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Identification and Genetic Mapping of Potential QTLs Conferring Heat Tolerance in Cotton (*Gossypium hirsutum* L.) by Using Micro Satellite Marker's Approach

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Abstract: High-temperature stress can cause serious abiotic damage that limits the yield and quality of cotton plants. Heat Tolerance (HT) during the different developmental stages of cotton can guarantee a high yield under heat stress. HT is a complex trait that is regulated by multiple quantitative trait loci (QTLs). In this study, the F_2 population derived from a cross between MNH-886, a heat-tolerant cultivar, and MNH-814, a heat-sensitive variety, was used to map HT QTLs during different morphological stages in cotton. A genetic map covering 4402.7 cm, with 175 marker loci and 26 linkage groups, was constructed by using this F_2 population (94 individuals). This population was evaluated for different 23 morpho-physiological HT contributing traits QTL analysis via composite interval mapping detected 17 QTLs: three QTLs each for Total Number of Sympodes (TNS), Length of Bract (LOB), and Length of Staminal-column (LOS); two QTLs for First Sympodial Node Height (FSH), and one QTL each for Sympodial Node Height (SNH), Percent Boll set on second position along Sympodia (PBS), Total Number of Nodes (TNN), Number of Bolls (NOB), Total Number of Buds (TNB), and Length of Petal (LOP). Individually, the QTLs accounted for 7.76%–36.62% of phenotypic variation. QTLs identified linked with heat tolerance traits can facilitate marker-assisted breeding for heat tolerance in cotton.

Keywords: *G. hirsutum*; molecular markers; morpho physiological characteristics; quantitative trait loci; heat tolerance

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1. Introduction

Cotton is a miracle of the plant realm as it fulfills most of the vital needs and provides more than 90% of the world's total production of fiber for the textile industry and edible oil for almost half of the world's population [1]. It has been observed that more than 50% of cotton around the globe is affected by abiotic stress such as salinity, drought, and heat stress that lead to deficient production of this field crop, especially when affected at the seedling stage [2]. Cotton growth requires sufficient fresh water for better fiber quality, but if it faces drought or heat stress the fiber production is reduced [3]. Many new drought tolerant cultivars of cotton have been introduced with improved plant growth, and even other genetically engineered genotypes of cotton by breeding techniques are being cultivated that can tolerate many abiotic stresses [4]. However, the genetic basics and amendments behind these stresses need to be evaluated more to combat these problems from the genetic roots. Cotton is divided into eight genomes (groups) from A to G and K including 45 diploids and the basic seven tetraploid [5,6]. Evolutionary data based on DNA sequencing suggested that about six to seven million years, ago due to trans-oceanic dispersal, D genome divergence gave rise to the A genome and in America (primarily Mexico), it became a separate lineage [7,8]. An incredible diversification occurred over this

time that resulted in the worldwide spread of the Gossypium species. Domestication of wild varieties of cotton by human beings resulted in lot of change in all phenotypic and genotypic characteristics.

In terms of production, Pakistan is at the fourth position among the cotton growers of the world; raw cotton exported from Pakistan holds third position in the world as per records of 2012–2013 [9]. Pakistan is more prone to climate changes due to its geographical location [10]. Heat stress is a combination of different intricate functions of intensity duration of temperature. Because of its geographical position, in Pakistan during the summer in some locations, the temperature reaches up to 50 °C and the scorching heat adversely affects cotton plants. Cotton is cultivated in hot areas in Pakistan [11]. High temperature affects growth and development of the plant as well as fiber quality traits [12,13]. Episodes of periodic heat stress and increase in average temperature for the full season enhances the detrimental effects on almost all the factors of plant growth, and that is the reason there is great reduction in the seed number, fiber quality, and content [14]. Cotton yield is suppressed when the plant faces heat and drought stress due to decreased plant transpiration and reduced biomass accumulation, resulting in an inadequate yield [15]; these stresses adversely affect cell elongation, differentiation, and division and also suppress stomatal conductance [16].

The cotton plant has a wide range of adaptability [17], but high temperature is one of the major constraints in cotton productivity and greatly reduces seed cotton yield and quality, which can be addressed by breeding methods. Marker-assisted selection fastens the breeding technology with an accurate approach towards the desired phenotypic traits among the breeding population [18], and it requires detection and analysis of genetic variations using advanced genetic approaches, leading to phenotypic traits of quantitative and agro-economic importance [19]. Genomic selection (GS) and MAS developed by molecular markers techniques has made it possible to map quantitative trait loci (QTL) and identifying QTLs for high-temperature stress and breeding heat-tolerant varieties is an effective way to address this issue. MAS methodology has been used globally to acquire ordered and swift ways for cotton improvement on large scales internationally, with both highly demanded attributes like high seed production and excellent quality of fiber [20]. For dissection of QTLs related to traits with complex genetic patterns of inheritance, molecular marker use has been an efficient tool and these markers have also facilitated MAS breeding [21].

Both agronomic and economically important traits are approached by researchers for obtaining the aim of better yield of cotton [22]. The main challenging goal for current cotton breeders is to further enhance cotton production. However, this aim is hindered by the use of locally available germplasm and extreme environmental fluctuations that influence yield attributing traits [23,24]. Certain different genes cause different expressions of characters regarding tolerance of heat stress at vegetative and reproductive growth stages [25]. Genes attributing to relative water content, stomatal conductance, especially along with Percent Boll set on the First Position along Sympodia (PBF), Percent Boll set on the second Position along Sympodia (PBS), Cell Injury (CIY), Boll Number (BON), Total number of Buds (TNB), Size of Petiole (SOP), Total number of Flowers (TNF), Length of Bract (LOB), Length of Petal (cm) (LOP), Length of Staminal Column (LOS), Length of Pistil (LPI), and Proline Con. ($\mu g\ mL^{-1}$) (PCO) have been reported as crucial for heat stress determination [26,27]. Therefore, during the selection of heat tolerant varieties, both vegetative and reproductive traits should be considered equally.

Molecular genetic methods, especially molecular markers, have been applied widely in cotton in recent couple of decades. Recently, the development of molecular markers was accelerated with the release of assembled genome sequences of *G. hirsutum* [28,29]. Numerous genetic linkage maps including the intraspecific map of *G. hirsutum* have been constructed using restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs). Thousands of quantitative trait loci (QTLs) for yield and fiber quality in cotton have been documented in Cotton

QTLdb, Release 2.3 [30,31]. However, there are few studies about the simultaneous dissection of the genetic basis underlying complex traits and their genetic correlations in multiple upland cotton populations by QTL mapping. In the situation of changing weather and elevating temperature around the globe, it is of the utmost importance to recognize QTLs for morphological, architectural, and physiological traits that are directly or indirectly affected by high heat stress at some stages of cotton plant development. This study was conducted to identify and map quantitative trait loci (QTLs) conferring heat tolerance in an Intraspecific cross and used microsatellite markers to identify polymorphism between two upland cotton cultivars in the scorching heat of Multan (Pakistan) during summer. QTL identified in this project could be helpful for future cotton growers of high-temperature regions in the world.

In this study, F_2 populations were used, which were derived from hybridization of two *G. hirsutum* normal lines (MNH-886 and MNH-814). The corresponding genetic linkage map was constructed using 175 polymorphic SSR markers. QTL mapping was implemented with the integration of the genotypic and phenotypic data of twenty-three agronomic and economic traits contributing towards heat tolerance; the aim of this study was to (a) screen cotton cultivars for heat tolerance, (b) select diverse cultivars as parental lines and then their assessment by SSRs for parental survey, (c) develop the segregating/mapping population (F_2) of selected parents and collect phenotypic trait data at different time intervals, (d) survey the F_2 population by polymorphic markers obtained from the parental survey, (e) evaluate phenotypic traits with the association of genotypic markers (SSR) data, (f) identify QTLs directing heat tolerance by QTL cartographer software, and (g) construct a genetic linkage map of *Gossypium* from the obtained information. The outcomes of this study will help plant breeders to produce heat-resistant varieties that will help farmers and countries with agriculture-dependent economies, especially in high-temperature areas around the globe.

