Mapping QTLs for physiological and biochemical traits related to grain yield under control and terminal heat stress conditions in bread wheat (*Triticum aestivum* L.)

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Abstract In order to detect genomic regions with different effects for some of the physiological and biochemical traits of wheat, four experiments were conducted at Research Farm of Agricultural and Natural Resources Research Center of Zabol in 2015–2016 and 2016–2017 growing seasons. The experiments were carried out using four alpha lattice designs with two replications under non-stress and terminal heat stress conditions. Plant materials used in this study included 167 recombinant inbred lines and their parents ('SeriM82' and 'Babax'). Six traits including grain yield (GY), proline content (PRO), water soluble carbohydrates (WSC), maximum efficiency of photosystem II (Fv/Fm), cytoplasmic membrane stability (CMS) and chlorophyll content (CHL) were evaluated. Genetic linkage map consisted of 211 AFLP marker, 120 SSR marker and 144 DArT markers with 1864 cm length and 4.4 cm mean distance. QTL analysis was carried out using a mixed-model-based composite interval mapping (MCIM) method. By the combined analysis of normal phenotypic values, 27 additive QTLs and five pairs of epistatic effects were identified for studied traits, among which two additive and one epistatic QTL showed significant QTL × environment interactions. By the combined analysis of stress phenotypic

values, a total of 26 QTLs with additive effects and 5 epistatic QTLs were detected, among which one additive and one epistatic QTL showed QTL × environment interactions. Six QTLs with major effects (*QGY-2B*, *QGY-2D*, *QPro-5B*, *QWSC-4A*, *QFv/Fm-6A* and *QCMS-4B*), which were common between two conditions could be useful for marker-assisted selection (MAS) in order to develop heat tolerant and high-performance wheat varieties.

Keywords Epistatic QTL · Heat stress · Recombinant inbred line · Grain yield

Introduction

One of the major factors limiting wheat production in the world is the heat stress that occurs during anthesis and the grain-filling period (Hays et al. 2007). Terminal heat stress can be mentioned as the most important abiotic stresses that reduce wheat yield in southern areas of Iran (Jalal Kamali and Duveiller 2008). In Iran, southern and south-eastern wheat growing regions experience high temperatures exceeding 30 °C during the grain development period due to delayed sowing. In these areas, 15 November to 15 December is the period when the wheat is usually planted and the crop faces a low rainfall and needs irrigation throughout the crop season. According to Mason et al. (2010), if the wheat grain growth period coincides with high temperatures, it can reduce grain yield by up to 28.3%. Therefore, one of the wheat breeding objectives in the world is modifying this crop to tolerate heat. Tolerance to heat is genetically and physiologically a complex trait, and significantly affected by environmental factors (Blum 1988). In previous studies, proline content (Mumtaz et al.

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1995), water soluble carbohydrates (McIntyre et al. 2010; Pinto et al. 2010), maximum efficiency of photosystem II (Pandey et al. 2015), cytoplasmic membrane stability (Talukder et al. 2014) and chlorophyll content (Tahmasebi et al. 2016) have been evaluated in control and heat stress conditions and described as indirect selection criteria for improving grain yield in heat prone environments. Understanding the genetic control of traits associated with grain yield is essential for the enhancing tolerance to heat in plants. It is now recognized that the effective method for genetic improvement of heat adaptive traits in wheat is marker-assisted selection (MAS) (Sadat et al. 2013). Various QTLs have already been reported by various researchers for wheat grain yield (Lopes et al. 2013), water-soluble carbohydrates (Yang et al. 2007), maximum efficiency of photosystem II (Azam et al. 2015), cytoplasmic membrane stability (Talukder et al. 2014) and chlorophyll content (Pinto et al. 2010) in heat stress condition. Four additive QTLs have been reported for chlorophyll content on chromosomes 1B, 2D, 3B and 7D in wheat in heat and drought stress conditions (Tahmasebi et al. 2016). Major QTLs for heat tolerance have also been reported on chromosomes 2A and 2B in wheat under heat stress condition (Bhusal et al. 2017). In complex traits, it is recognized that epistatic effects and QTL $\times$ environment interactions are important genetic components (Rebetzke et al. 2007). Relatively very few studies have been conducted in wheat to identify epistasis and Q $\times$ E interactions associated with grain yield and physiological and biochemical traits in terminal heat stress conditions.

