RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (1) 44-52, 2022
http://doi.org/10.54910/sabrao2022.54.1.5
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PHYSIOLOGICAL AND MOLECULAR RESPONSE OF COTTON (*Gossypium hirsutum* L.) TO HEAT STRESS AT THE SEEDLING STAGE

M. SAJID[1], M.A.B. SADDIQUE[1*], M.H.N. TAHIR[1], A. MATLOOB[2], Z. ALI[1], F. AHMAD[1], Q. SHAKIL[3], Z.U. NISA[4], and M. KIFAYAT[1]

[1]Institute of Plant Breeding and Biotechnology, MNS-University of Agriculture, Multan, Pakistan
[2]Department of Agronomy, MNS-University of Agriculture, Multan, Pakistan
[3]Fodder Research Sub-Station, Ayub Agricultural Research Institute, Faisalabad, Pakistan
[4]Cotton Research Institute, Multan, Punjab, Pakistan
*Corresponding author email: abubakarpbg@yahoo.com, abubakar.siddique@mnsuam.edu.pk
Email addresses of co-authors: sajidsans@gmail.com, abubakarpbg@yahoo.com
hammad.nadeem@mnsuam.edu.pk, amar.matloob@mnsuam.edu.pk, zulfiqarpbg@hotmail.com,
furqan.ahmad@mnsuam.edu.pk, shakil.qamar@yahoo.com, zaibsci.agri@gmail.com, mehwishkifayat@gmail.com

SUMMARY

The ideal temperature range for the optimal growth and development of cotton is 25 °C–32 °C and high temperature adversely affects the metabolic activities of plant cells. This study was aimed to screen heat-tolerant cotton genotypes based on physiological and molecular parameters. Experiments were carried out during 2019–2020 at the MNS-University of Agriculture, Multan, Pakistan. The research comprised two parts. In the first experiment, 30 cotton genotypes were sown in a completely randomized design with three replications under laboratory conditions for the determination of cell membrane thermostability. Principal component analysis was performed, and four genotypes, i.e., two heat-tolerant ('CRIS-5A' and 'VH-338') and two heat-sensitive ('FH-242' and 'VH-281') genotypes, were selected. In the second experiment, the screened cotton genotypes were sown in pots in a factorial complete randomized design with three replications and two treatments (normal and heat treatment). Heat stress was applied at the seedling stage, and eight leaf samples (one from each experimental unit) were collected. Two genes were used for molecular analysis and were amplified in all eight cDNA samples. Molecular analysis indicated the presence of *HSP70* and *HSP26* genes in the cotton genotypes, and the expression of these genes was measured by using ImageJ software. The gene expression level of *HSP70* was very high (16.41%) in 'VH-281', which is a heat-sensitive genotype under heat stress. The sensitive genotype 'FH-242' exhibited the highest gene expression level of *HSP26* (20.32%) under normal conditions. A similar sequence of *HSP70* gene of *Agave sisalana* was amplified for the first time in cotton. It is a good indicator for screening heat tolerant cotton genotypes at the molecular level.

Keywords: CMT, cotton, screening, heat shock proteins, high temperature, RCI%, oxidative damage

Key findings: Physiological and molecular parameters are helpful to breeders for screening heat-tolerant cotton genotypes. A gene in the cotton genome with a sequence similar to the sequence of the *HSP70* gene of *A. sisalana* can play an important role in the screening of cotton genotypes having resistance to heat stress.

Communicating Editor: Dr. B.P. Mallikarjuna Swamy
Manuscript received: June 10, 2021; Accepted: December 3, 2021.

To cite this manuscript: Sajid M, Saddique MAB, Tahir MHN, Matloob A, Ali Z, Ahmad F, Shakil Q, Nisa ZU, Kifayat M (2022). Physiological and molecular response of cotton (*Gossypium hirsutum* L.) to heat stress at the seedling stage. *SABRAO J. Breed. Genet.* 54(1): 44-52. http://doi.org/10.54910/sabrao2022.54.1.5

INTRODUCTION

Cotton is sensitive to heat stress, which is widely discussed worldwide. The rapid increase in the earth's temperature as a result of global warming is an alarming issue for the coming eras (Dabbert and Gore, 2014). From 2020 to 2080, the earth's temperature will rise by 0.5 °C–5.44 °C (IPCC, 2018). Although cotton originated from the hot climatic regions of the world, its growth and development are affected by prolonged periods of high temperature (HT) (Oosterhuis, 2002; Cottee *et al*., 2007). Although the temperature requirement of a cotton plant varies in accordance with growth stage, temperatures of 25 °C–32 °C are required for optimal growth (Reddy *et al.*, 1997; Burke and Wanjura, 2010).