2. Materials and Methods

This study was conducted to identify and map QTLs conferring heat tolerance in an Intraspecific cross and used microsatellite markers to identify polymorphism between two upland cotton cultivars in the scorching heat of Multan (Pakistan) during summer. QTL identified in this project could be helpful for future cotton growers of high temperature regions in the world. The research was arranged at Cotton Research Station (CRS) Multan to coincide the reproductive phase with higher temperature. The field work encompassed 14 cultivars sown in Randomized Complete Block Design (RCBD) replicated three times during the year 2012. All fourteen cultivars were tagged randomly altogether to evaluate 23 morphological and physiological parameters contributing to heat tolerance for identifying the genomic regions under plant breeding techniques; F2 generation was observed for screening purposes. The cultivars named as CIM-557, CIM-573, NN-3, Cyto-108, NIAB-852, CIM-588, BH-172, GH-102, NIAB-2008, MNH 886, CIM-554, Shahbaz-12, MNH-2007, and MNH 814 were chosen for screening of heat tolerance based on different agronomic traits related to heat, and their genomic basics were screened out. Different morpho-physiological characters included plant height (PH), fully dehiscent anther (FDA %), Total number of sympods (TNS), Total Number of Nodes (TNN), Pollen Viability (%) (POV), First Sympodial Node Number (FSN), First Sympodial Node Height (cm) (FSH), Sympodial Node Number bearing first effective boll (SNF), Sympodial Node Height (cm) bearing first effective boll (SNH), Sympodial Node Number bearing Last effective boll (SNL), Sympodial Node Height (cm) bearing last effective boll (SNB), Percent Boll set on First Position along Sympodia (PBF), Percent Boll set on second Position along Sympodia (PBS), Cell Injury (CIY), Boll Number (BON), Total number of Buds (TNB), Size of Petiole (SOP), Total number of Flowers (TNF), Length of Bract (LOB), Length of Petal (cm) (LOP), Length of Staminal Column (LOS), Length of Pistil (LPI), and Proline Con. ($\mu g\ mL^{-1}$) (PCO).

2.1. Heat Stress Estimation

Heat stress was measured in plants that were sown late in the month of April and traits were compared to plants sown earlier in May because temperature in the latter was higher than 46–48 °C during the research time period and the heat stress-related 23 morphophysiological traits were observed to be affected by temperature in late-sown irrigated conditions. The heat was estimated by a weather forecast taken from the automated metrological station of cotton research station, Multan, as given in the Table 1:

Table 1. Comparative Monthly Meteorological Data Recorded at CCRI, Multan.

Month	Air Temperature (°C)		Relative Humidity		Rainfall (mm)	Evapotranspiration (cm Day)	Soil Temperature (°C)	
	Max	Min	Max	Min			5 cm	10 cm
January	5.3	19.1	63	92	1.5	0.24	9.4	10.5
February	6.9	20.5	52	76	0.0	0.39	12.3	12.7
March	13.9	27.4	45	65	0.0	0.67	19.2	19.7
April	20.6	32.8	55	72	24.7	0.86	26.5	27.0
May	25.7	39.4	54	57	1.10	1.22	31.7	32.0
June	28.6	39.4	58	64	0.0	1.26	35.4	35.4
July	28.8	38.1	61	73	16.9	1.11	35.8	36.0
August	28.0	35.6	72	76	16.1	0.84	34.9	35.1
September	25.7	33.1	80	87	167.0	0.59	29.8	30.2
October	18.9	31.7	62	83	3.2	0.48	24.3	25.1
November	13.1	26.8	81	87	0.0	0.28	17.7	18.6
December	7.8	21.9	80	87	4.0	0.19	12.8	13.8

Heat-susceptible and -resistant varieties (MNH-814 and MNH-886 respectively) were selected on the basis of data for relative water content, osmotic potential, cell injury, and proline concentration. Relative water content was measured by the following [32] formula:

$$RWC = \frac{fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} \times 100$$

Cell Injury (CIY) was measured when the crop was 55–60 days old, and a sufficient number of leaves was taken from the upper portion and stored in a paper bag. By the use of a punching machine, 15–25 discs of 1 cm diameter were cut. With distilled water, leaf discs were washed three times, were put in test tubes, and then the test tubes were filled up to 40 mL with distilled water. Three sets were made each of 14 test tubes containing leaf discs of 14 cultivars. The first set of test tubes was kept at room temperature as control and the electrical conductivity of the water was noted. The second set was heated at 48 °C for 45 min in a water bath. When water was cooled after 6 h, its electrical conductivity was recorded, while the third set of test tubes was autoclaved at 15 lbs (pressure) for 15 min and electrical conductivity was noted when water was cooled.

The greater the EC, greater the damage caused to plant cells due to heat stress as the maximum number of electrolytes came out of the cell due to cell injury. Consequently, cell injury was also greater. Cell injury was expressed in percentage. Proline is an organic compound synthesized from glutamine. It is located in cytoplasm under stressed conditions as nontoxic compatible organic solute to compensate for the dehydrating effects of high osmotic pressure in the vacuole and in the external media. The proline concentration at 700 mol m^3 was not inhibitory to enzymes and develops in consequences of poor plant growth under toxic effects. Therefore, its exogenous application should promote

tolerance [33]. Different workers stated that upon heat stress, when starch and protein synthesis are inhibited, proline might be used by the plant for growth [34,35]. Proline from different tissues was measured by Spectrophotometry based on the method of ref. [36].

2.2. Parental Lines Screening

Fourteen tetraploid cotton cultivars were chosen, named CIM-557, CIM-573, NN-3, Cyto-108, NIAB-852, CIM-588, BH-172, GH-102, NIAB-2008, MNH 886, CIM-554, Shahbaz-12, MNH-2007, and MNH 814 for altogether 23 morphological and physiological characteristics, viz Total Plant Height (TPH), Fully Dehiscent Anther (%) (FDA), Total Number of Sympodes (TNS), Total Number of Nodes (TNN), Pollen Viability (%) (POV), 1st Sympodial Node number (FSN), 1st Sympodial node Height (cm) (FSH), Sympodial Node number having 1st effective boll (SNF), Sympodial Node Height (cm) having 1st effective boll (SNH), Sympodial Node Number having Last effective boll (SNN), Sympodial Node Height (cm) having last effective boll (SNH), Percent boll set on 1st position with sympodia (PBF), Percent boll set on 2nd position with sympodia (PBS), Cell Injury (CIY), Number of Bolls (NOB), Total number of buds (TNB), Size of Petiole (SOP), Total Number of Flowers (TNF), Length of Bract (LOB), Length of Petal (cm) (LOP), Length of Staminal column (LOS), Length of Pistil (LPI), and Proline Con. (μg mL^{-1}) (PCO), contributing to heat tolerance to identify the genomic regions. Arithmetic means of three replicates were calculated for fourteen cultivars for each characteristic. The data were compared. The variance and standard deviation were also calculated. The computation of trait correlation was carried out using Minitab Inc., University Park, PA, USA and the following shortlisted traits had considerably varying phenotypes among two genotypes, i.e., MNH 886 and MNH 814 (Figure 1).