The present study was designed (1) to identify QTLs with additive and epistatic effects for the physiological and biochemical traits associated with grain yield in the Seri M82/Babax population, (2) to detect the interactions of the additive and epistatic QTLs with environment and (3) to examine the associations of the heat adaptive traits with grain yield in control and terminal heat stress conditions.

Materials and methods

Plant material and field experiments

A mapping population consisting of 167 recombinant inbred lines (RIL) derived from the “Seri M82/Babax” cross was studied in four experiments at Zabol Agricultural Research Station (30°50'N, 60°15'E, and 483 m altitude) located at southeastern warm and dry area of Iran. “SeriM82” (KVZ/BUHO/KAL/BB) is recognized for its heat tolerance and high yield potential. “Babax” (BOW/NAC/VEE/3/BJY/COC) is a spring wheat, characterized by high grain yield potential (Olivares-Villegas et al. 2007; McIntyre et al. 2010; Pinto et al. 2010; Lopes et al. 2013).

Four experiments were carried out in 2015–2016 and 2016–2017 growing seasons. In each trial, 167 recombinant inbred lines and their parents (‘SeriM82’ and ‘Babax’) were planted in a 13 $\times$ 13 (13 genotypes in each of 13 sub-blocks) alpha lattice design with two replications. Experimental plots consisted of two 3 m long beds spaced at 60 cm between centers and comprising three rows per bed. To avoid yield reducing factors, appropriate fertilization and weed, disease, and pest controls were implemented. The experiments with normal planting date were conducted on 11 November 2015–16 and 16 November 2016–17 growing seasons and normal irrigation practices were performed throughout the crop cycle. The experiments with delayed sowing on January 21 were considered as terminal heat stress in 2015–16 and 2016–17 growing seasons. To avoid possible drought stress, the genotypes were irrigated throughout the growth cycle. Meanwhile, the rainfall for all trials was negligible. In the late sown experiments, plants experienced higher temperatures during anthesis and the grain-filling period. The average temperatures during post heading period under normally sown conditions were 25.3 and 26.2 °C in the 2 years, while under late sown, the temperatures were 33 and 35.3 °C, respectively (Table 1).

Phenotypic measurements and statistical analysis

The following traits were evaluated. Grain yield (GY) was calculated by weighing grains of harvested plants from an area of 2 m² in each plot (Sayre et al. 1997). Maximum quantum efficiency of photosystem II (Fv/Fm) was measured on flag leaves at the late boot stage using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, UK) (Baker and Rosenqvist 2004). Cytoplasmic membrane stability (CMS) was determined on flag leaves 7d after anthesis using the method described by Rizza et al. (1994). At the early milk stage, the leaf chlorophyll content (CHL) was measured on flag leaves in SPAD unit using a portable chlorophyll meter (Hansatech-Model-cl-ol, Hansatech Instruments Ltd, UK) (Yadawa 1986). Proline content (PRO) and water soluble carbohydrates (WSC) were measured on flag leaves 7d after anthesis using the methods described by Bates et al. (1973) and Yemm and Willis (1954), respectively. Combined analysis of variance was performed using the MIXED procedure in SAS v9.2 (SAS Institute 2009). The effects of replication and sub-block in the model were considered as random. The heritability (H^2) of each trait was estimated by:

$$H^2 = \sigma_g^2 / \sigma_g^2 + \sigma_{gy/y}^2 + \sigma_{ry}^2$$

where r is the number of replications, y is the number of years, σ_g^2 , σ_{gy}^2 and σ^2 represent the genotype, genotype $\times$ year, and error variances, respectively (Nyquist