Any fluctuation in temperature disturbs the whole growth pattern of cotton (Rahman *et al.*, 2004). HT affects the germination, root and shoot growth, sympodial and monopodial branches, inter-nodal distance, photosynthesis, respiration, ATP formation, biomass, boll formation per plants, boll size and weight, cellulose deposition, and fiber yield of cotton plants (Wahid *et al.*, 2007; Bibi *et al.*, 2008; Loka and Oosterhuis, 2016). HT affects cotton fiber quality (ITC, 2011). An increment in temperature affects fiber length, strength, and micronaire value, which are the main characteristics of fiber quality (Pettigrew, 2008). Its effects on the seed/boll ratio, seed size, and fiber/boll ratio are the main reasons for the reduction in cotton yield. HT causes a yield loss of 50% in cotton crops (Boyer, 1982; Sarwar *et al.*, 2017). Boll retention is directly connected to photosynthesis, minerals, and plant hormones, and metabolic activities are impaired at HT (Lobell and Field, 2007).

HT also causes oxidative stress, which directly damages different cellular compartments. Under heat stress, reactive oxygen species (ROS), particularly O_2•−, are produced in large amounts and damage the plant (McCarty and Jenkins, 2001; Halliwell, 2006). Oxidative stress causes proteolysis, membrane injury, DNA damage, and enzyme inhibition (Loka and Oosterhuis, 2010). In cotton, ROS causes the proteolysis of different proteins; this results in the shedding of premature leaves (Méndez-Natera *et al*., 2012; Hemantaranjan *et al.*, 2014).

HT affects the morphophysiological traits of the cotton crop. Therefore, a platform for developing heat-tolerant genotypes must be provided by exploring heat-tolerant genes. Numerous unexplored heat shock proteins (HSPs) that are responsible for heat tolerance are present in cotton (Wang *et al.*, 2008; Gupta *et al.*, 2010). Among the many screening techniques that have been used for cotton, relative cell injury% (RCI %) is the popular, authentic, and quick physiological technique for screening cotton germplasm for resistance to heat stress (Baker and Rosenqvist, 2004).

The RCI% is based on the measurement of electrolyte leakage from leaf discs in distilled water after heat treatment (Sullivan, 1992). This method has been widely used on many crops, e.g., rice, soybean, tomato, and cotton (Singh *et al.*, 2007). In cotton, the RCI% technique is widely used for screening heat-tolerant genotypes because RCI% is an authentic indicator of cell membrane thermostability (CMT) and is simpler and more cost-effective than other screening techniques (Rahman *et al.*, 2004; Azhar *et al.*, 2009; Emine *et al*., 2012). The objectives of this research were to screen the best heat-tolerant cotton genotypes on the basis of RCI % and study the molecular expression of the HSP gene in heat-tolerant and susceptible cotton genotypes. This research will provide one of the best ways for screening cotton germplasm for resistance to heat stress and exploring HSPs in cotton.

MATERIALS AND METHODS

This study was carried out during 2019–2020 at the MNS-University of Agriculture, Multan, Pakistan, with two experiments. Thirty cotton genotypes were used in the experiments. They were collected from the Cotton Research Institute, Multan, Pakistan. These genotypes were selected because they have been adapted to the local environment.

Experiment No. 1

The first experiment was performed to select heat-tolerant cotton genotypes on the basis of CMT. Thirty cotton genotypes were sown in pots in the laboratory by following a complete randomized design (CRD) with three replications. At the seedling stage, two sets of samples were collected from both sides of the midrib. One set was used as normal, and the other was subjected to heat treatment. Leaf disks were collected from the leaf samples and kept in Falcon tubes containing 2 ml of distilled water. RCI% was measured with an electric conductivity (EC) meter by following the formula of Sullivan (1972):

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

Where T = value of the heat-treated sample
C = value of the control samples
Digits 1 = first reading and 2 = final reading of the EC meter.

Principal component analysis

After measuring the CMT of all 30 genotypes, principal component analysis (PCA) was performed to screen the heat-tolerant genotypes.

Experiment No. 2

The second experiment was conducted to identify the role of HSPs in the screened genotypes against heat stress. The screened genotypes were sown in pots with three replications by following a factorial completely randomized design with two treatments (normal and heat stress). At the seedling stage, heat stress was applied by keeping the pots in a dry oven at 45 °C for 30 min, and eight leaf samples were taken (one from each experimental unit). The RNA was extracted from the leaf samples, and cDNA libraries were synthesized and stored at −80 °C.

The two primers of the HSP70 and HSP26 genes of *Agave sisalana* and *G. hirsutum* were used, respectively. The HSP70 gene sequence of *A. sisalana* was taken because *A. sisalana* is a heat-tolerant crop. The nucleotide sequence was BLAST against the cotton genome (https://cottonfgd.org/). The sequence that was similar to the HSP70 gene sequence of *A. sisalana* was used for primer designing (Table 1).

