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Abstract: High temperature stress affects tomato production both in tropical and sub-tropical environments worldwide. To explore genetic variation for heat tolerance in tomato, 329 transcontinental tomato varieties were evaluated at The Ministry of Municipality and Environment (MME) greenhouses near Doha, Qatar. Preliminary phenotypic analysis identified a diverse set of 71 hybrid and pure line tomato varieties for more detailed studies. The selected subset was examined in the greenhouse in a randomized complete block design under heat stress. The materials were phenotyped for fruit size, fruit weight, fruit hardness, fruit locules, fruit set (%), total soluble solids (TSS), and fruit yield. Significant phenotypic differences among genotypes were observed for all the traits assessed. To explore the genetic basis of variation among varieties the subset was genotyped using 104 SNP markers identified in previous heat tolerance genome wide association studies (GWAS). Nineteen QTL associated SNP markers could reliably select heat tolerant varieties in terms of better fruit yield, fruit set, and TSS. These markers are located on Chromosome 1, 5, 6, 8, 9, and 12. Interestingly, two clusters of markers on chromosome 6 were linked to significant effects on yield, fruit set, and TSS under high temperature. Some markers located within these QTL regions were also reported as associated with heat tolerance in other studies. Eighteen out of nineteen SNP markers were mapped within a gene body. Based on the phenotypic and the genotypic analysis, an elite set of five varieties was selected for approval for heat stress environments in Qatar. These findings have significance not only for Qatar, but they are also valuable for wider application at other locations worldwide. The 19 markers presented here could be adopted by breeders for marker-assisted selection (MAS) for heat tolerance.

Keywords: high temperature stress; QTL; SNP marker; genotypic selection; tomato

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1. Introduction

Global warming is increasing [1] and higher temperatures will progressively limit agricultural production, especially in tropical and subtropical regions [2-5]. A 28% reduction in tomato yield under high temperatures was reported in Australia [6]. There is an urgent need to improve adaptive management of crops and the selection of heat tolerant germplasm for current and future production environments [7, 8]. Qatar has a desert climate, and crops can only grow in winter and spring. However, global warming is reducing the duration of both winter and spring with consequences for vegetable production [9]. Heat tolerant crops, therefore, will play an important role in future agriculture in Qatar.

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop worldwide. It can be grown in both subtropical and tropical zones. The optimal daytime temperature for tomato production is 25 °C to 30 °C [10]. If temperature exceeds a critical point, productivity will fall significantly. For example, El Ahmadi and Stevens [11] reported that in several heat-tolerant tomato varieties, the number of flowers, pollen viability, fruit set, and yield were dramatically reduced under 38/27 °C day/night temperatures. Heat stress is defined as temperatures 10–15 °C higher than optimal [2]. High temperature stress can cause negative impact on plant development, including morphology, physiology, biochemistry, and molecular pathways at all vegetative and reproductive stages, which leads to loss of yield. During anthesis, tomato is very sensitive to temperature fluctuations, which impairs anther, pollen, and pistil development, leading to reduced fertilization, lower fruit set, and poorer quality fruit and yield [2, 12-14]. At the physiological level, heat stress impacts photosynthesis, respiration, and membrane plasticity [15-17]. Damage to cell membranes results in electrolyte leakage [18, 19]. Electrolyte leakage is commonly used to assess tolerance and sensitivity to heat stress [6, 20]. Studies of the tomato transcriptome under normal and heat stressed conditions, identified hundreds of genes that changed expression, including heat shock proteins (HSPs) and their related transcription factors (HSFs) [21-24]. High temperature stress also causes biochemical changes, including changes to the levels of sugars, fatty acids, proline, salicylic acid, and abscisic acid. In addition, reactive oxygen species (ROS) accumulate and enzymes in chloroplasts and mitochondria are inactivated [17, 25].

Heat tolerance is controlled by multiple genes which induce physiological and biochemical changes. Several studies have identified quantitative trait loci (QTL) linked to reproductive traits under heat stress using biparental QTL mapping, introgression lines, multiparent advanced generation intercross (MAGIC) populations [26-29] and genome-wide association studies (GWAS) [6]. Candidate genes linked to the heat stress response have also been identified [23, 24, 30]. Recently, genomic selection was applied to tomato [30-32] with some success. Heat tolerant genotypes were successfully predicted with good accuracy for yield (0.729) and total soluble solids (SCC, 0.715) [30]. Whole genome sequencing of a heat tolerant line revealed highly variable chromosome regions (QTL) compared to a reference genome and a high number of candidate genes [24]. While genomic selection and whole-genome sequencing may not be cost effective for a small breeding program, traditional marker-assisted selection (MAS) for key traits remains viable.

In this study, previously identified tomato QTL markers for agronomic traits under heat stress [6, 26] were validated in a totally different set of 71 tomato varieties grown in Qatar. The aim was to identify and validate QTL for genotypic selection for heat tolerance. The identified markers would be useful to breeders for MAS.

2. Materials and Methods

2.1. Plant Materials and Growth Condition

Transcontinental tomato varieties were evaluated at the Ministry of Municipality and Environment (MME) net greenhouse (25.518031°N, 51.208001°E) at the Al-Utouriya research station, Doha, Qatar. In the first season 2019 – 2020, 329 varieties were grown in the greenhouse. Ten plants per variety were sown in October and seedlings were transplanted at the end of October following a completely randomized design in three replicates. Fruits were harvested from February to May. Plants were spaced 0.5 m between rows and 0.3 m within rows and watered via a fully automated drip irrigation system. In the second season 2020 - 2021, 71 transcontinental tomato varieties (Table 1) were grown in the same conditions in a completely randomized block design with three replicates. These varieties included different tomato types: globe, plum, cherry, oxheart, and beefsteak. Bumble bees were used in all seasons and greenhouses to help pollinate flowers. Mid-day temperature (11:00am) was recorded from December to May 2000 - 2021 (Figure 1). In the third season 2021 – 2022, 10 selected varieties were evaluated in a randomized complete block designs of three replicates.

Table 1. Tomato varieties selected from year 1 testing for evaluation in years 2 and 3.