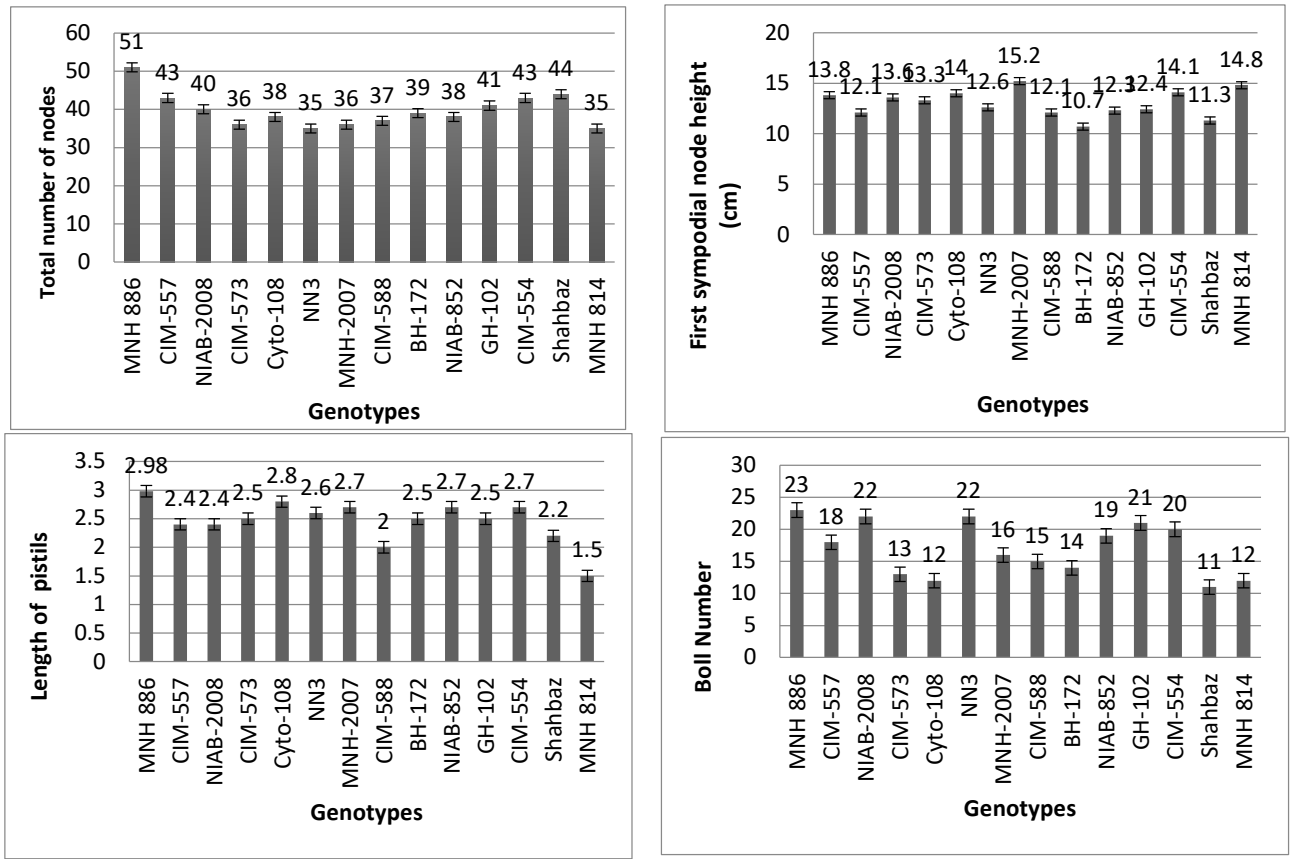


Figure 1. *Cont.*

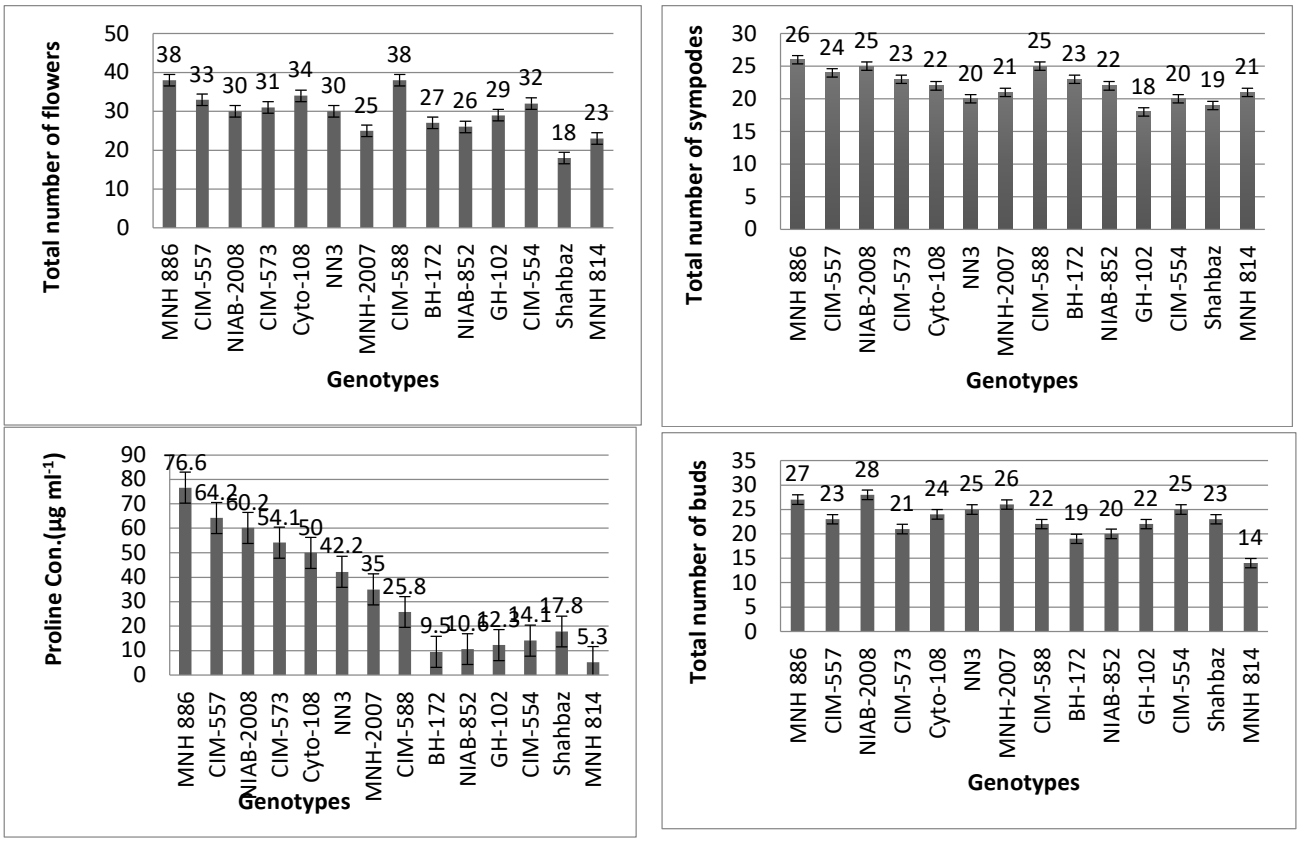


Figure 1. Mean values of phenotypic variations of morpho-physiological traits related to the heat stress of 14 cotton genotypes.

2.3. Mapping Population

Based on highly significant differences between two parental lines, the F_2 population was developed by self-pollinating F_1 plants from a cross between upland cotton line MNH-886 (a heat-tolerant cultivar), and MNH-814 (a heat-sensitive cultivar), and was used to map HT-QTLs during different morphological stages in cotton. Five plants were tagged at random in each line for recording physiological traits data. Ninety-four plants from the F_2 population were selected to derive phenotypic and molecular data along with two parents. The experimental field area of Cotton Research Station Multan under natural conditions was selected for experiment to coincide the reproductive phase with higher temperature.

2.4. Phenotypic Data Collection Statistical Analysis

Selected parental lines and 94 F_2 individuals' phenotypic data were collected from fields at different time intervals. Arithmetic means of 3 replicates were calculated for each parent for each characteristic. The data for heat characteristics were compared. The computation of trait correlation was carried out using Minitab Inc., University Park, PA, USA.

2.5. Microsatellite Analysis

Laboratory techniques for DNA extraction were performed as described by Peterson. Amplification reactions were carried out in 15 uL reaction volumes containing 30 mg genomic DNA, 1.0 µM each of SSR primers sequences, which were drawn from the following sources: BNL primers from the Research Genetics Co. (Huntsville, AL, USA, http://www.resgen.com, accessed on 7 April 2022); JESPR primers [37]; CIR primers [37]; and NAU primers [38,39], 100 uM each of dATP, dCTP, dGTP, and dTTP, 1 unit of Taq DNA Polymerase (Fermentas), 1x*Taq* Polymerase Buffer, and 2.5 mM $MgCl_2$. PCR amplifications were performed as described [40] using a Peltier Thermal Cycler (MJ Research, Waltham,

MA, USA) programmed as follows: an initial denaturation of 5 min at 94°; 35 cycles of 94° for 1 min (denaturation), 55° for 1 min (annealing), and 72° for 2 min (extension). One additional cycle of 10 min at 72° was used for final extension. The amplified products were electrophoresed on a 10% non-denatured polyacrylamide gel using a DYCZ-30 electrophoresis apparatus (Beijing WoDeLife sciences instrument company, Beijing, China).