Table 1 Peak temperature, average maximum and average minimum temperature, rain, relative humidity and pan evaporation during the cropping seasons (2015–16 and 2016–17)

Season	Trial	Peak Temp (month) (°C)	Planting date	Harvesting date	AvgMax/avgMin (°C)	Rain (mm)	RH (%)	Pan evaporation (mm)
2015–2016	Normal sowing date	33 (Apr)	11 Nov	10 Apr	26.2/12.2	17	48.6	6.3
	Late sowing date	41 (May)	21 Jan	20 May	35.3/16.2	17	42.2	7.2
2016–2017	Normal sowing date	31 (Apr)	16 Nov	15 Apr	25.3/11.3	20	45.3	5.5
	Late sowing date	39 (May)	21 Jan	25 May	33.2/14.2	20	39.2	6.8

1991). In each of the environmental condition, genetic correlations between studied traits were also estimated using the MIXED procedure (SAS Institute 2009).

Map construction and QTL mapping

A genetic linkage map comprising 120 simple sequence repeat (SSR), 211 amplified fragment length polymorphism (AFLP) and 144 diversity array technology (DART) markers distributed over 29 linkage groups (LGs) was constructed using JoinMap 4.0 Software (Van Ooijen 2006). Segregation ratio for each marker within a LG was examined using $P < 0.01$. Single markers exhibiting a segregation distortion from a 1:1 ratio were removed if their presence affected marker order or marker distances in the linkage group (McIntyre et al. 2010; Lopes et al. 2013). The best order of markers within each linkage group was determined according to the maximum likelihood method and the distance between markers in centi Morgan (cM) was estimated using the Kosambi (1944) equation. Mixed linear model-based composite interval mapping (MCIM) was undertaken using QTLNetwork ver. 2.0 (Yang et al. 2008) with the program's default values: a walk speed of 1 cm, testing window and filtration window size of 10 cm for 1-Dimensional genome scans to detect a main effect QTLs (MQTLs), epistatic QTLs (EQTLs) and their environmental interactions. The critical F-value was determined by 1000-permutation tests (Doerge and Churchill 1996) with $P \leq 0.05$ as an experimental-wise significance level for putative QTL identification and determination of QTL effects.

Results

Phenotypic variation and correlations among traits

The RIL population showed normal distribution for all studied traits (Fig. 1). The combined analysis of the four

environments' data showed very significant differences among the recombinant inbred lines for all studied traits. The effect of the environment and the environment $\times$ genotype interaction was also very significant ($P \leq 0.01$) for all investigated traits (Online Resource 1). The simple statistics of all the studied traits in 167 'SeriM82/Babax' recombinant inbred lines of wheat and their parents for non-stress and heat stress conditions (mean of two growing seasons) are shown in Online Resource 2. In both environmental conditions, the difference between means of recombinant inbred lines and parental means was not significant for most traits. Transgressive segregation in both positive and negative directions was significant for almost all studied traits in the two different environmental conditions. Broad sense heritabilities (h^2) ranged from 33.06% for chlorophyll content to 90% for proline content and from 24% for grain yield to 87.4% for proline content in non-stress and heat stress conditions, respectively (Online Resource 2). Correlation analysis showed that in non-stress condition, there was a positive significant association between GY with CHL and Fv/Fm. CMS is negatively correlated with GY, WSC and Fv/Fm. In the heat stress condition, GY is positively associated with PRO, WSC and Fv/Fm and is negatively correlated with CMS (Table 2).