ID	Company code	Variety name	ID	Company code	Variety name
1	TF-0014	Amish Gold	36	TF-0141	Ding Wall Scotty
2	TF-0017S	Amy's Apricot	37	TF-0147	Double Rich
3	TF-0018	Amy's Sugar Gem	38	TF-0176	Florida Pink
4	TF-0027	Arkansas Marvel	39	Pakistan	Salar F1
5	TF-0032	Aunt Lucy's Italian Paste	40	Pakistan	Surkhail F1
6	TF-0268	Japanese Oxheart	41	Pakistan	Sundar F1
7	TF-0367	Ozark Pink	42	Pakistan	Saandal F1
8	TF-0449	Shenghaung Cherry	43	Pakistan	Tomato seed -2198
9	TF-0450	Shirley Amish Red	44	Pakistan	Tomato seed -2199
10	TF-0173	Fence Row Cherry	45	Pakistan	Tomato seed -2217
11	TF-0187	German Johnson	46	Pakistan	Tomato seed -2218
12	TF-0197	Giant Syrian	47	Pakistan	Tomato seed -2230
13	TF-0213F	Goose Creek	48	AVRDC	AVTO90304
14	TF-0227	Gregori's Altai	49	AVRDC	AVTO9801
15	TF-0227G	Grightmire's Pride	50	AVRDC	AVTO1007
16	TF-0235	Hazel Mae	51	AVRDC	AVTO1010
17	TF-0561	Homer Fike's Yellow Oxheart	52	AVRDC	AVTO9001
18	TF-0035A	Austin's Red Pear	53	DRW7806	hybrid tomato
19	TF-0036	Australia	54	Bright Star F1	Bright Star F1
20	TF-0070A	Bloody Butcher	55	Roenza	Roenza
21	TF-0077M	Brandy Sweet Plum	56	619	619
22	TF-0078	Brandywine	57	SV7846TH	SV7846TH
23	TF-0106	Chadwick Cherry	58	syngenta	Tomato: 413485
24	TF-0109	Chapman	59	syngenta	Jarawa Ind tomato
25	TF-0129	Creole	60	syngenta	Tomato -Beef Vikllio
26	TF-0135	Dad's Sunset	61	syngenta	Pilavy Ind tomato
27	TF-0137	Debarao	62	syngenta	T415271 Ind tomato
28	TF-0142	Dinner Plate	63	syngenta	Tomato Dafnis
29	TF-0146	Dona	64	syngenta	Tomato Commondo
30	TF-0149	Dr. Lyle	65	syngenta	Tomato Izmono
31	TF-0150	Dr. Neal	66	TF-0004	Ace 55
32	TF-0167	Ethiopia Roi Humbert	67	TF-0021	Anahu
33	TF-0024	Angora Super Sweet	68	TF-0330	Mrs. Houseworth
34	TF-0093	Bulgarian Triumph	69	TF-0404J	Punta Banda
35	TF-0121	Clint Eastwood's Rowdy Red	70	TF-0486	Sweet Organic tomato
			71	Banana Legs	Banana Legs

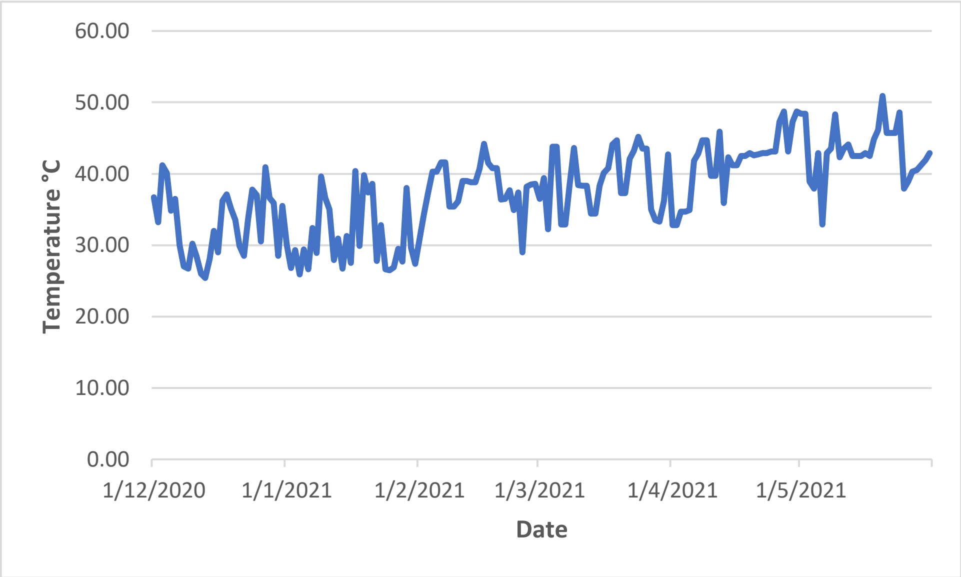

Figure 1. Temperature at mid-day during the growth period of year 2.

2.2. Phenotyping and Statistical Analysis

Harvesting was conducted every 10 days in the first month, and twice per week afterwards. Yield was calculated as the weight of all harvested fruits per plant. Final yield was expressed as yield/3 plants (kg). Five fruits from each plot were taken at ripeness from the fourth harvest to determine fruit characteristics. Fruit length and diameter was determined using a digital caliper (cm). Fruit firmness (hardness) was measured on the two opposite sides of the fruit using a pressure test (lb/cm^2). Total soluble solids Brix (TSS) in the fruit juice was determined using a handheld refractometer. Fruits were cut crosswise to determine the number of locules. Flowers/inflorescence were counted every 2 – 4 days. Fruits/inflorescence were also recorded. Fruit set (%) was then calculated as fruits/inflorescence divided by flowers/inflorescence.

Data were analysed for each year independently using a linear mixed model and the REML function of the statistical software package Genstat (64-bit release 22.1, VSN International Ltd). Genotype was considered a fixed effect and plot position within each replicate a random effect. The 10 selected genotypes were analysed across years. In this instance, year and genotype were considered fixed effects and plot position within replicates within years as random effects.

2.3. QTL Marker Selection and Genotyping

Previous GWAS of tomato identified more than 100 SNP markers [6] associated with various traits under heat stress. To validate these markers in a different set of tomato varieties, 96 markers were selected by filtering out those with low PIC, high percentage missing data and/or sequence uncertainty. Eight SNP markers from Xu et al. [26] were also included. All markers were projected on to the tomato genome SL4.0 (https://solgenomics.net/organism/Solanum_lycopersicum/genome). The names of 104 markers were designated as genome version (SL4-0) + chromosome number (ch01) + position, e.g., SL4-0ch01_1477682, to assist identification. All the marker sequences are listed in the supplementary document (Table S1). DNA extraction and genotyping (SeqSNP) of 71 tomato varieties was conducted by LGC Biosearch Technologies. The genotype and phenotype (fruit_set, yield and TSS) association study was done using Box plotting. A Student T-test was used to determine significance.

2.3. Comparison among Published QTL and QTL Identified in this Study

Published QTL loci from Gonzalo et al. 2020 [27] and Bineau et al. 2021[28] were retrieved and projected onto genome SL4.0, so that all QTL from different studies can be compared (Table S2).

3. Results

3.1. Phenotypic Data Analysis

Phenotypic traits varied significantly among the 71 selected varieties. However, many of these varieties included different types and fruit shapes including globe, plum, cherry, oxheart, and beefsteak. This variation must be accounted for if the set was to be used in an association for heat tolerance. Therefore the differences among fruit types was assessed (Table 2). Results showed that TSS, fruit_set and yield (without beefsteak) were not significant among types. These three traits were subsequently used in the association study.

Table 2. The probability values of Wald statistics for different tomato types for various traits assessed on 71 tomato varieties.

	Frt_Width (cm)	Frt_Length (cm)	TSS (%)	Flowers /inflo	Fruit_set (%)	yield (kg)	yield* (kg)
Tomato_type	<0.001	<0.001	0.058	<0.001	0.237	0.002	0.056

*yield excluding Beefsteak.