The primers of the HSP70 and HSP26 genes were optimized at 55 °C and 60.6 °C, respectively. These optimized primers were utilized for annealing on cDNA samples. The expression rates (%) of the HSP70 and HSP26 genes in all heat-treated and normal samples were calculated by using ImageJ software (https://www.imageJsoftware.net/).

Table 1. Primers designed for molecular analysis through AmplifX software.

No.	Gene	Gene ID	CDS	Forward prime	Reverse primer
1	HSP70	>XM_016815688.1	1941	ATGCTGGTGTCATTGCTGG TCT	GCAGTGGCCTTCACCTCAA AGATA
2	HSP26	>XM_016874442.1	714	TGGCAACAGCTACTGATAA GGACT	TCCCAAGGCCTGATGGGAA TAACT

RESULTS

Cell Membrane Thermostability (CMT)

The CMT values of all 30 cotton genotypes were recorded. Under normal conditions, the highest mean value of 46.51% and the lowest value of 14.55% were shown by the 'FH-242' and 'CRIS-5A' genotypes, respectively. Under heat stress, the highest mean value of 94.53% and the lowest value of 57% were exhibited by the 'FH-242' and 'CRIS-5A' genotypes, respectively. The average CMT values were 32.07% and 76.81% under normal and heat stress conditions, respectively. The RCI performance of the genotypes is presented in Figure 1 and differed among the genotypes.

Principal Component Analysis (PCA)

PCA was also performed for the selection of heat-responsive and heat-sensitive genotypes on the basis of RCI%. Four genotypes were selected on the basis of their performance and vector OP length. Two genotypes, namely, 'CRIS-5A' (10) and 'VH-338' (9), were selected as heat-tolerant genotypes, whereas genotypes 'VH-281' (16) and 'FH-242' (7) were selected as sensitive genotypes. The biplot based on PCA was divided into two components. The first and second components accounted for 95.5% and 4.5% of the variability, respectively. The genotypes that were located away from the origin on the right side were considered as good performers compared with the genotypes that were close to the origin. The opposite side of the biplot indicated low RCI% values. Therefore, these genotypes were selected as heat-tolerant (Figure 2).

Molecular analysis

The heat-tolerant genotypes 'CRIS-5A' and 'VH-338' and the heat-sensitive genotypes 'FH-242' and VH-281 were used to analyze the expression of heat-responsive genes. The picture of the gene expression on gel is

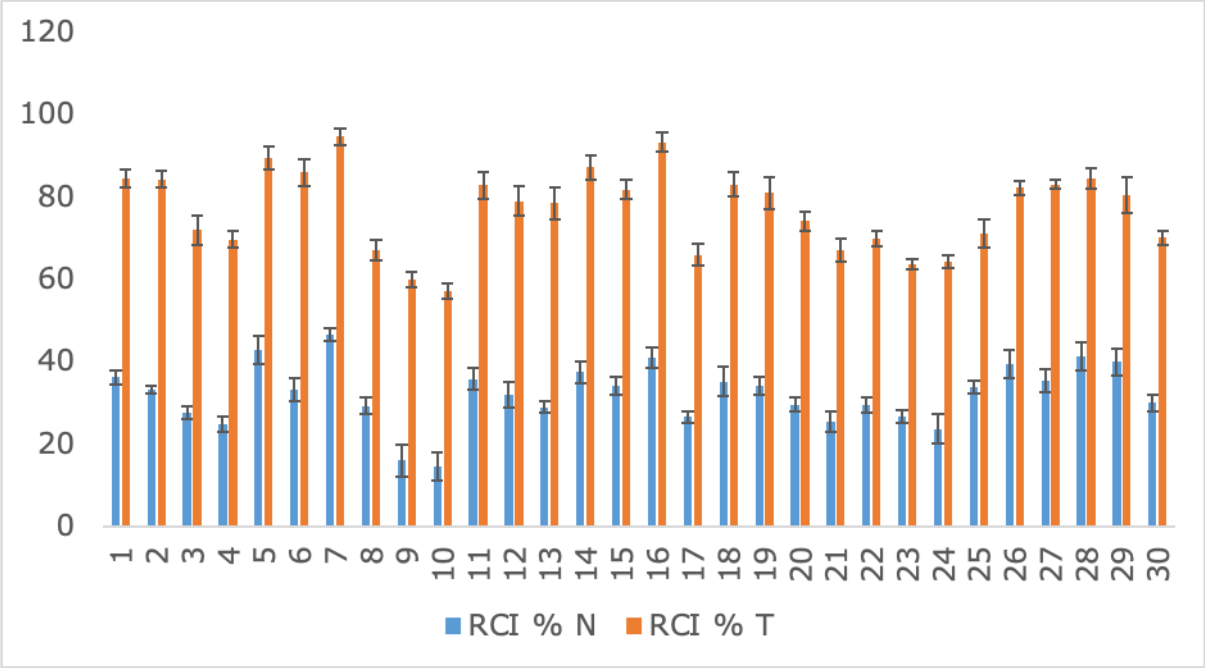

Figure 1. RCI performance of cotton genotypes under normal and heat stress conditions.