2.6. QTL Mapping

Genetic mapping and QTL analysis were performed on each population separately and combined across populations. Linkage maps were constructed using MAPMAKER/Exp Version 3.0b software [41]. QTLs were identified by composite interval mapping [42] using Windows QTLs Cartographer 2.5 [43]. A LOD threshold of 3.0 was used [44]. Marker's order was confirmed with the "ripple" command. Recombination frequencies were converted into map distances (cm) using the Kosambi mapping function [45].

Tests for independence of QTLs were also conducted when 2 or more QTLs of a trait were located on the same chromosome [46]. QTLs were declared significant if the corresponding LR score were greater than 11.5 (equal to a LOD score of 2.5). The proportion of the phenotypic variation explained by each QTL was calculated as R^2 (%) = Phenotypic variability explained by QTL/all of the variation in the population × 100. The total phenotypic variance explained together by all the putative QTLs for each trait was estimated by fitting a multiple-QTL model in the Mapmaker/QTL program.

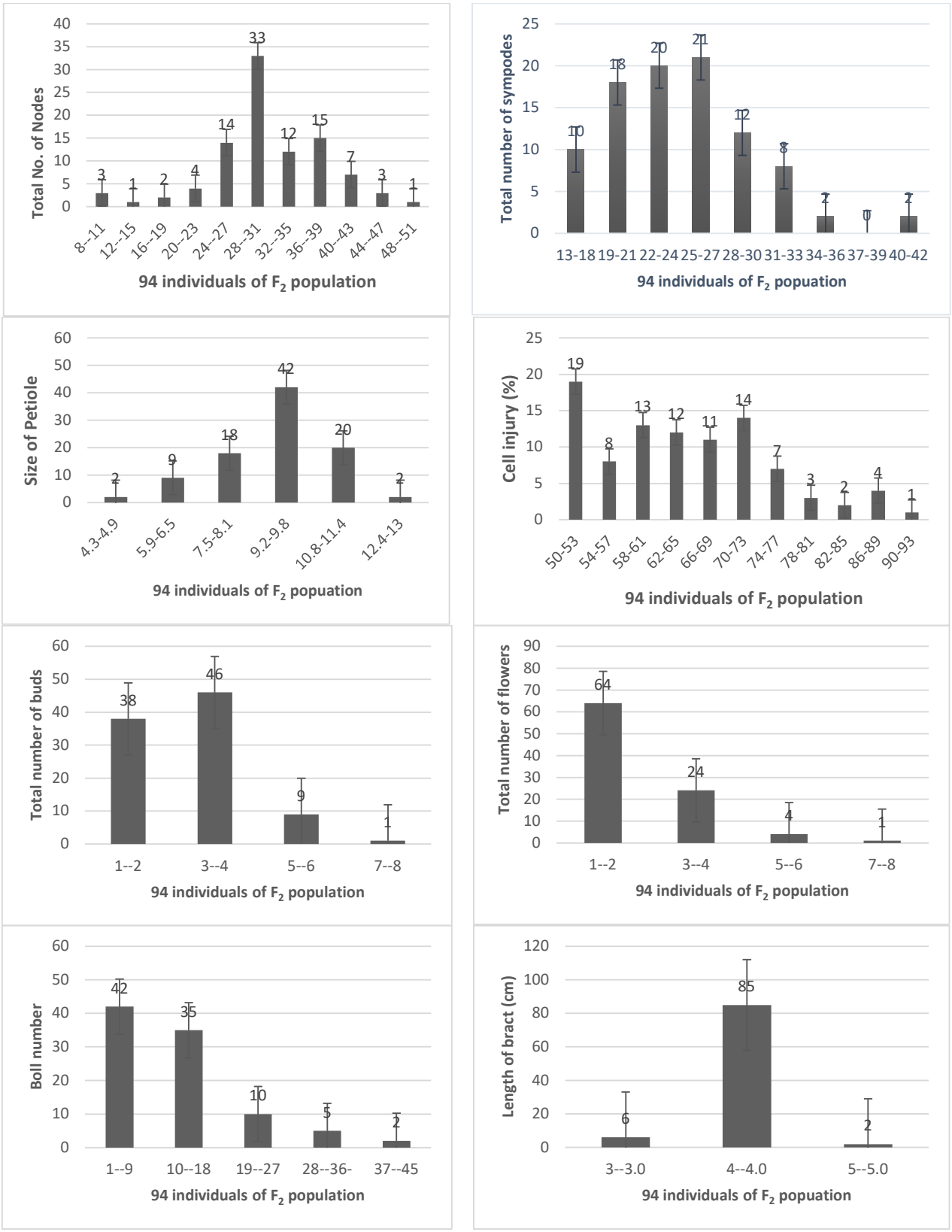
3. Results

3.1. Average Performance of Cotton Varieties Based on Morpho-Physiological Traits

Based on statistically significant differences for various morpho-physiological characteristics, two cultivars, MNH-886 and MNH-814, were selected. Significant variations in heat tolerance characteristics were observed among both varieties. The mean value for fully dehiscent anthers (FDA) was 92 and 64 for MNH-886 and MNH-814 respectively. MNH-814 showed less pollen viability (66.4) than MNH886 (88.3). SNF was 34 for MNH-886 and for MNH-814 its average value was 27 (Figure 2a). The trait PBF average data of both parents were 51 and 38. MNH-886 showed less CIY while exposed to high temperatures, with an average value of 65, while MNH-814 was susceptible to extreme temperatures and the CIY was greater, with a value of 80.

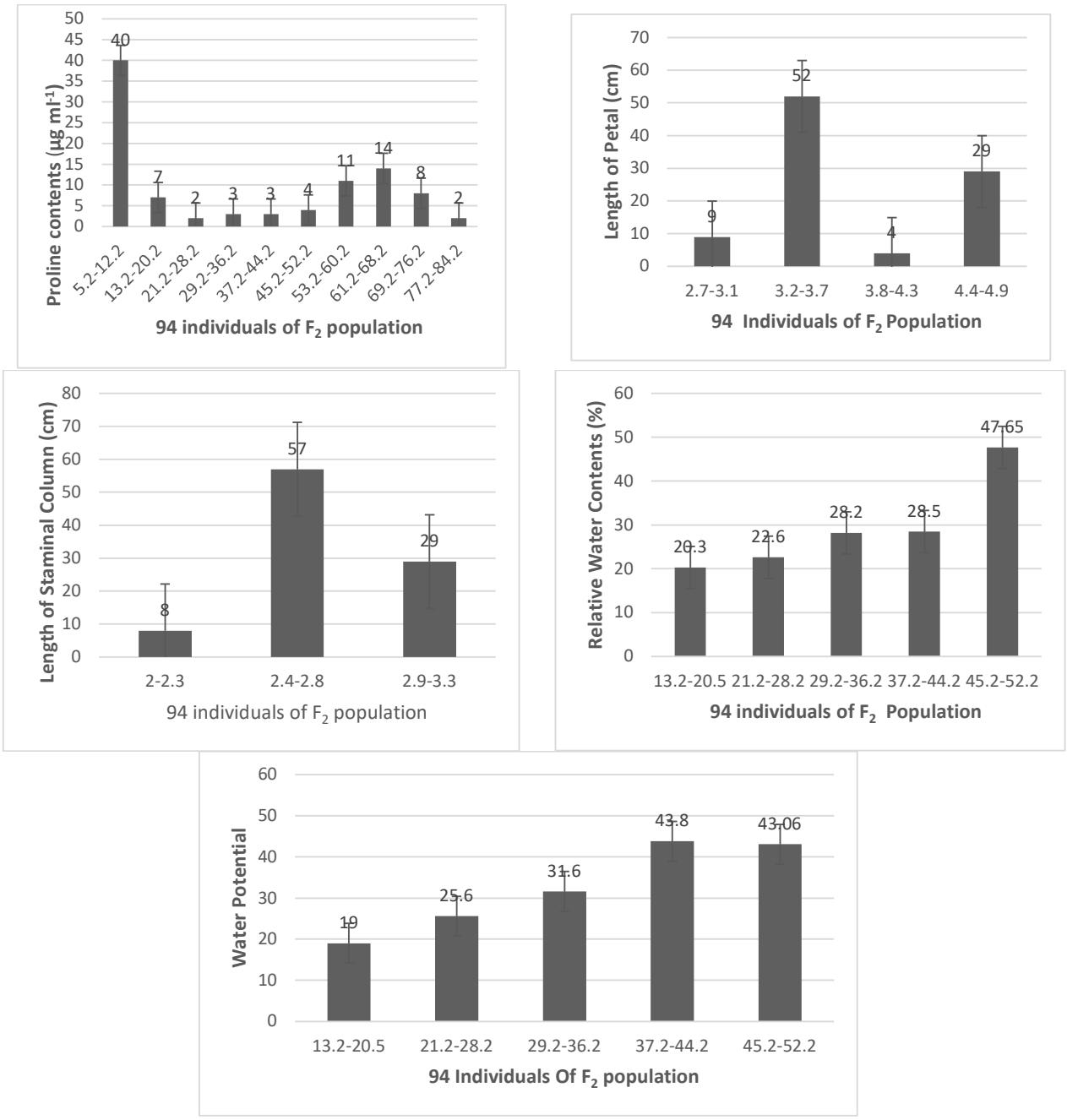
Likewise, MNH 886 excelled in NOB with an average value of 23 while MNH-814 showed 12 TNN under heat-stress conditions. MNH 886 showed TNF even under heat stress with an average value of 35 while MNH 814 showed retention of a smaller number of flowers with an average value of 23. Maximum variation was observed in trait PCO; its value was 5.3 for MNH-886 and 76.6 for MNH 814. The average values of morpho-physiological traits showed that both varieties vary in most of the traits and showed that MNH-886 excelled in heat tolerance considering each trait compared with other cultivars, while MNH-814 was the most susceptible as compared with other varieties (Table 2).