QTLs with additive (A) and additive $\times$ environment (AE) interaction effects

QTLs with additive and additive $\times$ environment interaction effects detected by mixed model-based composite interval mapping implemented in QTLNetwork for all studied traits in two environmental conditions are represented in Table 3. In total, 53 additive QTLs were identified for all studied traits, 27 of them were detected in non-stress condition and 26 of them were identified in the heat stress condition, among which two and one showed significant additive $\times$ environment interaction effects in non-stress and heat stress conditions, respectively (Table 3; Fig. 2). For GY, three additive QTLs were mapped on

Fig. 1 Frequency distribution of the means of traits and parents positions among 167 recombinant inbred lines (RIL) under stress and normal stress environments in 2015 and 2016. B and S are Babax and Serim82, respectively

chromosomes 1B, 2B and 2D in non-stress condition. Of these QTLs, one (*QGY-1B*) showed significant additive $\times$ environment interaction effects, explaining 1.13% of the phenotypic variation. The *QGY-2B* contributed the largest phenotypic variation (10.64%) with Babax alleles that increased grain yield. In heat stress condition, six

additive QTLs on chromosomes 1A, 1B, 2B, 2D, 3A and 7A were detected for GY. The *QGY-2D* accounted for 11.58% of the phenotypic variation, with Babax alleles corresponding to higher grain yield. Six additive QTLs were detected for PRO on chromosomes 2B, 2D, 3B, 5B, 6A and 6B in the non-stress condition. The *QPro-5B*

Table 2 Genetic correlations of 6 physiological and biochemical traits in a population of 167 recombinant inbred lines of wheat and their parents for non-stress (below diagonal) and stress (above diagonal) conditions

Trait	GY (gm^{-2})	PRO (μgg^{-1})	WSC (mgg^{-1})	CHL (SPAD value)	Fv/Fm	CMS
GY (gm^{-2})		0.265**	0.225**	− 0.19 ^{ns}	0.82**	− 0.215*
PRO (μgg^{-1})	− 0.15 ^{ns}		0.225*	0.325**	0.244**	0.277*
WSC (mgg^{-1})	− 0.16 ^{ns}	0.16 ^{ns}		0.315**	− 0.17 ^{ns}	0.34**
CHL (SPAD value)	0.213*	0.315**	0.47**		− 0.15 ^{ns}	0.315**
Fv/Fm	0.86**	− 0.17 ^{ns}	− 0.31**	0.18 ^{ns}		− 0.37**
CMS	− 0.27**	0.14 ^{ns}	− 0.271**	0.41**	− 0.33**	

^{ns} Non-significant; *GY* grain yield; *PRO* proline content; *WSC* water soluble carbohydrates; *CHL* Chlorophyll content; *Fv/Fm* maximum efficiency of photosystem II; *CMS* cytoplasmic membrane stability

* and **, significant at 0.05 and 0.01 probability level, respectively

explained the largest percentage (11.03%) of the phenotypic variation with Babax alleles had increased proline content. In the heat stress condition, three QTLs on chromosomes 5A, 5B and 7A were detected for PRO. Seri M82 alleles at *QPro-5B* and *QPro-7A* had positive effects on the trait, whereas the Babax allele at *QPro-5A* had increased proline content. The *QPro-5B* explained the largest phenotypic variation (12.38%) in this condition.

For WSC, four QTLs were detected on chromosomes 1B, 4A, 4B and 7D, accounting for 28.95% of the phenotypic variance, in non-stress condition. The Babax allele at *QWSC-4A* was associated with an increased WSC at this locus, while the Seri M82 alleles at *QWSC-1B*, *QWSC-4B* and *QWSC-7D* showed positive effects on WSC. Four QTLs were detected for WSC on chromosomes 3B, 4A, 5A and 7D in the heat stress condition. One additive effect (*QWSC-3B*) had significant additive $\times$ environment interactions. The *QWSC-4A* was contributed by Babax alleles and explained the largest percentage (10.47%) of the phenotypic variance and increased WSC. Three additive QTLs were identified for Fv/Fm on chromosomes 1D, 3B and 6A in non-stress condition, among which one (*QFv/Fm-6A*) showed significant additive $\times$ environment interactions. At *QFv/Fm-1D* and *QFv/Fm-6A*, the Babax alleles increased Fv/Fm, whereas the allele at *QFv/Fm-3B* from Seri M82 was associated with an increased value of Fv/Fm. In the heat stress condition, six QTLs were detected for Fv/Fm on chromosomes 1D, 2B, 3B, 6A, 6B and 7B. The *QFv/Fm-6A* explained the largest phenotypic variation (11.23%) with the alleles from Babax that increased Fv/Fm.