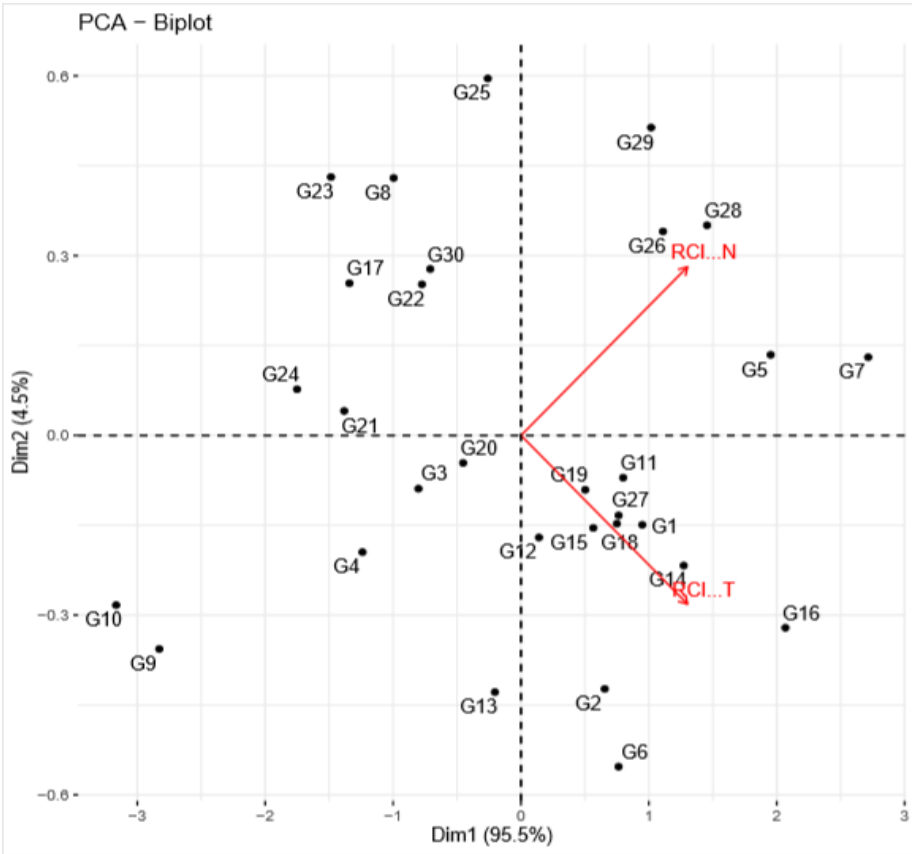

Figure 2. Bi-plot analysis based on the RCI% of cotton genotypes.

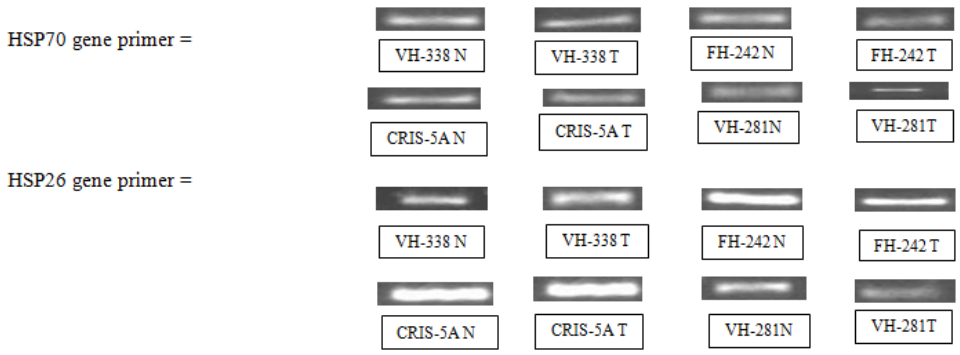

Figure 3. Representative PCR results of the optimized primers for the screened genotypes.