Phenotypic distribution of F_2 population for morpho-physiological traits is shown in Figure 2a,b. The phenotypic values of morpho-physiological traits are presented in Table 3. Twenty-one morpho-physiological traits displayed a normal distribution (skewness did not exceed 1.0), while two traits, TNF and NOB, showed a non-normal distribution. These results indicated the trend of having major QTL involvement in this population and it was thus suitable for QTL analysis.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. (**a**,**b**) Frequency distribution of morpho-physiological traits related to heat stress across F_2 population.

Table 2. Mean values data for 23 morpho-physiological traits of fourteen cotton cultivars.

Cultivars	Total Plant Height (cm)	Fully Dehiscent Anther (%)	Pollen Viability (%)	First Sympodial Node Number	First Sympodial Node Height (cm)	Sympodial Node Number Bearing First Effective Boll	Sympodial Node Height (cm) Bearing First Effective Boll	Sympodial Node Number Bearing Last Effective Boll	Sympodial Node Height (cm) Bearing Last Effective Boll	Percent Boll Set on First Position along Sympodia	Percent Boll Set on Second Position along Sympodia
MNH 886	90	92	88.3	7	13.8	8	15	34	114.2	51	32
CIM-557	67	91	87.4	7	12.1	8	14.8	32	93.4	49	31
NIAB-2008	55	89	86.1	7	13.6	8	15.2	33	82.5	50	32
CIM-573	54	88	85.8	8	13.3	9	15.9	31	112.3	48	31
Cyto-108	67	87	85.1	7	14	8	16.3	33	108.9	49	30
NN3	88	85	83.5	7	12.6	8	14.5	32	105.6	47	31
MNH-2007	67	83	82.2	8	15.2	9	17.9	31	87.3	47	30
CIM-588	77	82	80.5	7	12.1	8	15.6	33	92.7	45	29
BH-172	80	80	79.3	7	10.7	8	13.8	32	84.5	44	29
NIAB-852	85	79	77.5	7	12.3	8	14.9	33	113.8	45	28
GH-102	77	77	76.3	7	12.4	8	15.3	31	103.2	43	27
CIM-554	66	75	74.1	7	14.1	8	16.8	31	121.2	42	29
Shahbaz	88	73	71.2	7	11.3	8	13.7	29	81.7	41	26
MNH 814	54	64	66.4	8	14.8	9	17.3	27	106.5	38	23
Max	90	92	88	8	15	9	17	34	121	51	32
Min	54	64	66	7	10	8	13	27	81	38	23
Variance	219	60	42	181	1.70	181	1.52	3.34	176	14	6
Std. Dev.	±14.79	±7.80	±6.53	±42	±1.30	±42	±1.23	±1.82	±13.2	±3.75	±2.50

Cultivars	Cell Injury (%)	Total Number of Sympodes	Total Number of Nodes	Size of Petiole	Total Number of Flowers	Number of Bolls	Total Number of Buds	Length of Bract (cm)	Length of Petal (cm)	Length of Staminal Column	Length of Pistil	Proline Con. ($\mu g\ mL^{-1}$)
MNH-886	65	26	51	9.3	38	23	27	5	4	2.90	2.98	76.7
CIM-557	66	24	43	7.3	33	18	23	4.5	2.9	2.4	2.4	64.2
NIAB-2008	67	25	40	7.6	30	22	28	3.6	2.8	2.3	2.4	60.2
CIM-573	67	23	36	8.1	31	13	21	3.7	2.6	2.6	2.5	54.1
Cyto-108	68	22	38	8.3	34	12	24	3.9	2.7	2.7	2.8	50
NN3	68	20	35	7.5	30	22	25	3.4	2.6	2.1	2.6	42.2

Table 2. *Cont.*

Cultivars	Total Plant Height (cm)	Fully Dehiscent Anther (%)	Pollen Viability (%)	First Sympodial Node Number	First Sympodial Node Height (cm)		Sympodial Node Number Bearing First Effective Boll	Sympodial Node Height (cm) Bearing First Effective Boll	Sympodial Node Number Bearing Last Effective Boll	Sympodial Node Height (cm) Bearing Last Effective Boll	Percent Boll Set on First Position along Sympodia	Percent Boll Set on Second Position along Sympodia
MNH-2007	68	21	36	7.8	25	16	26	3.3	2.5	2.5	2.7	35
CIM-588	70	25	37	9.0	38	15	22	3.7	2.2	2	2	25.8
BH-172	71	23	39	8.0	27	14	19	3.1	2.8	2.2	2.5	17.8
NIAB-852	72	22	38	8.7	26	19	20	3.2	2.8	2.6	2.7	14.1
GH-102	73	18	41	8.5	29	21	22	3.5	2.7	2.7	2.5	12.3
CIM-554	74	20	43	8.3	32	20	25	3.8	2.4	2.5	2.7	10.6
Shahbaz	76	19	44	7.9	18	11	23	3.9	2.9	3	2.2	9.5
MNH-814	80	21	35	7	23	12	14	3	2	4.5	1.5	5.3
Max	80	26	51	9	38	23	28	5	4	4	2	76
Min	65	18	35	7	18	11	14	3	2	2	1.5	5
Variance	18	5	19	0.41	27	19	13	0.29	0.20	0.36	0.13	56
Std. Dev.	±4.25	±2.40	±4.41	±0.64	±5.2	±4.4	±3.6	±0.54	±0.45	±0.60	±0.37	±23

Table 3. Phenotypic values for heat tolerance traits of F_2 population and their parents.