The 10 selected varieties were analysed across years to assess main effects and genotype x environment interaction (Table 3). Year and genotype effects were significant at P<0.05 for all traits. The genotype x environment interaction was also significan for all traits except locule number and fruit_set. When tomato types Globe and Plum were separated and analysed, difference in main and interaction effects were observed (Table 4). Globe genotype effects were all significant, as was the genotype x environment effect for yield and Frt_Width. However, the plum type genotype effects were only significant for Frt_Width, although a genotype x environment effect was observed for yield. The envionment main affects were all significant across types except for globle Locule_number and yield.

Table 3. The probability values of Wald statistics from the 2 year analysis of 10 varieties.

	Frt_Width (cm)	Frt_Length (cm)	Hardness (lb/cm^{2})	TSS (%)	Locule_ number	Flowrs /inflo	Fruits /inflo	Fruit_set (%)	yield (kg)
Year	0.005	0.043	<0.001	<0.001	0.01	0.466	<0.001	<0.001	0.005
Genotype	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Year x Genotype	0.029	<0.001	<0.001	<0.001	0.354	0.016	0.013	0.132	<0.001

Table 4. The probability values of Wald statistics from the 2 year analysis of Globe only and Plum only.

	Globe				Plum			
	Frt_Width (cm)	Frt_Length (cm)	Locule_ number	yield (kg)	Frt_Width (cm)	Frt_Length (cm)	Locule_ number	yield (kg)
Year	0.066	0.008	0.154	0.467	0.027	0.026	0.015	0.001
Genotype	<0.001	<0.001	<0.001	<0.001	<0.001	0.369	0.215	0.316
Year x Genotype	0.031	0.433	0.49	<0.001	0.116	0.252	0.32	0.015

3.2. QTL Marker Genotyping and Association Study

A total of 104 QTL SNP markers selected from previous GWAS studies [6, 26] were used to genotype the 71 varieties selected for evaluation in the second season. The marker profiles of the 71 varieties were listed in Table S3. Twenty-one markers had a minor allele frequency larger than 9% and were selected for genotypic and phenotypic association analysis. Among these 21 markers, 19 had a significant effect on tomato fruit_set, yield, and TSS under heat stress in Qatar. These markers are located on chromosomes 1, 5, 6, 8, 9, and 12 (Figures 2 and 3). Two markers were located on each of chromosomes 1, 5, 9, and 12, one marker on chromosome 8, and 10 markers on chromosome 6. One marker located on chromosome 3 and another on chromosome 4 had no significant effect on the traits analysed.

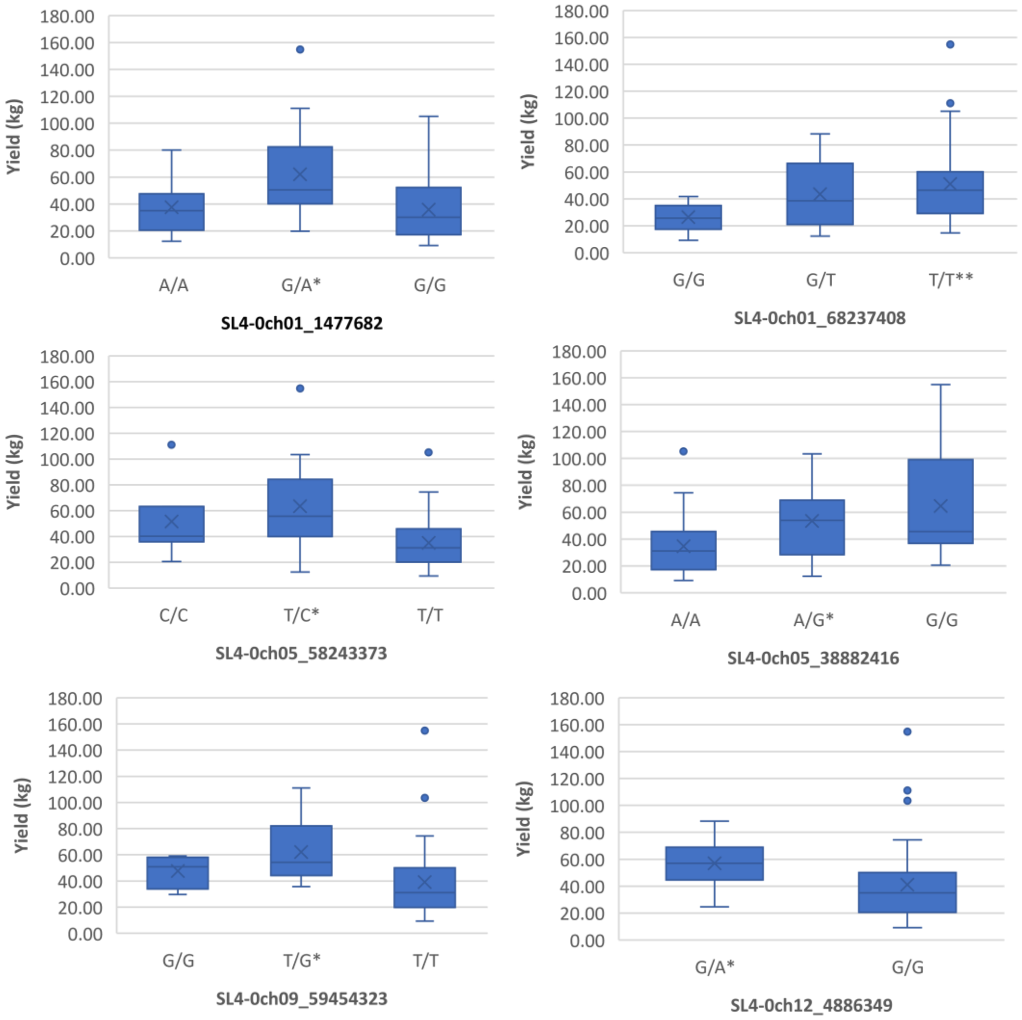

Figure 2. Boxplots showing markers on chromosome 1, 5, 9 and 12 associated with yield under heat stress. The mean is represented by the x sign, while the median is represented by the horizontal line that divide the box. The lower and upper box boundaries represent 25th percentile and 75th percentile, respectively. ** and * indicate statistical significance $p<0.01$ and $p<0.05$, respectively.

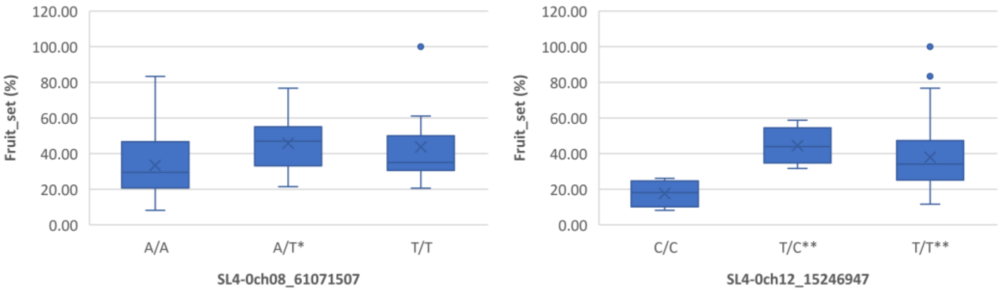

Figure 3. Boxplots showing markers on chromosome 8 and 12 associated with fruit_set under heat stress. The mean is represented by the x sign, while the median is represented by the horizontal line that divide the box. The lower and upper box boundaries represent 25th percentile and 75th percentile, respectively. ** and * indicate statistical significance $p<0.01$ and $p<0.05$, respectively.