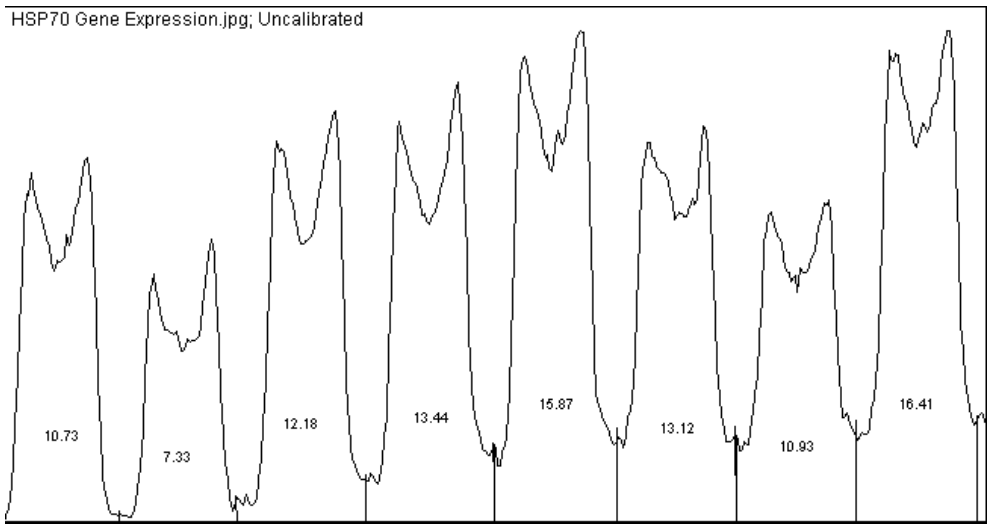

Figure 4. Expression rate of the HSP70 gene in the cDNA samples of the screened genotypes.

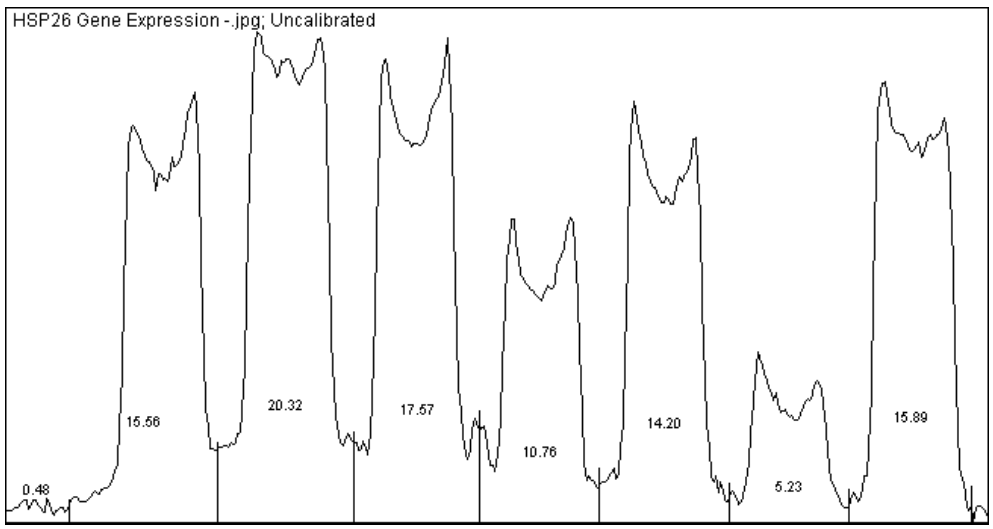

Figure 5. Expression rate of the HSP26 gene in the cDNA samples of the screened genotypes.

presented in Figure 3. The peaks in Figures 4 and 5 indicate the expression percentage of the responsive genes, and each peak indicated each band of the genotype. In 'VH-281', which was a heat-sensitive genotype, the expression rate of the HSP70 gene was very high (16.41%) under heat stress compared with that in other genotypes. The lowest (7.33%) expression level was observed in 'VH-338', which is a heat-tolerant genotype (Figure 4).

Under normal conditions, the expression of the HSP26 gene was very high (20.32%) in the sensitive genotype 'FH-242' compared with that in other genotypes. The lowest (0.48%) expression rate was observed in the tolerant genotype 'VH-338' in the control environment (Figure 5). The results revealed that the HSP70 and HSP26 genes are involved in inducing heat tolerance in cotton and can be used to screen heat-tolerant cotton genotypes.

DISCUSSION

Cotton is widely cultivated as a cash crop all over the world, including Pakistan. Cotton faces many challenges, including biotic and abiotic stresses. Among abiotic stresses, heat stress is the major emerging issue that significantly affects cotton growth and development (Reddy *et al*., 1997; Oosterhuis, 2002; Burke and Wanjura, 2010). Heat stress reduces yield potential. This effect has been reported in many crops, including cotton. Sarwar (2017) reported that a change of a single degree in temperature leads to a reduction of up to 110 kg/ha in cotton yield.

Heat-tolerant cotton genotypes that can survive under heat stress and provide high yields must be developed to overcome yield losses due to heat stress. The tolerant genotypes could be screened out through physiological techniques, i.e., RCI%. This screening technique is widely used by many scientists to screen heat-tolerant genotypes in many crops, including cotton.

Electrolyte leakage from the cell membrane is very common under heat stress conditions and indicates poor membrane stability (Azeem *et al*., 2008). The present study investigated the physiological and molecular responses of cotton genotypes. CMT was measured at the seedling stage of the cotton genotypes. In agreement with the present study, the previous research found that CMT is the major physiological indicator of a plant's response to heat stress. Chaudhary *et al*. (2020) reported that under heat stress, cotton genotypes with high CMT tend to have a better phenotypic appearance than those with low CMT.