Population Size	Traits	Parents		F_2 Population Statistical Data				
		MNH-886	MNH-814	Max	Min	Mean	SD	Skew
94	TPH	90	54	48	105	74.37	12.07	0.390
	FDA	92	64	60	92.	75.03	9.98	0.365
	POV	88.3	66.4	58	666	78.48	61.89	0.391
	FSN	7	8	7	9	7.82	0.824	0.328
	FSH	13.8	14.8	10	16.10	13.64	1.134	−0.224
	SNF	8	9	6	11	8.64	0.912	0.934
	SNH	15	17.3	11.10	17.30	14.30	1.568	0.211
	SNL	34	27	22	34	28.92	3.26	0.002
	SNB	114.2	106.5	80.5	115	100.19	9.63	−0.315
	PBF	51	38	31	51	43.44	5.30	−0.210
	PBS	32	23	23	3191	62.47	326.15	0.691
	CIY	65	80	50	90	64.60	10.36	0.477
	TNS	26	21	13	39	21.77	5.33	0.732
	TNN	45	51	6	48	30.93	7.12	−0.577
	SOP	9.3	7	4.30	12.30	8.69	1.51	−0.428
	TNF	35	23	1	8	2.13	1.25	1.62
	NOB	23	12	1	45	12.10	8.80	1.26
	TNB	27	14	1	7	2.92	1.32	0.796
	LOB	5	3	3	5	3.94	0.33	−0.770
	LOP	4	2	2.70	4	3.65	0.317	−0.760
	LOS	2.90	4.5	2.0	3.10	2.64	0.261	−0.434
	LPI	2.98	1.5	2.30	4.00	2.99	0.308	0.240
	PCO	5.3	76.6	5.20	76.7	33.43	27.05	0.274

3.2. Stress Determining Physiological Traits

Physiological traits measure the response of plants to different phenomena taking place internally, such as cell injury and production of certain proteins, such as proline, in response to heat stress. MNH-886 showed less CIY while exposed to high temperatures, with an average value of 65, while MNH-814 was susceptible to extreme temperatures and CIY was higher with a value of 80. CIM-557 showed 64.2, while MNH-886 showed a significant value of 76.6 for proline content in heat stress. MNH-814 was found as the most susceptible among fourteen experimental cultivars and showed a proline content value of 5.3 under stress (Table 4).

Table 4. Stress determining physiological traits (Relative Water Content = RWC, water potential = WP, Osmotic Potential = OP, CIY = Cell injury = CIY, proline Contents = PCO).

Population Size	Traits	Parents (Means)		F_2 Population Statistical Data				
		MNH-886	MNH-814	Max	Min	Mean	SD	Skew
94	RWC	47.65	43.06	54.91	40.28	47.65308	3.815905	0.321
	WP	20.30	19.00	27	15	19.65	2.511	0.283
	OP	860.76	805.92	975	727	833.34	65.07	0.382
	CIY	65	80	50	90	64.60	10.36	0.477
	PCO	5.3	7.66	7.66	5.3	6.48	2.05	0.274

3.3. Correlation

Correlation (Figure 3) was observed by OriginPro 8.5 software and it was observed that plant height showed a positive correlation with the number of fruiting branches per plant, total number of nodes, size of petiole and balls, length of bracts, length of petals, and length of pistil but it had no correlation with total number of flowers, whereas plant height was negatively correlated with total number of nodes, first sympodial node height, sympodial nose number bearing first effective boll, sympodia node height, bearing last effective boll, cell injury, total number of sympods, length of staminal column, and proline content. Fully dehiscent anther had a positive correlation with sympodial node number, percent boll set on first position, percent boll set on second position along sympodia, total number of sympods, total number of nodes, size of petiole, boll number, total number of bolls, and length of bract. Hence, the length of petiole, proline contents, sympodial node number, percent boll set on first and second position along sympodia, total number of nodes, size of petiole, branch number, total number of bolls, length of bract, and length of petiol were all positively correlated with each other and a significant effect was observed among the traits.

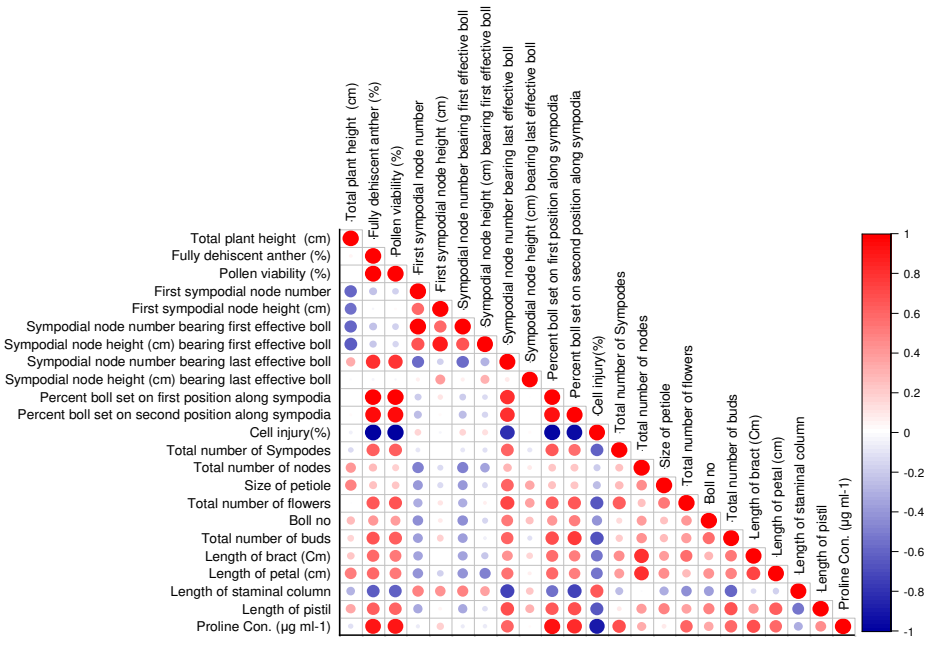


Figure 3. Pearson correlation among phenotypic traits of cotton under heat stress (= + ve = - ve).

3.4. Construction and Characterization of Intra Specific Linkage Map

Among the 1450 SSR primer pairs tested on parental lines, 175 markers were found to be polymorphic. These markers were applied on population. Using a LOD score > 3.0, these markers were assigned to 26 chromosomes for population based on the information on the cotton SSR map [47]. The linkage map was constructed for the F_2 population. Each linkage group was assigned to specific chromosome (Figure 4). The linkage maps covered approximately 4402.7 cm (Table 5) with an average distance of 20 cm within the markers which, according to the position of SSR markers, is common with the cotton map [48]. We estimate that we surveyed close to 70% of the cotton map, comparing the length of our map with that of the cotton map. The genetic map for the population was generated by MAPMAKER/version 3.0. Genotypic frequencies deviation from the expected segregation ratio of 1:2:1 for the co-dominant locus or 3:1 for the dominant locus was detected with the legitimacy of the additive-dominance model by means of the Chi square (χ^2) method [49].