Interestingly, clusters of markers were identified on chromosome 6, most of them on the short arm. Figure 4 shows the markers on chromosome 6 that associated with both fruit_set and yield, while those associated with yield only are shown in Figure 5.

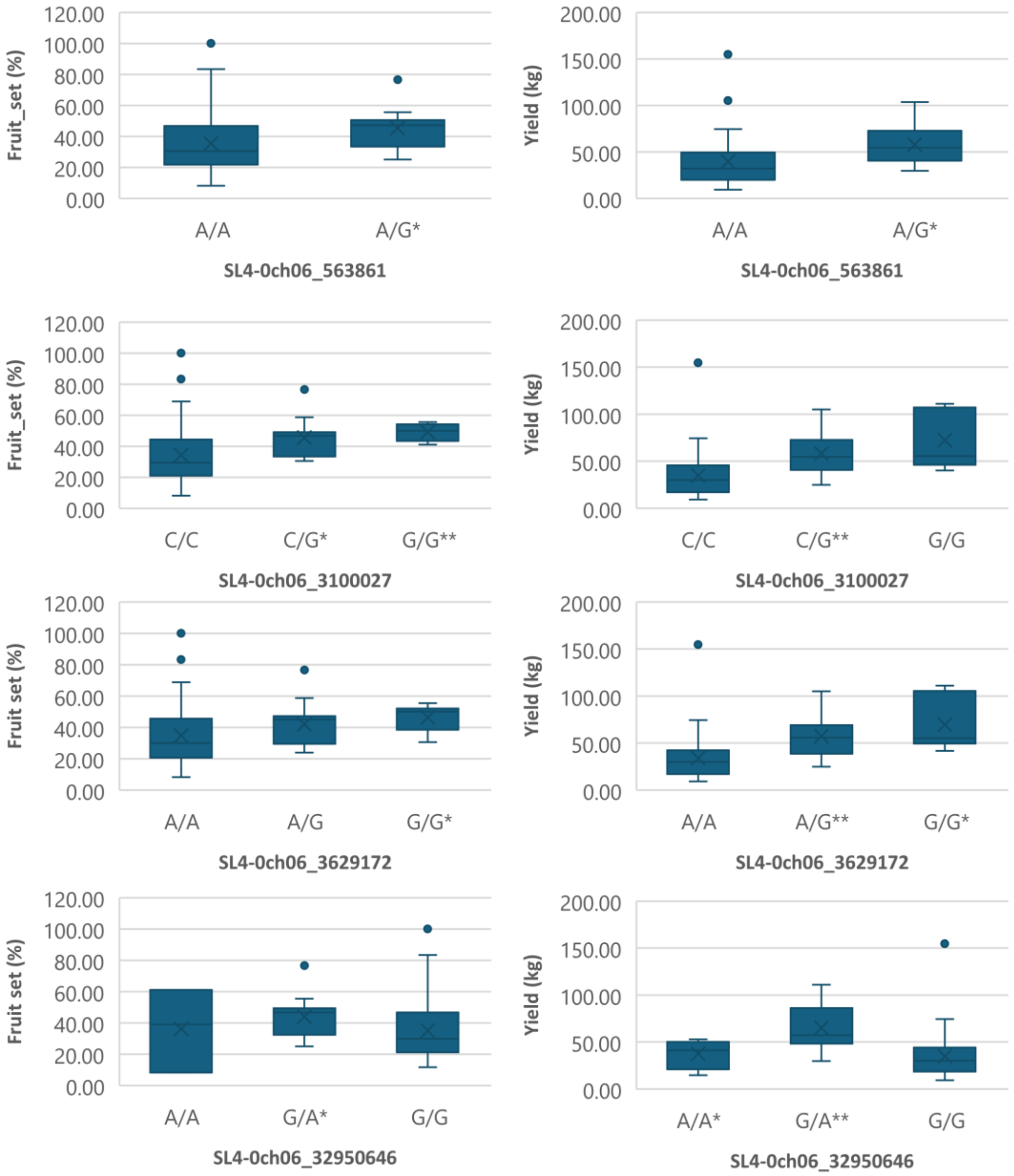

Figure 4. Boxplots showing markers on chromosome 6 associated with both fruit_set and yield under heat stress. The mean is represented by the x sign, while the median is represented by the horizontal line that divide the box. The lower and upper box boundaries represent 25th percentile and 75th percentile, respectively. ** and * indicate statistical significance $p<0.01$ and $p<0.05$, respectively.