CMT is one of the important parameters for screening heat-tolerant genotypes. In this study, heat stress applied at the seedling stage of plants significantly affected the CMT. Lidon and Dias (2010) also reported that heat stress adversely affects CMT. In this study, the CMT values ranged from 57.0% to 93.53% under heat stress and from 14.51% to 46.5% under normal conditions; these findings are in agreement with the results of Abro *et al*. (2015). Under heat stress and normal conditions, 'CRIS-5A' and 'VH-338' had the lowest RCI%, and 'FH-242' and 'VH-281' had the highest RCI%. The present results were similar to the findings of Asha and Ahamed (2013) and Shavkiev et al. (2021). Abro *et al*. (2015) screened out various heat-tolerant cotton genotypes with high CMT rates. Karademir *et al*. (2012) and Singh *et al*. (2018) also used RCI% to screen heat-tolerant cotton genotypes in their research.

Physiological and molecular mechanisms play an important role and promote plant growth to address heat stress. Moreover, these mechanisms repair damaged enzymes and proteins and stabilize the cell environment (Zhang *et al*., 2016). Molecular approaches provide a vital platform for developing heat-tolerant genotypes. At the molecular level, HSPs are mainly responsible for responding to heat stress (Usman, 2014). In this study, the screened genotypes ('CRIS-5A', 'VH-338', 'FH-242', and 'VH-281') were used for molecular analysis. The molecular findings of this work are similar to the results reported by Susan and Mary (1989), Mohamed and Hamid (2013), and Zhang *et al*. (2016). Under heat stress, the expression of the HSP70 gene was very high (16.41%) in 'VH-281', a sensitive genotype, relative to that in other genotypes, whereas the lowest (7.33%) expression level was observed in 'VH-338', which is a heat-tolerant genotype.

The expression level of the HSP26 gene was found to be very high (20.32%) in the sensitive genotype 'FH-242' relative to that in other genotypes, whereas the lowest (0.48%) expression rate was observed in the tolerant 'VH-338' genotype. The results revealed the activity of HSP70 and HSP26 genes inside the screened genotypes under heat stress. Parallel results were also reported by many scientists (Easton *et al*., 2000; Krishna and Gloor, 2001; Young, 2010). Kotak *et al.* (2007) and Zhang *et al.* (2016) also demonstrated the presence of HSP genes in cotton genotypes and used

them to develop heat-tolerant genotypes. These experiments obviously showed that the CMT and expression of HSP genes are reliable parameters for the screening of heat-tolerant cotton genotypes.

CONCLUSIONS

In Pakistan, temperatures fall within the range of 45 °C–50 °C during the cotton-growing season and badly affect all the morpho-physiological traits of the cotton crop. RCI% is a good indicator for screening heat-tolerant genotypes. Our results revealed that the heat-tolerant genotypes 'CRIS-5A' and 'VH-338' performed well in terms of RCI%. Under heat stress, the expression of the HSP70 and HSP26 genes changed in heat-tolerant and susceptible genotypes and therefore can be used to screen cotton genotypes under heat stress. In the current research, the HSP70 gene sequences of *A. sisalana* were taken because this crop is a heat-tolerant plant. The nucleotide sequences were BLAST against the cotton genome. This gene was amplified for the first time in cotton. It is a good indicator for screening heat-tolerant cotton genotypes at the molecular level because it shows high expression when heat-tolerant genotypes are exposed to heat stress. The further exploration of the HSP genes is needed to develop heat-tolerant cotton genotypes. This research may provide a way for plant breeders to develop heat-tolerant cotton genotypes.

ACKNOWLEDGMENT

The research activities were financed by the Annual Development Plan (ADP-22036-Agriculture).

REFERENCES

Abro S, Rajput MT, Khan MA, Sial MA, Tahir SS (2015). Screening of cotton (*G. hirsutum* L.) genotypes for heat tolerance. *Pak. J. Bot.* 47: 2085-2091.

Asha R, Ahamed L (2013). Screening of cotton for heat tolerance. *Int. J. Bio-Resour. Stress Manag.* 4: 599-604.

Azeem IK, Iftikhar KA, Sadaqet HA (2008). Heat tolerance is variable in cotton (*G. hirsutum* L.) and can be exploited for breeding better yielding cultivars under high-temperature regimes. *Pak. J. Bot.* 40: 2053-2058.

Azhar FM, ALI Z, Akhtar MM, Khan AA, Trethowan R (2009). Genetic variability of heat tolerance, and its effect on yield and fiber quality traits in upland cotton (*G. hirsutum* L.). *Plant Breed.* 128: 356-362.

Baker NR, Rosenqvist E (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J. Exp. Bot.* 55: 1607-1621.