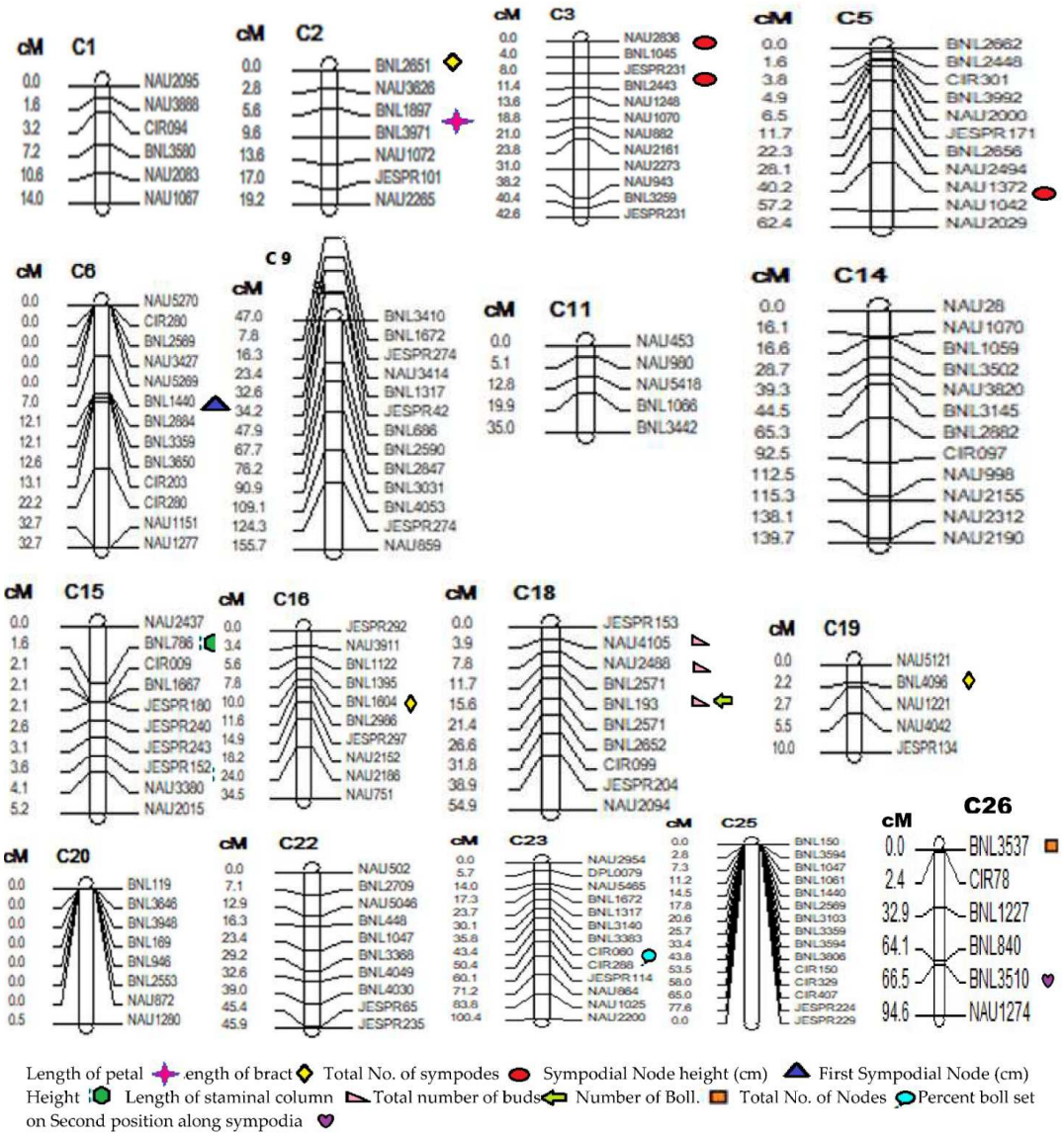


Figure 4. Linkage map and QTLs for heat stress tolerance determined in an F_2 population made from cross among Intraspecific MNH-886 and MNH-814 (*G. hirsutum*). The gap indicates that the linkage distance of the primer loci > 50 (cm) indicate significant QTLs (Kosambi).

Table 5. Basic characteristics of the genetic map.

Item	Field Exp. Pop
Total no. of SSR loci	175
No. of mapped loci	171
No. of individuals	94
No. of linkage groups	17
No. of unlinked loci	4
Length of map (cm)	4402.7
Total no. of skewed loci	24

3.5. QTLs Mapping for Traits Associated with Heat Tolerance in Cotton

A summary of statistically important QTLs is shown in Table 6. All QTLs for First Sympodial node height, Sympodial node height, percent boll set along sympodia at 2nd position along sympodia, Total number of Sympodes, Total no. of nodes, number of bolls, Total no of buds, Length of bract, Length of Staminal column, and and Length of petal are shown in Figure 3. A total of 17 regions were recognized that contain the QTLs with LOD value 3.0 and above. The most noteworthy QTLs are described in Table 6.

Table 6. QTLs related to heat tolerance in Intraspecific cross among MNH-886 and MNH-814. (LOD = Logarithm of odds, Additive = Additional effects, Dominance/additive = ratio between dominance and additive effects, PV% = Phenotypic variance).

QTLs	Chr. No.	SSR Markers	LOD Value	Additive	Dominance	Dominance/Additive	PV% Age
First Sympodial Node Height (cm)							
qFSHa1	**15**	**BNL786-CIR009**	6.10	0.59	−0.80	−1.36	36.62
qFSHa2	**15**	**JESPR152-NAU3380**	6.09	0.58	−0.81	−1.39	35.98
Sympodial Node Height (cm)							
qSNH1	**6**	**BNL1440-BNL2884**	3.42	0.77	−0.31	−0.40	17.59
Percent Boll Set on Second Position Along Sympodia							
qPBS1	**26**	**BNL3510-NAU1274**	18.19	0.69	0.35	0.50	14.56
Total No. of Sympodes							
qTNSa1	**03**	**NAU2836-BNL1045**	3.59	6.00	0.41	0.07	10.05
qTNSa2	**03**	**JESPR231-BNL2443**	3.71	6.27	0.38	0.06	10.12
qTNSa3	**05**	**NAU1372-NAU1042**	3.98	2.89	−0.50	−0.17	16.93
Total No. of Nodes							
qTNN1	**23**	**CIR080-CIR288**	4.05	0.18	0.03	0.17	12.91
Number of Bolls							
qNOB1	**26**	**BNL3537-CIR078**	3.80	4.25	−3.15	−0.74	21.52
Total Number of Buds							
qTNB1	**18**	**BNL193-BNL2571**	3.79	1.05	−0.74	−0.70	17.67
Length of Bract							
qLOBa1	**02**	**BNL2651-NAU3626**	3.24	0.18	0.04	0.20	8.59
qLOBa2	**16**	**BNL1604-BNL2986**	3.01	−0.13	−0.03	0.23	7.76
qLOBa3	**19**	**NAU5121-BNL4096**	4.05	0.18	0.03	0.17	12.91
Length of Staminal Column							

Table 6. *Cont.*

QTLs	Chr. No.	SSR Markers	LOD Value	Additive	Dominance	Dominance/Additive	PV% Age
qLOSa1	**18**	**JESPR153-NAU4105**	3.78	0.52	0.11	0.20	16.30
qLOSa2	**18**	**NAU2488-BNL2571**	3.76	0.52	0.11	0.20	15.84
qLOSa3	**18**	**BNL193-BNL2571**	3.07	0.30	0.11	0.36	14.57
			Length of Petal				
qLOP1	**02**	**BNL1897-BNL3971**	3.56	0.45	-0.02	-0.05	19.46

3.5.1. QTLs for First Sympodial Node Height (FSH)

Two QTLs, *qFSHa1* and *qFSHa2*, for first sympodial node height were detected on chromosome 15 with LOD $\geq$ 6.0, which explain 35% and 36% of phenotypic variance in F_2 respectively. These Loci detected 35%–36% of the PV value. When two QTLs were assembled together, they explained 71% of the PV value. The additive values for both QTLs were 0.59 and 0.58 respectively (Table 6). Position of the QTLs on the linkage map is shown in Figure 4.

3.5.2. QTLs for Sympodial Node Height Bearing First Effective Boll Set (SNH)

One QTL, *qSNH1*, influencing Last Effective Boll Set with a LOD score of 3.42 was detected in the F_2 population and it was located on chromosome 6. Putative QTL in this region accounted for 17% of phenotypic variance. So, this QTL explained 17% of the total phenotypic variance (Table 6 and Figure 4).

3.5.3. QTLs for Percent Boll Set on Second Position along Sympodia (PBS)

In the F_2 population of one QTL, *qPBS1*, the total influencing number of nodes was identified with a LOD score of 18.21 and it was located on chromosome 26. Phenotypic variance in this region was 14.56% (Table 6). The additive value for this QTL was 0.69.

3.5.4. QTLs for Total No of Sympodes (TNS)

Two QTLs, *qTNSa1* and *qTNSa2*, on chromosome 03 were detected for a total number of sympodes with LOD values 3.59 and 3.71 respectively. Phenotypic variance was observed between 10.05% and 10.12%, and the additive effect was 6.00 and 6.27 respectively. A total of 22% of phenotypic variance was seen when two QTL were fitted simultaneously. The third QTL*qTNSa3* was detected on chromosome 05 with LOD value 3.98. The additive effect was 2.89. Phenotypic variance observed was 16.93%. (Table 6).

3.5.5. QTL for Total No of Nodes (TNN)

On chromosome 23 single QTL *qTNN1*was detected for total no of nodes with LOD value 4.05. Positive additive effect was seen with value 0.18. Phenotypic variance seen was 12.91% (Table 6).

3.5.6. QTLs for Number of Bolls (NOB)

In experiment, one QTL, *q NOB1*, for Length of bract was identified on chromosome 26 with accumulative phenotypic variance of 21.52%. The LOD value was 3.80. So, this QTL showed phenotypic variance of 22%. Additive positive effect of *q NOB1* was 4.25 (Table 6).