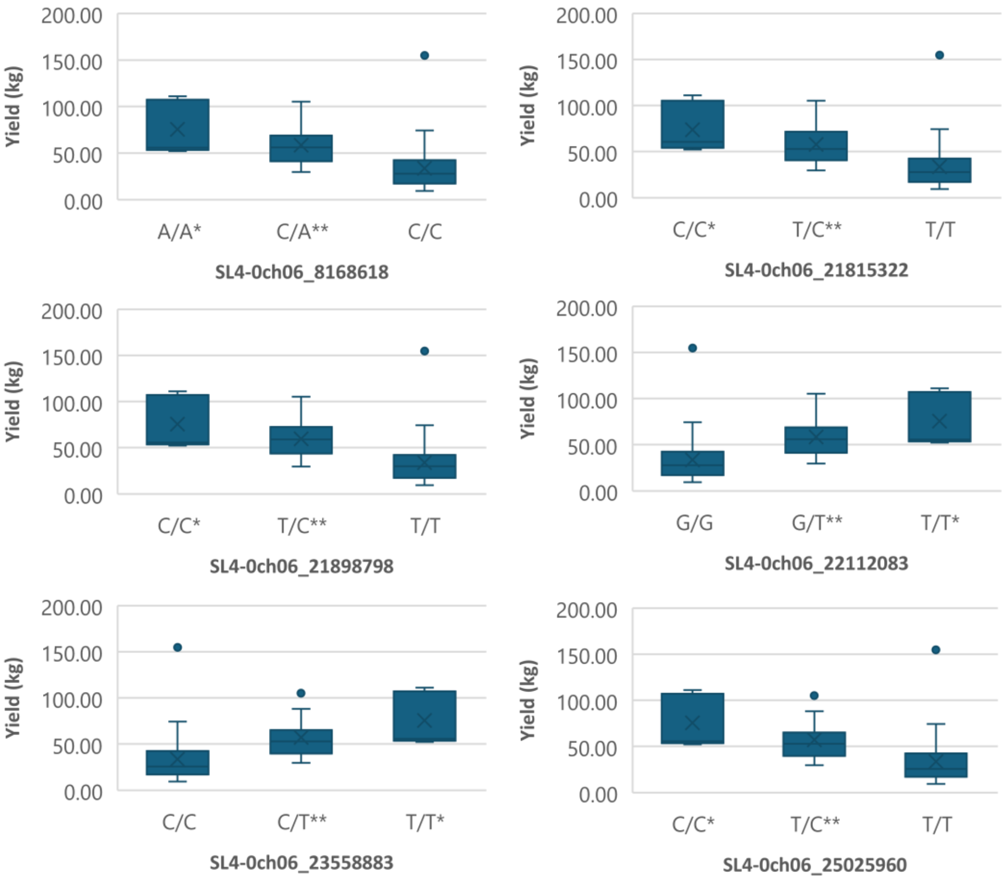

Figure 5. Boxplots showing markers on chromosome 6 associated with yield under heat stress. The mean is represented by the x sign, while the median is represented by the horizontal line that divide the box. The lower and upper box boundaries represent 25th percentile and 75th percentile, respectively. ** and * indicate statistical significance $p<0.01$ and $p<0.05$, respectively.

The markers on chromosome 6 that associated with yield, fruit_set and TSS are shown in Figure 6. Another marker on the short arm of chromosome 9 was very significantly associated with TSS (Figure 7). Alleles that are favorable for yield and fruit_set often had the opposite effect on TSS. The 19 markers associated with traits and their favorable alleles are summarized in Table 5.

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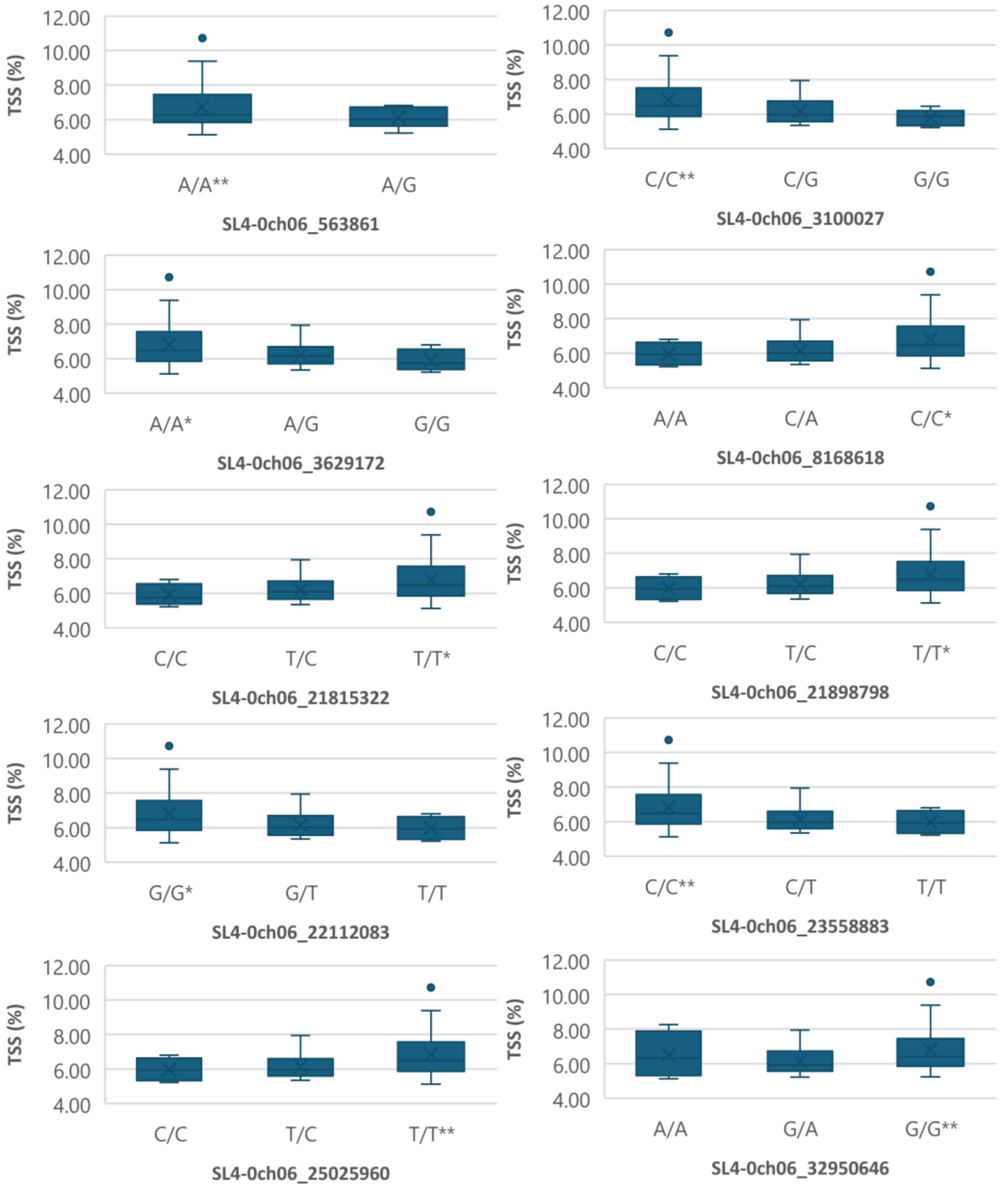

Figure 6. Boxplots showing markers on chromosome 6 associated with TSS. The mean is represented by the x sign, while the median is represented by the horizontal line that divide the box. The lower and upper box boundaries represent 25th percentile and 75th percentile, respectively. ** and * indicate statistical significance p<0.01 and p<0.05, respectively.

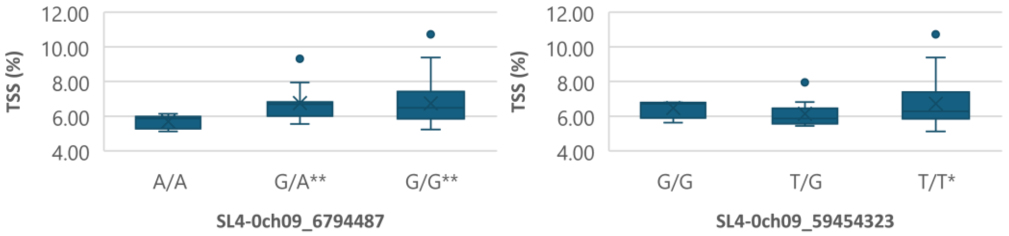

Figure 7. Boxplots showing markers on chromosome 9 associated with TSS. The mean is represented by the x sign, while the median is represented by the horizontal line that divide the box. The lower and upper box boundaries represent 25th percentile and 75th percentile, respectively. ** and * indicate statistical significance p<0.01 and p<0.05, respectively.

Table 5. List of favorable alleles of SNP markers for yield, fruit_set, and TSS.