In the non-stress condition, five QTLs were found for CMS on chromosomes 2D, 4B, 6A, 6B and 6D. The *QCMS-4B* was contributed by Seri M82 alleles and accounted for the largest percentage (11.30%) of the phenotypic variance and increased CMS. Five QTLs were also identified for CMS on chromosomes 2B, 3B, 4B, 7A and 7B in the heat stress condition. At *QCMS-2B* and *QCMS-*

7B, the positive alleles were from Babax, whereas at *QCMS-3B*, *QCMS-4B* and *QCMS-7A* loci, the positive alleles were from the Seri M82. For CHL, six QTLs were detected on chromosomes 2D, 3B, 4A, 6A, 6B and 7D in non-stress condition. QTL *QChl-4A* accounted for the largest percentage (10.27%) of the phenotypic variation with Babax allele that increased Fv/Fm in this condition. Two QTLs were also detected for CHL on chromosomes 2B and 7A in the heat stress condition, explaining 17.96% of the CHL phenotypic variation. At *QChl-2B*, the Babax allele increased CHL, whereas at *QChl-7A* locus, the allele from Seri M82 had increased chlorophyll content. Considering all the QTLs found in the two conditions, there was one common QTL for each trait in the two conditions, except for CHL (Table 3; Fig. 2). It was also observed that the positive alleles of these common QTLs were derived from both parents.

Epistasis (AA) and epistasis $\times$ environment (AAE) interactions

Five pairs of QTLs with epistatic effects were identified for CHL, WSC and PRO in non-stress condition (Table 4; Fig. 2). The phenotypic variances explained by these varied from 0.55% (*QWSC-3B/QWSC-4A*) for WSC to 1.37% (*QPro-5B/QPro-6B*) for PRO. One pair of epistatic QTLs (*QChl-3B/QChl-4D*) was identified for CHL with the alleles from Seri M82/Babax which reduced chlorophyll content. The *QChl-4D* did not show a main effect on CHL. For WSC, two pairs of epistatic QTLs were detected. One pair of these QTLs (*QWSC-4B/QWSC-7D*) with the alleles derived from Seri M82 increased WSC, whereas the other pair (*QWSC-3B/QWSC-4A*) reduced WSC. One of these QTLs (*QWSC-3B*) did not show a main effect on WSC. One pair of WSC epistatic QTLs (*QWSC-3B/QWSC-4A*) showed significant epistasis $\times$ environment (AAE) interactions, accounting for 0.78% of the WSC phenotypic variation. Two pairs of QTLs were involved in epistatic

Table 3 Estimated additive and additive $\times$ environment interaction effects of QTLs for evaluated traits in the two environmental conditions