3.5.7. QTLs for Total No of Buds (TNB)

In the F_2 population, one QTL, *qTNB1*, influencing the Total No. of buds was identified with a LOD score of 3.79 and it was located on chromosome 18. Phenotypic variance in this region was 17.67%. Additive effect was positive with value 1.05 (Table 6).

3.5.8. QTLs for Length of Bract (LOB)

Three QTLs, *qLOBa1*, *qLOBa2*, and *qLOBa3*, for length of bract were detected during analysis. The first QTL was on chromosome 2 with LOD $\geq$ 3.24 and with a positive additive effect of 0.18. Phenotypic variance observed was 8.59%. The second QTL was detected on chromosome 16 with LOD $\geq$ 3.01 and a negative additive effect of 0.13 and phenotypic variance 7.76%. The third QTL was detected on chromosome 19 with a positive additive effect of 0.18 and phenotypic variance of 12.91%. When three QTLs were fitted together simultaneously the phenotypic variance was 28% (Table 6).

3.5.9. QTL for Length of Staminal Column (LOS)

Three QTL, s*qLOSa1*, *qLOSa2*, and *qLOSa3*, were detected on chromosome number 18 for length of staminal column. The LOD values were 3.78, 3.76, and 3.07 respectively. Results showed positive additive effects of 0.52, 0.52, and 0.30 for the three QTLs, while 16.30, 15.84, and 14.57 were the values for phenotypic variance for all QTLs. When the three QTL's were fitted together simultaneously the phenotypic variance was 47% (Table 6).

3.5.10. QTLs for Length of Petal (LOP)

One QTL, *qLOP1*, was identified that influenced Length of petal trait. The QTL was located on chromosome number 2. The LOD value for QTL was 3.56. Phenotypic variance was 19.46. The QTL showed a positive additive effect of 0.45 (Table 6).

4. Discussion

The current study was carried out to identify the genetic basis responses of cotton plants under heat stress. The data collected were at the parental line and then after by F_2 generation from which heat-susceptible and heat-tolerant genotypes were selected for the screening process. Initially, the emergence of the first sympodial branch at lower nodes determined the early maturity of cotton plants. Theoretically, it is implicated for the 1st sympodial branch to appear on lower nodes as it is highly correlated with earliness and heat tolerance [50,51]. The strong relationship between early maturity and lower sympodial branch node number was reported in previous studies [52]. It was reported that there was a strong association of the 1st sympodial branch node number and heat tolerance. Highly significant differences were found in analysis of variance for the 1st sympodial node number (Table 4). The data of correlation (Figure 3) showed a positive correlation of the 1st sympodial node number expressed with all the traits except sympodial node number, present boll set on first position along sympodia, cell injury, and length of pistil. Node number to set the initial fruiting sympodia is a reliable and realistic morphological trait of heat tolerance [53]. Minimum and maximum temperature significantly affected the first sympodial branch with 1st boll [54]. All genotypes under study differed significantly for this trait (Table 4). Hussain et al. (2000) revealed similar results for plant height under heat stress, presenting a familiar correlation among traits that plant height has a positive correlation with the morphological traits under study [55]. Boll development was affected by the high temperature stress as compared with vegetative phase and a similar reduction in boll weight was observed when the temperature fluctuated [56]. Morris (1964) also reported a reduction in cotton boll maturity time at high temperature stress [57]. After screening the genotypes on morphological parameters, one genotypes was selected as tolerant against heat stress and another one was selected as heat susceptible, among others, on the basis of physiological characteristics, i.e., relative water contents, water potential, osmotic potential, cell injury, and proline contents. Highly significant differences were perceived by analysis of variance for all the physiological traits among the genotypes, except photosynthesis rate, which is significant (Table 3). The membrane structure of plant cells was distorted under severe temperature stress, which caused the increased permeability of membrane. As a result, electrolyte leakage increased and eventually led to cell death [58]. Azhar et al. (2009) measured the heat tolerance in term of relative cell injury percentage in cotton and found that thermal stress-tolerant genotypes were more stable

for seed cotton yield and also maintained fiber quality as compared with heat-susceptible genotypes. A significant decrease was observed in leaf relative water content % (RWC) for heat-susceptible genotypes when exposed to heat stress, and similar findings were also obtained by Rahman et al. (2000), Siddique et al. (2000), and Parida et al. (2007) under stress conditions [59–61]. Higher leaf relative water content (RWC) could be a criterion for selection of a parent for hybridization to develop stress-tolerant genotypes [62,63]. On the basis of grand mean attained from normal and heat-stress situations, the protein contents was variable among genotypes and Raison et al. (1982) revealed that for temperature conditions above the optimum, significant reticence of photosynthesis takes place, resulting in substantial reduction in protein formation [64].

Finally, it was observed that high heat tolerance is a multigenic trait and its expression is controlled by many QTLs. Almost all the vegetative and floral characteristics of cotton plants were affected adversely because of this stress. The identification of QTLs activated to combat heat stress allowed the estimation of genetic architecture and improvement of heat-tolerance traits by molecular marker-assisted selection (MAS). A total of 1450 markers were applied, among which 175 SSR markers were observed to be polymorphic and were found to be significant; the observations were also in accordance with some other researchers [65]. In order to dissect the genetic basis of heat tolerance, two upland cotton cultivars (MNH-886 and MNH-884) were selected as parents and an F_2 population was developed. A high LOD (logarithm of odds) value provided strong evidence that the reported QTLs are actually associated with the respective traits. We only reported QTLs whose LOD score values were greater than three and which showed a significant additive or dominance genetic effect. A total of 17 QTLs with different effects on ten morphological and physiological traits such as First sympodial node height (FSH), sympodial node height (SNH), Percent boll set along sympodia on 2nd position (PBS), total no. of sympods (TNS), total no. of nodes (TNN), number of bolls (NOB), total no. of buds (TNB), length of bracts (LOB), length of staminal column (LOS), and length of petal (LOP) were detected in the present study. These QTLs were mapped on chromosome numbers 2, 3, 5, 6, 15, 16, 18, 19, 23, and 26. QTLs for length of petal and length of bracts were located on Chr. 2 while QTLs for total no. of buds and length of staminal column were located on Chr. 18 [66,67]. Likewise, QTLs for Boll no. and Percent boll set along sympodia on 2nd position were located on Chr. 26. Our findings are in accordance with work carried out by [68,69].

5. Conclusions

The purpose of cotton breeding is to boost and stabilize its yield in abiotic and biotic stress environments and to make cultivars with such physiological and architectural characteristics that can tolerate heat stress conditions. A low level of polymorphism is one of the major constraints for plant breeders and geneticists that can be attributed to the different processes like selection and domestication. It resulted in narrowing genetic shuffling in cotton. The use of an enormous number of SSRs can overcome the constraint of low polymorphism. In this study project, more than 1450 SSRs were assessed and the polymorphism rate was 12%, meaning the genetic diversity level was low owing to Intraspecific cross and segregation distortions. In spite of Intraspecific cross, 17 QTLs were detected by evaluating earlier-used and some novel traits. QTL detection can be attributed to a high rate of diversity in both parents. SSR markers were found best to deal with and easiest to assess polymorphism. The main goal of cotton breeding is to help increase and stabilize its productivity in stress environments and to develop cultivars with morphological traits which can withstand heat conditions. Our data suggest that favorable alleles for morphological traits can be combined to improve heat stress tolerance in cotton. Comparisons could be made to evaluate the consistency of QTL detection for the same trait in various backgrounds, which will help to determine the value of targeting these loci for selection in breeding programs.

6. Future Recommendation

Such coverage in the localization of QTLs controlling different quantitative traits suggested a close genotypic correlation among these traits or a pleiotropic effect of a single gene. It remains to be tested whether these common genomic regions have pleiotropic effects or there are clusters of tightly linked genes for some related traits in these regions. A more numerous mapping population and more closely spaced markers in the map are needed to determine whether the QTLs correspond to a gene with pleiotropic effects or to several separate but closely linked genes, each controlling a single character.

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