Marker	Traits	Favorable alleles
SL4-0ch01_1477682	Yield	GA* > GG, AA
SL4-0ch01_68237408	Yield	TT**, GT > GG
SL4-0ch05_38882416	Yield	GA*, GG > AA
SL4-0ch05_58243373	Yield	CT*, CC > TT
SL4-0ch06_563861	Fruit set	GA* > AA
SL4-0ch06_563861	Yield	GA* > AA
SL4-0ch06_563861	TSS	AA** > GA
SL4-0ch06_3100027	Fruit set	GG**, GC* > CC
SL4-0ch06_3100027	Yield	GC**, GG > CC
SL4-0ch06_3100027	TSS	CC** > GC, GG
SL4-0ch06_3629172	Fruit set	GG*, GA > AA
SL4-0ch06_3629172	Yield	GA**, GG* > AA
SL4-0ch06_3629172	TSS	AA* > GA, GG
SL4-0ch06_8168618	Yield	CA**, AA* > CC
SL4-0ch06_8168618	TSS	CC* > CA, AA
SL4-0ch06_21815322	Yield	CC**, CT* > TT
SL4-0ch06_21815322	TSS	TT* > CT, CC
SL4-0ch06_21898798	Yield	CT**, CC* > TT
SL4-0ch06_21898798	TSS	TT* > CT, CC
SL4-0ch06_22112083	Yield	GT**, TT* > GG
SL4-0ch06_22112083	TSS	GG* > GT, TT
SL4-0ch06_23558883	Yield	CT**, TT* > CC
SL4-0ch06_23558883	TSS	CC** > CT, TT
SL4-0ch06_25025960	Yield	CT**, CC* > TT
SL4-0ch06_25025960	TSS	TT** > CT, CC
SL4-0ch06_32950646	Fruit set	GA* > GG, (AA)
SL4-0ch06_32950646	Yield	GA** > AA* > GG
SL4-0ch06_32950646	TSS	GG** > GA, (AA)
SL4-0ch08_61071507	Fruit set	AT*, TT > AA
SL4-0ch09_6794487	TSS	GG**, GA* > AA
SL4-0ch09_59454323	Yield	GT*, (GG) > TT
SL4-0ch09_59454323	TSS	TT*, (GG) > GT
SL4-0ch12_4886349	Yield	GA* > GG
SL4-0ch12_15246947	Fruit set	CT**, TT** > CC

**p<0.01, *p<0.05, () indicates the number of the individuals in the population is too small. TSS is total soluble solids.

3.3. Genotypic and Phenotypic Selection

Favourable alleles for each marker were identified based on association with desirable phenotype. According to the genotype of each variety, favourable varieties for high fruit_set and yield were selected (Table 6). Based on phenotypic data, five varieties were selected for heat stress environments in Qatar (Table 7).

Table 6. The fifteen varieties selected for high yield based on genotype (favourable alleles > 15 out of total 19 markers).

ID	Company code	Variety name	Favorable allele %
43	Pakistan	Tomato seed -2198	89.5
44	Pakistan	Tomato seed -2199	100
45	Pakistan	Tomato seed -2217	78.9

46	Pakistan	Tomato seed -2218	89.5
53	DRW7806	hybrid tomato	100
55	Roenza	Roenza	89.5
56	619	619	84.2
57	SV7846TH	SV7846TH	89.5
59	syngenta	Jarawa Ind tomato	94.7
60	syngenta	Tomato -Beef Vikllio	84.2
61	syngenta	Pilavy Ind tomato	100
62	syngenta	T415271 Ind tomato	84.2
63	syngenta	Tomato Dafnis	94.7
64	syngenta	Tomato Commondo	100
65	syngenta	Tomato Izmono	89.5

Table 7. Genotypes of heat tolerant lines selected by phenotyping based on yield and quality.

	#43	#44	#45	#47	#58
SL4-0ch01_1477682	GA	GA	GA	AA	GG
SL4-0ch01_68237408	TT	TT	TT	TT	TT
SL4-0ch05_38882416	GG	GG	GG	GA	GA
SL4-0ch05_58243373	CC	C/C	C/C	T/T	CT
SL4-0ch06_563861	GA	GA	AA	AA	AA
SL4-0ch06_3100027	GC	GC	GC	GC	GG
SL4-0ch06_3629172	GA	GA	GA	GA	GG
SL4-0ch06_8168618	CA	CA	CA	CC	AA
SL4-0ch06_21815322	CT	CT	CT	TT	CC
SL4-0ch06_21898798	CT	CT	CT	TT	CC
SL4-0ch06_22112083	GT	GT	GT	GG	TT
SL4-0ch06_23558883	CT	CT	CT	CC	TT
SL4-0ch06_25025960	CT	CT	CT	T/T	CC
SL4-0ch06_32950646	GA	GA	GA	GG	GA
SL4-0ch08_61071507	AA	AT	AA	AT	AT
SL4-0ch09_6794487	GA	GG	AA	GA	AA
SL4-0ch09_59454323	TT	GT	TT	TT	TT
SL4-0ch12_4886349	GA	GA	GA	GA	GG
SL4-0ch12_15246947	TT	TT	TT	CT	TT
Allele for yield/total markers	89.5%	100%	78.9%	42.1%	73.7%

Yellow highlighted is the less favorable allele for yield; green highlight is favorable for TSS.

Five tomato cultivars selected phenotypically for growing under Qatari conditions had genotypes favorable for yield (#43, #44, #45, #58) and yield and quality (TSS, #47).

3.4. QTL Markers Comparsion

Tomato QTL linked to reproductive traits affected by high temperature, such as flower number (FLN) and fruit number (FRN) per truss and percentage of fruit set (FRS), pollen viability (PV), and yield, have been recently investigated [26-28]. The chromosome locations of the reported QTL were in the genome sequence of SL2.5. These reported QTL positions were projected into genome SL4.0, so that the QTL identified in this study can be compared with those reported. Some of the QTL identified in this study are in the same regions of reported QTL, while others are new (Figure 8).