Trait	QTL name	Marker interval	QTL position (cm)	Range	Additive effect (A) ^a	R _a ^{2b}	AE ₁ ^c	AE ₂ ^d	R _{ac} ^{e2}
<i>Normal environment</i>									
GY (gm ⁻²)	<i>QGY-1B</i>	wPt-0944-aac/caa-4	8.2	0.0–23.6	– 0.054**	8.14	– 0.03*	0.03*	1.13
	<i>QGY-2B</i>	aac/cta-1-wPt-0615	91.9	89.7–94.9	0.04**	10.64			
	<i>QGY-2D</i>	wPt-6657-gdm035	1	0.0–15.0	– 0.023*	10.11			
Proline content (μgg ⁻¹)	<i>QPro-2B</i>	agg/cac-7-gwm301c	91.1	90.7–91.6	1.8**	10.21			
	<i>QPro-2D</i>	gdm035-acg/cta-6	8.1	0.0–71.4	– 1.3**	6.12			
	<i>QPro-3B</i>	aac/cta-6-wPt-7186	84.7	84.4–85.4	– 1.9**	9.15			
	<i>QPro-5B</i>	aag/ctg-6-gwm274	4.1	0.2–6.0	2.14**	11.03			
	<i>QPro-6A</i>	wPt-7599-gwm518b	51.8	42.9–54.8	– 2.88**	8.26			
	<i>QPro-6B</i>	agg/cac-12-agg/cta-4	70.6	66.1–73.9	1.49*	5.39			
	<i>QWSC-7D</i>	gwm130-acc/cat-10	0.0	0.0–2.0	0.0004**	10.09			
WSC (mgg ⁻¹)	<i>QWSC-4B</i>	wPt-8650-wPt-0246	69.6	61.8–69.6	0.0004**	8.10			
	<i>QWSC-4A</i>	gA/Ba44-gwm397	15.2	13.1–21.2	– 0.0003**	10.39			
	<i>QWSC-1B</i>	wPt-0944-aac/caa-4	12.2	2.2–21.6	0.0002*	5.0			
	<i>QFv/Fm-1D</i>	barc0062-gdm0111	128.5	128–130.5	– 0.006*	6.49			
(Fv/Fm)	<i>QFv/Fm-3B</i>	agg/cat-3-gwm389	91.9	88.7–98.5	0.006*	7.04			
	<i>QFv/Fm-6A</i>	barc0003-wmc0163	63.7	54.8–75.5	– 0.005*	10.84	0.006*	– 0.006*	2.22
	<i>QCMS-2D</i>	wmc0018-wPt-0298	68.7	46.5–74.3	5.8**	6.43			
CMS	<i>QCMS-4B</i>	aac/ctg-7-wPt-0391	36.3	19.1–47.8	5.7*	11.30			
	<i>QCMS-6A</i>	wPt-4016-wPt-7330	3.6	0.0–26.4	6.02**	4.53			
	<i>QCMS-6B</i>	acc/ctg-9-acg/cta-5	14.7	6.0–19.1	7.6**	10.11			
	<i>QCMS-6D</i>	gdm132-gwm469	0.0	0.0–24.3	– 5.4*	3.27			
	<i>QChl-2D</i>	wmc0018-wPt-0298	68.7	59.5–74.3	0.53**	6.46			
Chlorophyll content (SPAD value)	<i>QChl-3B</i>	aca/ctg-5-gwm644	123	120.5–133	– 0.79**	6.49			
	<i>QChl-4A</i>	barc070-acg/cta-10	99.5	97.8–103.1	0.68**	10.27			
	<i>QChl-6A</i>	aca/caa-5-wPt-7599	43.9	27.9–61.5	– 0.73**	7.44			
	<i>QChl-6B</i>	wPt-3774-acc/ctg-9	0.0	0.0–18.0	0.47*	4.47			
	<i>QChl-7D</i>	gwm130-acc/cat-10	0.0	0.0–6.7	– 0.56**	10.02			
<i>Stress environment</i>									
GY (gm ⁻²)	<i>QGY-1A</i>	agg/cac-6-agg/cac-6	45.3	36.2–62.8	0.02**	5.04			
	<i>QGY-1B</i>	aag/ctc-6-gwm301b	61.8	61.7–62.2	– 0.02**	7.30			
	<i>QGY-2B</i>	aag/ctc-13-acc/ctc-9	91.1	90.7–91.6	0.02**	10.12			
WSC (mgg ⁻¹)	<i>QGY-2D</i>	wPt-6657-gdm035	4.0	0.0–9.0	– 0.01**	11.58			
	<i>QGY-3A</i>	wPt-2478-gwm369	25.5	19.8–30.1	– 0.02**	6.43			
	<i>QGY-7A</i>	wPt-4748-aca/cag-8	20.7	19.0–28.7	0.008**	3.83			
	<i>QWSC-3B</i>	wPt-2757-barc147	86.8	86.0–87.7	0.0005**	10.07	0.0003*	– 0.0003*	1.04
	<i>QWSC-4A</i>	act/cag-3-agg/cta-12	13.1	11.1–14.8	0.0005**	10.47			
	<i>QWSC-5A</i>	wPt-3563-gwm617a	74.0	68.6–75.0	0.0003**	6.64			
	<i>QWSC-7D</i>	cfid0014-gwm473	41.8	20.7–51.9	0.0002*	5.76			
Proline content (μgg ⁻¹)	<i>QPro-5A</i>	aac/ctc-12-gwm304	38.8	37.3–39.8	– 2.53**	5.0			
	<i>QPro-5B</i>	acc/ctc-3-gwm133	7.0	6.0–8.9	1.88**	12.38			
	<i>QPro-7A</i>	wPt-2260-aca/cac-8	19.0	18.8–19.3	1.91**	6.99			
(Fv/Fm)	<i>QFv/Fm-1D</i>	wPt-5503-wPt-9380	41.0	32.6–45.8	– 0.01**	10.06			
	<i>QFv/Fm-2B</i>	act/ctc-1-wPt-0047	61.5	52.9–79.5	– 0.009**	6.81			
	<i>QFv/Fm-3B</i>	aag/ctc-9-wPt-8238	142.2	137–142.2	0.008**	3.13			