Ban YG, Nawalkar D, Mote B, Kumar V, Narwade A (2015). Crop phenology, thermal requirement, yield, and fiber properties of cotton *(G. hirsutum*) genotypes as influenced by different environments. *Int. J. Plant Physiol.* 20: 137-144.

Bibi A, Oosterhuis D, Brown R, Gonias E, Bourland F (2003). The physiological response of cotton to high temperatures for germplasm screening. Summaries of Arkansas Cotton Research pp. 87-93.

Bibi A, Oosterhuis D, Gonias E (2008). Photosynthesis, the quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. *J. Cotton Sci.* 12: 150-159.

Boyer JS (1982). Plant productivity and environment. *Sci.* 218: 443-448.

Burke JJ, Wanjura DF (2010). Plant responses to temperature extremes. In: Physiology of cotton. *Springer* pp. 123-128.

Chaudhary S, Devi P, Bhardwaj A, Jha UC, Sharma KD, Prasad PVV, Siddique KHM, Bindumadhava H, Kumar S, Nayyar H (2020). Identification and characterization of contrasting genotypes/cultivars for developing heat tolerance in agricultural crops: Current status and prospects. *Front. Plant Sci.* 11: 587264.

Cottee NS, Tan DKY, Cothren T, Bange MP, Campbell LC (2007). Screening cotton cultivars for thermotolerance under field conditions. Fourth World Cotton Research Conference at Lubbock, Texas pp. 2234-2246.

Dabbert T, Gore MA (2014). Challenges and perspectives on improving heat and drought stress resilience in cotton. *J. Cotton Sci.* 18: 393-409.

Dehghani H, Ebadi A, Yousefi A (2006). Biplot analysis of genotype by environment interaction for barley yield in Iran. *Agron. J.* 98: 388-393.

Easton D. P, Kaneko Y, Subjeck J. R (2000). The Hsp110 and Grp170 stress proteins: Newly recognized relatives of the Hsp70s. *Cell Stress Chaperones* 5: 276-290.

Ekinci R, Başbag S, Karademir E, Karademir C (2017). The effects of high-temperature stress on some agronomic characters in cotton. *Pak. J. Bot.* 49: 503-508.

Emine K (2012). Screening cotton varieties (*G. hirsutum* L.) for heat tolerance under field conditions. *Afr. J. Agric. Res.* 7: 6335-6342.

Gupta SC, Sharma A, Mishra M, Mishra RK, Chowdhuri DK (2010). Heat shock proteins in toxicology: how close and how far? *Life Sci.* 86: 377-384.

Halliwell B (2006). Oxidative stress and neurodegeneration: where are we now? *J. Neurochem.* 97: 1634-1658.

Hemantaranjan A, Bhanu AN, Singh M, Yadav D, Patel P, Singh K, Katiyar D (2014). Heat stress responses and thermotolerance. *Adv. Plants Agric. Res.* 1: 1-10.

IPCC. 2018. Climate change 2018. Synthesis report. Version ingles. Page Climate Change 2018: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

ITC (2011). Cotton and climate change impacts and options to mitigate and adapt. Technical Paper. International Trade Centre (ITC), Geneva: Doc. No. MAR-11-200.E. pp. 32.

Karademir E, Karademir C, Ekinci R, Başbag S, Başal H (2012). Screening cotton varieties (*G. hirsutum* L.) for heat tolerance under field conditions. *Afr. J. Agric. Res.* 7: 6335-6342.

Kotak S, Larkindale J, Lee U, Doring PVK, Vierling E, Scharf KD (2007). Complexity of the heat stress response in plants. *Curr. Opin. Plant Biol.* 10: 310-316.

Krishna P, Gloor G (2001). The Hsp90 family of proteins in *Arabidopsis thaliana*. *Cell Stress Chaperones.* 6: 238-246.

Lidon FC, Dias AS (2010). Bread and durum wheat tolerance under heat stress: A synoptical overview. *Emir. J. Food Agric.* 22: 412-436.

Lobell DB, Field CB. (2007). Global scale climate-crop yield relationships and the impacts of recent warming. *Environ. Res. Lett.* 2: 014002.

Loka D, Oosterhuis D (2010). Effect of high night temperatures on cotton respiration, ATP levels, and carbohydrate content. *Environ. Exp. Bot.* 68: 258-263.

Loka DA, Oosterhuis DM (2016). Effect of high night temperatures during anthesis on cotton (*G. hirsutum* L.) pistil and leaf physiology and biochemistry. *Aust. J. Crop Sci.* 10(5): 741-748.

Lokhande S, Reddy KR (2014). Quantifying temperature effects on cotton reproductive efficiency and fiber quality. *Agron. J.* 106: 1275-1282.

Maxwell K, Johnson GN (2000). Chlorophyll fluorescence is a practical guide. *J. Exp. Bot.* 51: 659-668.

McCarty JC, Jenkins JN (2001). Primitive cotton germplasm: yield and fiber traits for 16 Day-Neutral Accessions. Mississippi Agricultural and Forestry Experiment Station.