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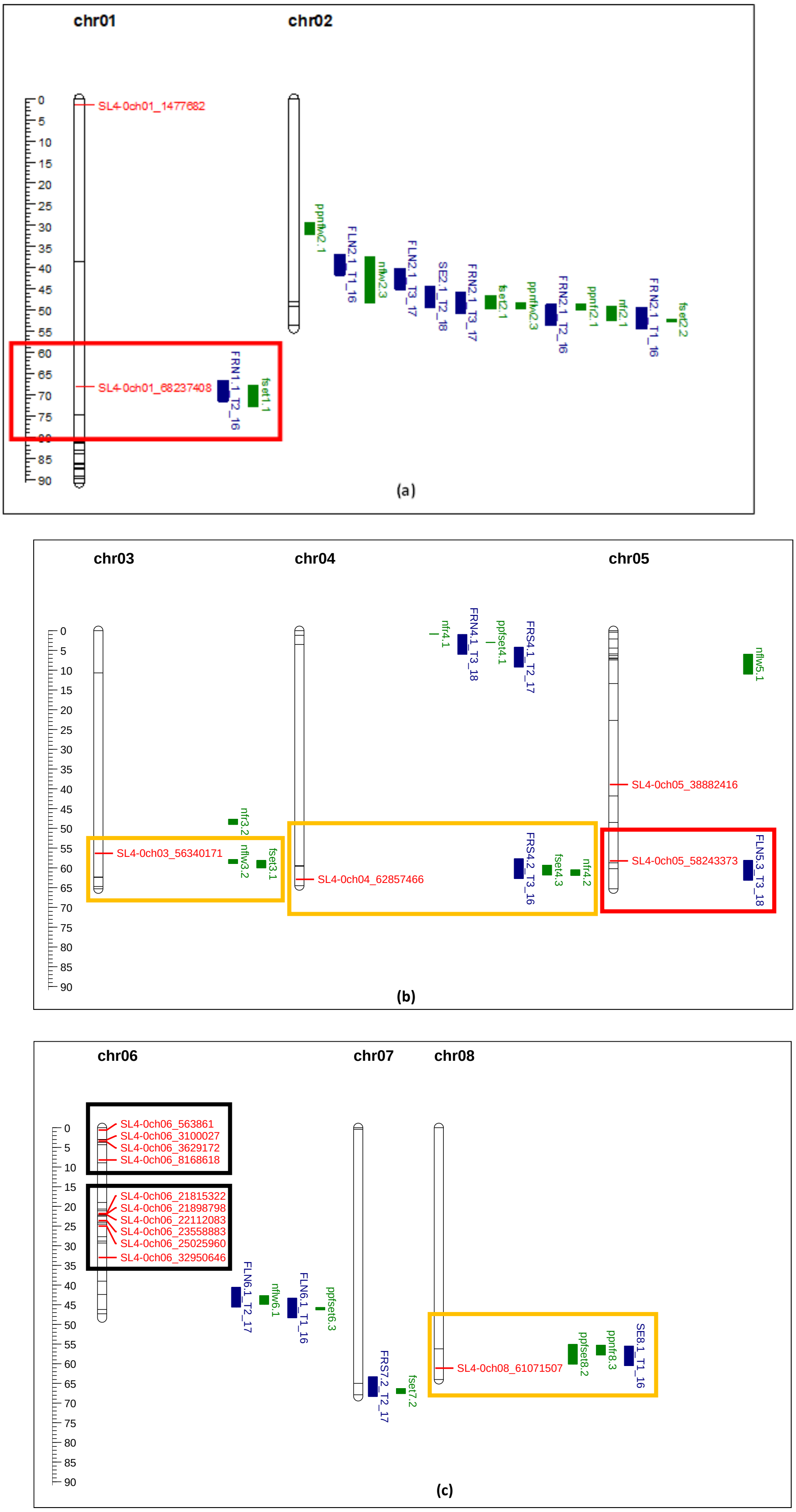

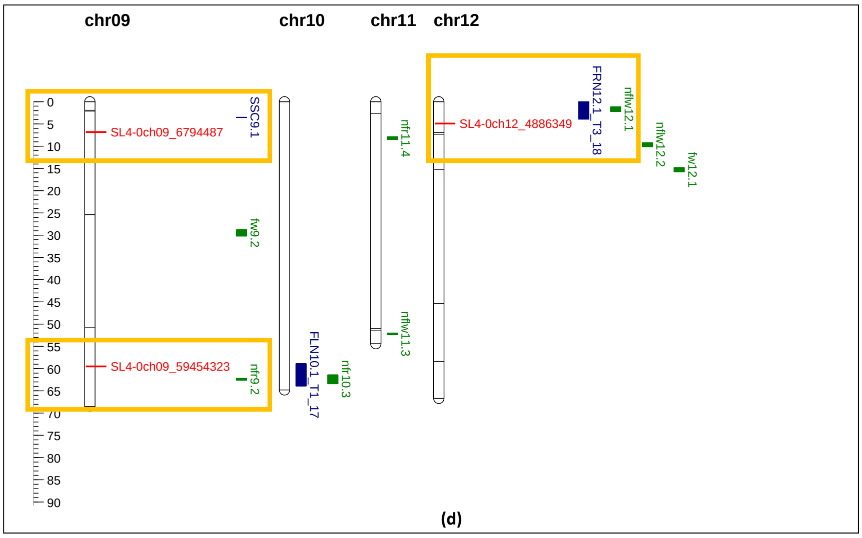

Figure 8. comparison of QTL markers identified in this study (red) with QTL identified in Gonzalo et al. (2020, blue) and Bineau et al. (2021, green). Red rectangle shows the makers of interest are aligned in the same chromosome position of other QTL. Yellow rectangle shows the markers of interest are located within 4 Mbp of other QTL. Black rectangle shows clusters of markers identified in this study significantly associated with fruit_set, yield, and TSS under heat stress. (**a**) Chromosome 1 and 2; (**b**) Chromosome 3, 4, and 5; (**c**) Chromosome 6, 7, and 8; (**d**) Chromosome 9, 10, 11, and 12.

Mapping the position of markers in the genome (SL4.0) showed that the majority of markers (18 out of 19 markers) were locate within a gene body (Table 8). SNPs locate either in the coding region or in the intron, 5′ untranslated region (UTR), 3′ UTR, and downstream (Table 8). Variation in the coding region could result in dysfunctional protein if it is a synonymous or missense mutation. Whereas sequence changes in the intron, 5′ UTR, 3′ UTR, and up- or downstream of a gene could cause transcriptional or translational change.

Table 8. Locations of markers in gene body.

Marker	Genes SNP located	SNP position	gene function
SL4-0ch01_1477682	Solyc01g006890	coding	EEIG1/EHBP1 N-terminal domain (C2 domain superfamily)
SL4-0ch01_68237408	Solyc01g067100	coding	Cullin
SL4-0ch05_38882416	Solyc05g025955	intron	ABC transporter B family member 11
SL4-0ch05_58243373	Solyc05g047450	intron	Methyl-CpG-binding domain-containing protein 2
SL4-0ch06_563861	Solyc06g005520	coding	Protein kinase superfamily
SL4-0ch06_3100027	Solyc06g009160	3′ UTR	Glyoxysomal fatty acid β-oxidation multifunctional protein MFP-a
SL4-0ch06_3629172	Solyc06g009680	coding	BRCT domain-containing protein
SL4-0ch06_8168618	Solyc06g011570	coding	Haloacid dehalogenase-like hydrolase family protein
SL4-0ch06_21815322	Solyc06g034330	3′ UTR	unknown
SL4-0ch06_21898798	intergenic region		
SL4-0ch06_22112083	Solyc06g035450	5′ UTR	DEAD-box ATP-dependent RNA helicase
SL4-0ch06_23558883	Solyc06g036240	3′ UTR	Pleiotropic drug resistance protein
SL4-0ch06_25025960	Solyc06g036690	intron	unknown
SL4-0ch06_32950646	Solyc06g051630	intron	Phosphoinositide phospholipase C
SL4-0ch08_61071507	Solyc08g079440	coding	UDP-glucuronate 4-epimerase 4
SL4-0ch09_6794487	Solyc09g014720	coding	Protein kinase domain
SL4-0ch09_59454323	Solyc09g065300	coding	spindle pole body-associated protein
SL4-0ch12_4886349	Solyc12g014010	coding	Glycosyltransferase
SL4-0ch12_15246947	Solyc12g021280	downstream	Serine/threonine-protein kinase STN7, chloroplastic

4. Discussion

Qatar has a desert climate. It has very low annual rainfall and a hot and long summer. The experiment was carried out in a net greenhouse to assess tomato production under high temperature.