Table 3 continued

Trait	QTL name	Marker interval	QTL position (cm)	Range	Additive effect (A) ^a	R _a ^{2b}	AE ₁ ^c	AE ₂ ^d	R _{ae} ^e
CMS	<i>QFv/Fm-6A</i>	wmc0256-acc/ctg-6	68.8	67.5–71.5	– 0.01**	11.23			
	<i>QFv/Fm-6B</i>	wPt-2899-wPt-4764	66.1	33.2–90.0	0.009**	7.83			
	<i>QFv/Fm-7B</i>	wPt-3723-gwm132c	12.7	3.0–15.1	– 0.01**	4.43			
	<i>QCMS-2B</i>	act/ctc-1-wPt-0047	60.5	51.9–77.5	– 7.63**	10.58			
	<i>QCMS-3B</i>	wPt-1804-aca/cag-9	72.1	64.0–75.1	6.94**	5.86			
	<i>QCMS-4B</i>	wPt-1708-wmc048a	9.3	8.8–23.8	6.09**	10.02			
	<i>QCMS-7A</i>	cfa2049-wPt-4553	43.4	33.7–49.4	5.78**	4.66			
	<i>QCMS-7B</i>	wPt-3723-gwm132c	12.7	0.0–15.1	– 5.46*	3.77			
Chlorophyll content (SPAD value)	<i>QChl-2B</i>	gwm301a-acc/ctg-3	93.9	91.1–98.9	– 0.85**	10.25			
	<i>QChl-7A</i>	wPt-6460-aag/cta-7	0.0	0.0–1.0	0.49*	9.05			

ns non-significant; *a* additive effect of the QTL. *b* percentage of phenotypic variation explained by additive effect of the QTL. *c* additive and environment interaction in E1 (2015 year). *d* additive and environment interaction in E2 (2016 year). *e* percentage of phenotypic variation explained by additive and environment interaction

* and ** significant at 0.05 and 0.01 probability level, respectively

QTLs (AA) for PRO; one pair of QTL (*QPro-3B/QPro-6B*) which carried the alleles from Seri M82/Babax reduced PRO, whereas the other pair (*QPro-5B/QPro-6B*) carrying the Babax alleles increased PRO. In the heat stress condition, five pairs of QTLs with epistatic effects were also identified for Fv/Fm, GY and WSC, among which one showed significant epistasis × environment (AAE) interactions. Two pairs of epistatic QTLs were identified for Fv/Fm; one pair of QTL (*QFv/Fm-2B/QFv/Fm-7B*) with the alleles from Babax reduced Fv/Fm, whereas the other pair (*QFv/Fm-1A/QFv/Fm-1D*) carrying the alleles from Babax/Seri M82 increased Fv/Fm and showed significant epistasis × environment (AAE) interactions. From these QTLs, *QFv/Fm-1A* did not show a main effect on Fv/Fm. Two pairs of QTLs with epistatic effects were identified for GY, both pairs of epistatic QTLs (*QGY-1A/QGY-2D* and *QGY-1A/QGY-3A*) carrying the alleles from Seri M82/Babax reduced GY. One pair of epistatic QTLs was also identified for WSC with the alleles from Seri M82 which increased WSC.

