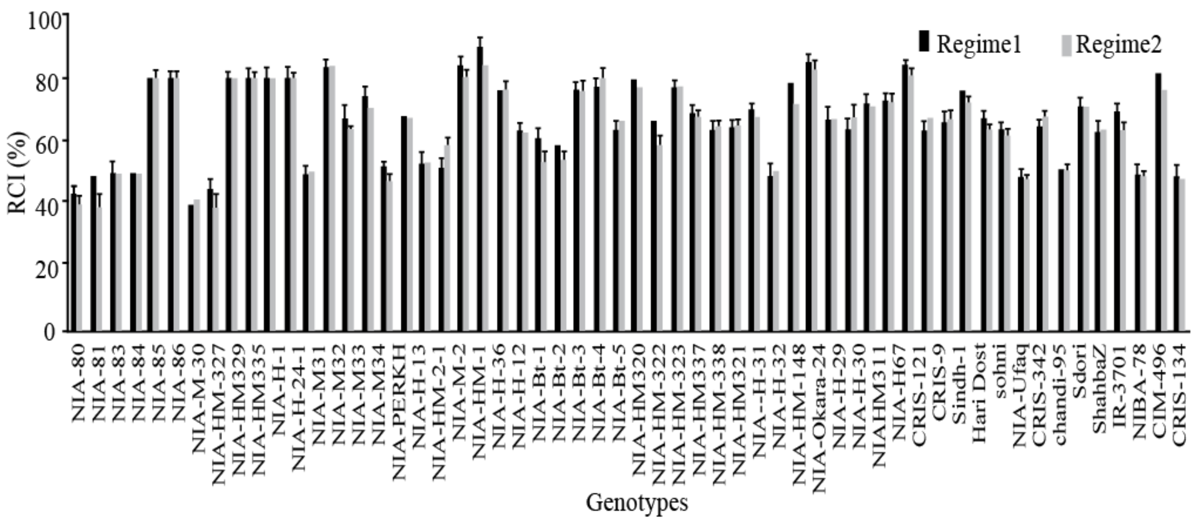

Fig. 2. Screening of different genotypes of cotton through cell thermo stability (CMT) under heat stress and non-stress regimes.

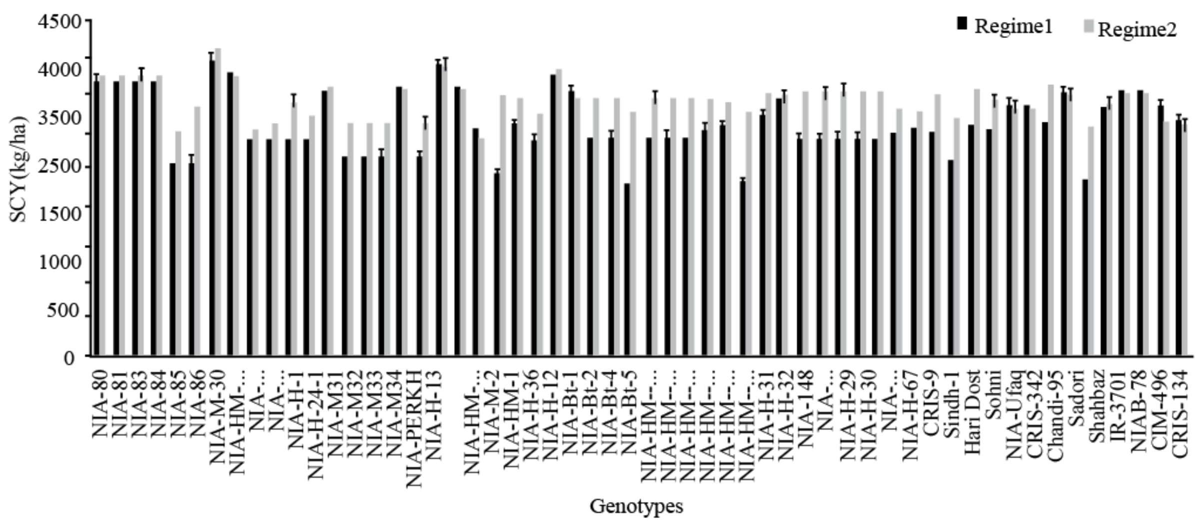

Fig. 3. Seed cotton yield (kg/ha) of 58 cotton genotypes under heat stress and non-stress regimes.