Méndez-Natera JR, Rondón A, Hernández J, Merazo-Pinto JF (2012). Genetic studies in upland cotton. III. Genetic parameters, correlation and path analysis. *SABRAO J. Breed. Genet*. 44(1): 112-128.

Mohammed HI, Hamid AMEA (2013). Molecular and biochemical studies for heat tolerance on four cotton genotypes. *Roman. Biotechnol. Lett.* 18: 8823-8831.

Oliveira P, Munita C (2011). Comparatives studies of the biplot and multidimensional scaling analysis in experimental data. *In* Proc. 58th World Statistical Congress. International Statistical Institute: (Session CPS008) pp. 6024.

Oosterhuis D (2002). Day or night high temperatures: A major cause of yield variability. *Cotton Grower* 46: 8-9.

Pettigrew W (2008). The effect of higher temperatures on cotton lint yield production and fiber quality. *Crop Sci.* 48: 278-285.

Rahman H, Malik S, Saleem M (2005). Inheritance of seed physical traits in upland cotton under different temperature regimes. *Span J. Agric. Res.* 3(2): 225-231.

Rahman H, Malik SA, Saleem M (2004). Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. *Field Crops Res*. 85: 149-158.

Reddy KR, Hodges HF, McKinion JM (1997). Modeling temperature effects on cotton internode and leaf growth. *Crop Sci*. 37: 503-509.

Roussopoulos D, Liakatas A, Whittington W (1998). Cotton responses to different light–temperature regimes. *J. Agric. Sci.* 131: 277-283.

Sarwar M, Saleem M, Najeeb U, Shakeel A, Ali S, Bilal M (2017). Hydrogen peroxide reduces heat‐induced yield losses in cotton (G. hirsutum L.) by protecting cellular membrane damage. J. Agron. Crop Sci. 203: 429-441.

Shavkiev J, Azimov A, Nabiev S, Khamdullaev S, Amanov B, Kholikova M, Matniyazova H, Yuldashov U (2021). Comparative performance and genetic attributes of upland cotton genotypes for yield-related traits under optimal and deficit irrigation conditions. *SABRAO J. Breed. Genet.* 53(2): 157-171.

Singh K, Wijewardana C, Gajanayake B, Lokhande S, Wallace T, Jones D (2018). Genotypic variability among cotton cultivars for heat and drought tolerance using reproductive and physiological traits. *Euphytica* 214: 1-22.

Singh RP, Prasad PV, Sunita K, Giri S, Reddy KR (2007). Influence of high temperature and breeding for heat tolerance in cotton: A review. *Adv. Agron.* 93: 313-385.

Song G, Wang M, Zeng B, Zhang J, Jiang C, Hu Q, Geng G, Tang C (2015). Anther response to high-temperature stress during development and pollen thermotolerance heterosis as revealed by pollen tube growth and in vitro pollen vigor analysis in upland cotton. *Planta* 241: 1271-1285.

Steel RGD, Torrie JH, Dicky DA (1997) Principles and procedures of statistics, A biometrical approach. 3rd Edition, McGraw Hill, Inc. Book Co., New York, 352-358.

Sullivan CY (1972). Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. Sorghum in Seventies.

Oxford and IBH Pub. Co. Heat shock protein expression in thermotolerant and thermosensitive lines of cotton.

Susan EF, O'Connell MA (1989). Heat shock protein expression in thermotolerant and thermosensitive lines of cotton. *Plant Cell Reports*. 8: 37-40.

Usman MG (2014). Heat shock proteins: Functions and response against heat stress in plants. *Int. J. Sci. Tech. Res.* 3: 204-218.

Wahid A, Gelani S, Ashraf M, Foolad MR. (2007). Heat tolerance in plants: an overview. *Environ. Exp. Bot.* 61: 199-223.

Wang L, Cai H, Bai X, Li LW, Li Y, Zhu YM (2008). Cultivation of transgenic rice plants with OsCDPK7 gene and its salt tolerance. *Yi chuan = Hereditas* 30: 1051-1055.

Young JC (2010). Mechanisms of the Hsp70 chaperone system This paper is one of a selection of papers published in this special issue entitled - Canadian society of biochemistry, molecular and cellular biology 52nd annual meeting-protein folding: Principles and diseases and has undergone the Journal's usual peer review process. *Biochem. Cell Biol*. 88: 291-300.

Zhang J, Vibha S, Stewart JM, Underwood J (2016). Heat-tolerance in cotton is correlated with induced overexpression of heat-shock factors, heat-shock proteins, and general stress response genes. *J. Cotton Sci.* 20: 253-262.

Zhao D, Reddy KR, Kakani VG, Koti S, Gao W (2005). Physiological causes of cotton fruit abscission under conditions of high temperature and enhanced ultraviolet - B radiation. Physiol. Plantarum 124:189-199.