The temperature inside the net greenhouse was higher than the ambient temperature. Figure 1 demonstrated that day temperature during the tomato growth period was fluctuated severely. During the vegetative growth (December), the plants experienced a few days of over 40 °C. During the reproductive period (January and February), more days exceeded 40 °C. The effects of heat stress on vegetative development were evident at high temperature (i.e., 40 °C) [22], whereas reproductive traits are often affected by long-term mild heat stress (i.e., 31 °C) [26], or short periods of high heat stress (over 40 °C) [2]. Plant response to heat stress is complex and controlled by multiple genes. Phenotypic traits, such as flower number, fruit number, percentage of fruit set, stigma exsertion, pollen viability, electrolyte leakage, soluble solid content, were used for QTL analysis by others [6, 26-28]. It was shown that fruit set is an important trait that directly affects yield. In this report, fruit set and yield were the focus of the genetic analysis. As TSS was an important trait for the fruit quality it was also included in the analysis.

Previous GWAS [6] used 144 tomato accessions and DArTseq (Diversity Arrays Technology by sequencing) for association study and identified 142 QTL markers (SNP) that had high log scores associated with heat tolerance. In the previous report, the arbitrary number from DArTseq were used as the marker name, which was not meaningful. In this study, 96 markers were selected, and the SNP position was converted from tomato genome SL2.4 to SL4.0. The name of the markers was converted to show genome sequence version, chromosome number, and position. This will allow the research community and breeders to use these markers easily. The QTL markers/positions identified in other studies [26-28] were also converted according to SL4.0. Thus, these QTL were comparable (Figure 8).

Comparison of markers identified in this study with the QTL reported by other researchers showed general agreement for several QTL. Markers SL4-0ch01_68237408 and SL4-0ch05_58243373 perfectly aligned with QTL associated with fruit number, flower number, and fruit set [27, 28]. Other markers, SL4-0ch8_ 61071507, SL4-0ch09_59454323, SL4-0ch12_4886349, located within 4 Mbp of other QTL related to flower and fruit traits [27, 28]. Marker SL4-0ch09_6794487 showed strong association with TSS, which is also located within 4 Mbp of a SSC QTL [27]. Three markers from Xu et al. (2017) showed correlation with yield and fruit set in this study. SL4-0ch01_1477682 (solcap_snp_sl_8704) was associated with style length [26]. SL4.0ch01_68237408 (solcap_snp_sl_13762) was associated with flower number per inflorescence [26]. These two traits were related to productivity and final yield. Another marker, SL4.0ch08_61071507 (solcap_snp_sl_15446), was associated with inflorescence number [26], which in this study was related to fruit set. Although 104 markers were used to genotype the 71 tomato varieties/accessions, only 21 markers showed enough polymorphism (minor allele frequency larger than 9%) for association analysis, and 19 markers showed significant association with yield, fruit set, and TSS. Two markers, SL4-0ch03_56340171 and SL4-0ch04_62857466, which mapped within the QTLs related to flower number, fruit number, and fruit set (Figure 8), were not significant for fruit set and yield in this study. This was probably due to the small population size and reduced power of the association study.

Interestingly 10 markers on chromosome 6 had a significant impact on yield, fruit set and TSS. Alsamir et al. (2017) [6] identified markers on chromosomes 1 and 6 that significantly impacted electrolyte leakage (EL). The EL trait was indicative of heat stress impact which was reflected in yield in this study. Cappetta et al. (2021) [30] found a high density of SNPs on chromosome 6 linked to heat tolerance. A major QTL was found on chromosome 6 (in a similar region to that reported here) which explained 86% of the phenotypic variance related to yield [30]. This QTL region contains Solyc06g006057, Solyc06g007310, Solyc06g007530, Solyc06g008720, Solyc06g009920, Solyc06g036260, Solyc06g036485, Solyc06g051190 variant genes, coding for Leucine-rich receptor-like protein kinase family protein, Deoxyribonuclease tatD, B3 domain-containing protein (Os05g0481400), Zinc ion binding protein, ATPase E1-E2 type family protein, Beta-carotene hydroxylase 1, Kinase family protein, and RNA-dependent RNA polymerase family protein. Using differential gene expression analysis of tolerant and sensitive accessions under high temperature, Gonzalo et al. (2021) [23] identified genes on chromosome 6 that upregulated during heat stress in tolerant accessions, including heat shock proteins, gibberellin-3-β-dioxygenase 1, and indeterminate-domain 16-like protein, which is plant specific transcription factor regulating in sugar homeostasis, leaf and root architecture, inflorescence and seed development.

The most interesting finding was that the markers identified in this study are all located in a gene body except one. Table 8 lists the SNP markers and their associated genes and gene functions. These genes may be important for conveying heat tolerance in tomato. For example, ABC transporter (SL4-0ch05_38882416) is a transmembrane protein; its function is to import essential nutrients to the cell and to export toxic molecules out. The role of ABC transporter in the defence of multiple plant pathogens has been demonstrated [33-35]. The role of ABC transporters in abiotic stress response, such as heat stress, could also be important, but have yet to be studied. Another protein, Cullin (SL4-0ch01_68237408) and its protein family is involved in protein degradation. Involvement of Cullin in the heat stress response is also possible. Multiple markers on chromosome 6 are located in different genes, including protein kinase (same finding as in [30]), Glyoxysomal fatty acid β-oxidation multifunctional protein (lipid metabolism), hydrolase, pleiotropic drug resistance protein, and phosphoinositide phospholipase C (signal transduction). Another chloroplastic Serine/threonine-protein kinase (SL4-0ch12_15246947) may play roles in photosynthesis during heat stress. Indeed, SNP location is important for genetic selection. Cappetta et al. (2021) [30] used a subset of 2,278 SNPs mapped in gene body regions to perform genomic selection (GS). They obtained similar accuracy to the full dataset of 10,648 SNPs. Overall, the markers/genes identified in this study are of importance for selection of heat tolerant tomato varieties/accessions.

5. Conclusions

Validation of previously identified QTL makers was successfully achieved by using different genetic resources. Nineteen SNP markers had significant effects on fruit set, yield, and total soluble solids of tomato under heat stress and are recommended for MAS in breeding programs. Mapping of SNPs identified eighteen candidate genes for heat tolerance. They are valuable for further study to explore the molecular mechanism of plant response to high temperature. Fundings in this study are of significance for the tomato industry and research community.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: The list of marker sequences; Table S2: Projection of published QTLs to genome SL4.0; Table S3: Marker profiles of 71 varieties used in this study.

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