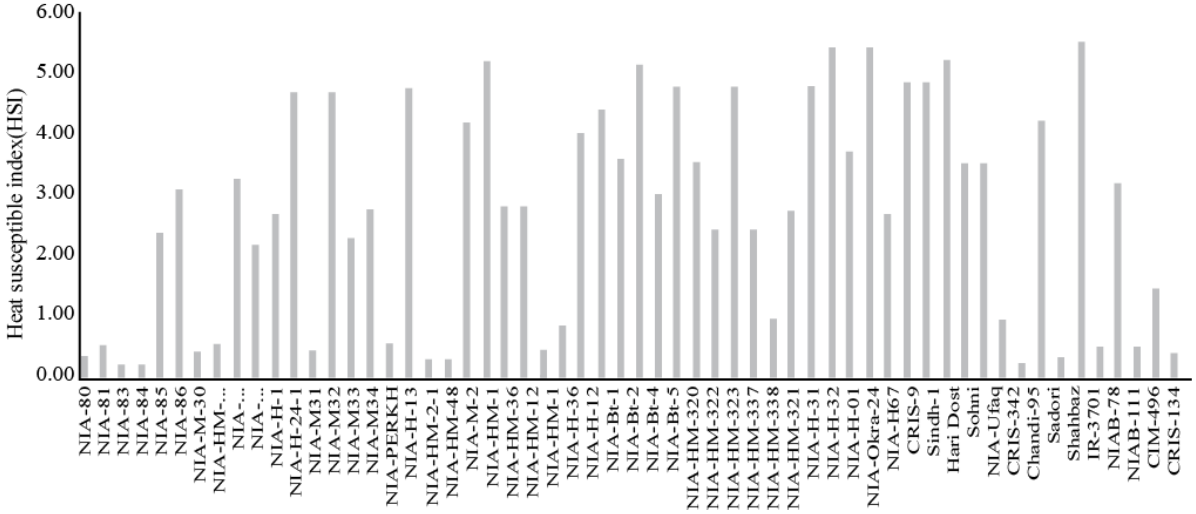

Fig. 4. Heat susceptible Index (HSI) for seed cotton yield (kg/ha).

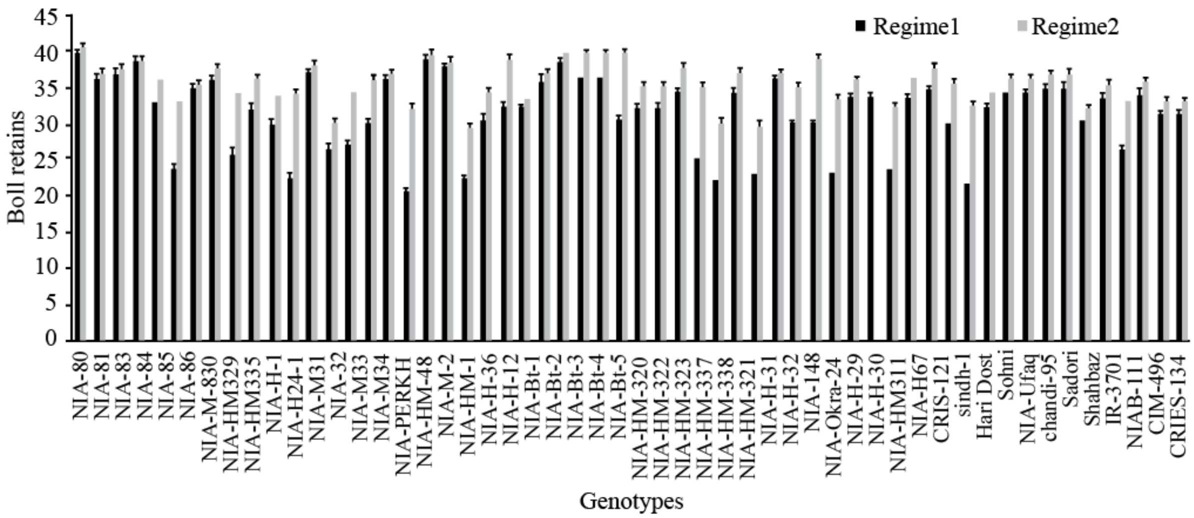

Fig. 5. Boll retains of 58 cotton genotypes under heat stress and non-stress regimes.

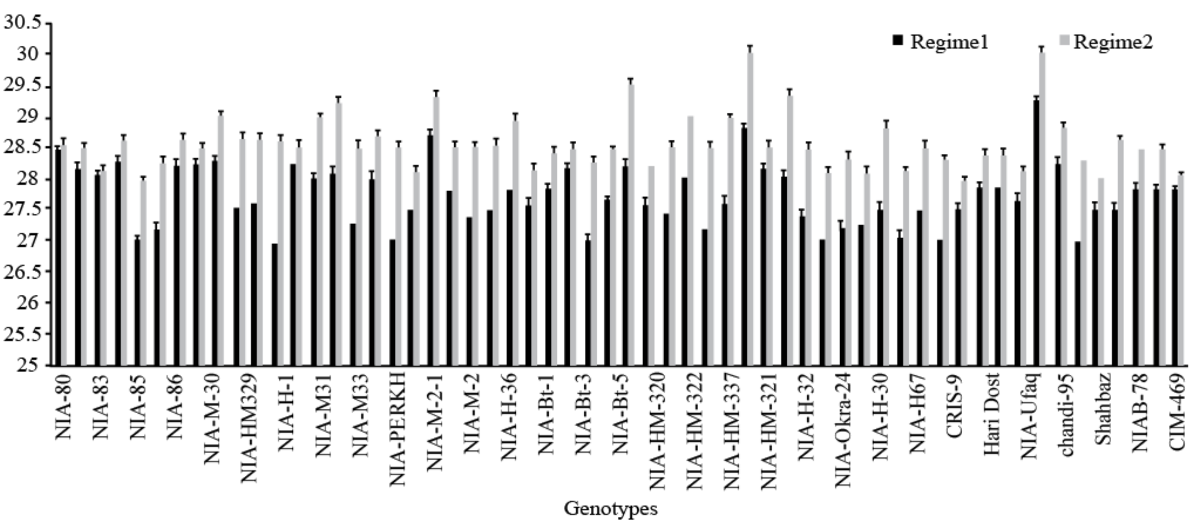

Fig. 6. Staple length (mm) of 58 cotton genotypes under heat stress and non-stress regimes.

Table 2. Mean analysis of variance for RCIL%, Boll retains, SCY (kg/ha) and Staple length (mm).

Source	DF	CMT	Bolls retains	Seed cotton yield (kg/ha)	Staple length (mm)
Replicate	2	244.6	2186.05	0.7	228.4
Genotypes	57	755.0**	785596.0**	80.8**	187.7
Sowing dates	1	360.1*	1.4508**	1996.8**	63.1 *
Sowing dates *genotypes	57	70.8	136227**	17.0**	188.9
Error	230	59.7	3668.50	3.45	187.9

*, ** Significance levels at 5% and 1%, respectively and ns = Non-significant

Table 3. List of the germplasm after screening.

Total No. of genotypes	Heat tolerant germplasm	Heat susceptible germ plasm
58	NIA-Perkh,	NIA-85
	CRIS-342,	NIA-86
	CRIS-134,	NIA-H-329,
	NIAB-111	NIA-H-335,
	Sadori	NIA-148,
	NIA-80,	NIA-H-32
	NIA-81,	NIA-H-35,
	NIA-83	NIA-M-2,
	NIA-84,	NIA-HM-320,
	NIA-M-30,	NIA-Bt-3,
	NIA-HM-327,	NIA-Okra
	NIA-M31,	CRIS-121
	NIA-H-13,	CIM-496
	NIA-HM-2-1,	
	NIA-HM-48,	
	NIA-Bt-1,	
	NIA-Bt-2	

Conclusion

The high temperature (>44°C) was observed during cotton growing season which significantly affected the growth and development of plants. Genotypes NIA-80, NIA-81, NIA-83, NIA-84, NIA-M-30, NIA-M31, NIA-HM-48, NIA-HM-327, NIA-H-32, NIA-HM-2-1, NIA-Bt1 ,NIA-Bt2, NIA-Perkh, CRIS-342, CRIS-134, NIAB-111 and Sadori in both regimes (heat stress and non-stress regimes) had the higher yield and lowest relative injury level (higher cell membrane thermo stability) at 50°C and their usefulness could be used as a source of heat-tolerant germplasm in future cotton breeding programs. These studies also highlighted the use of cell membrane thermo-stability as a useful technique in identifying the heat tolerant genotypes. Our studies may provide valuable informations regarding the identification and selection of potentially high yielding genotypes with better tolerance to heat stresses. The selected heat-tolerant genotypes could be used as a heat-tolerant germplasm in future breeding program. Such stable genotypes with better tolerance could be widely grown over heat prone areas of the region.

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(Received for publication 27 November 2014)