Co-location of QTLs for studied traits

The results indicated that six QTL clusters were identified for different traits in the non-stress condition. One QTL cluster on chromosome 1B affected GY and WSC. The GY QTL on chromosome 2B was co-located with the QTL of PRO. The QTL cluster mapped on chromosome 2D influenced GY, PRO, CMS and CHL. The QTL cluster found on chromosome 6A included PRO, Fv/Fm and CHL. QTLs associated with CMS and CHL (*QCMS-6B* and *QChl-6B*, respectively) on chromosome 6B were also co-located.

QTLs for WSC and CHL were detected in the same location on 7D (Table 5; Fig. 2). In the heat stress condition, four QTL clusters were also observed for different traits on chromosomes 2B, 7A and 7B. The QTL cluster which was detected on chromosome 2B included QTLs for Fv/Fm and CMS. The GY QTL on chromosome 7A coincided in its genomic regions with the QTL of PRO. QTLs for CMS and Fv/Fm were identified in the same location on 7B. QTLs associated with GY and CHL (*QGY-2B* and *QChl-2B*, respectively) on chromosome 2B were also co-located. These common genomic regions for different traits can be explained by the linkage between two or more genes or the pleiotropic effect of one gene.

In conclusion, the result verified that WSC, PRO, CMS and Fv/Fm could be used as criteria for the selection of heat tolerant wheat varieties in breeding programs and the stable QTLs related to these traits allow rapid screening of genotypes in pre-breeding programs to increase their efficiency through marker-assisted selection.

Discussion

QTLs for investigated traits with A, AE, AA and AAE interaction effects

We could detect 27 and 26 main-effect QTLs with additive effect (A) for all studied traits in non-stress and terminal heat stress conditions, respectively. Two and one of these QTLs showed significant additive × environment (AE) interaction effects. 5 and 5 pairs of QTLs with epistatic effects (AA) were also identified for studied traits, among

Fig. 2 Location of QTLs with additive effects, epistatic effects, and QTL × treatment interaction on linkage groups detected by QTLNetwork

Fig. 2 continued

Table 4 Estimated epistasis (AA) and epistasis $\times$ environment interaction (AAE) effects of QTLs for evaluated traits in two growing seasons for two environmental conditions

Trait	QTLi	Marker interval	QTL position	QTLj	Marker interval	QTL position	AA ^a	R _{aa} ^{b2}	AAE _i ^c	AAE _j ^d	R _{aae} ^{e2}
<i>Normal environment</i>											
Chlorophyll content	<i>QChl-3B</i>	aca/ctg-5-gwm644	123	<i>QChl-4D</i>	cfd023-cfd071	12.3	– 0.47*	0.94			
WSC (mg g ⁻¹)	<i>QWSC-3B</i>	wPt-1804-aca/cag-9	76.1	<i>QWSC-4A</i>	gA/Ba44-gwm397	15.2	– 0.0002*	0.55	0.001*	– 0.001*	0.78
	<i>QWSC-4B</i>	wPt-8650-wPt-0246	69.6	<i>QWSC-7D</i>	gwm130-acc/cat-10	0.0	0.0003**	0.88			
Proline content	<i>QPro-3B</i>	aac/cta-6-wPt-7186	84.7	<i>QPro-6B</i>	agg/cac-12-agg/cta-4	70.6	– 0.91*	0.65			
	<i>QPro-5B</i>	aag/ctg-6-gwm274	4.1	<i>QPro-6B</i>	agg/cac-12-agg/cta-4	70.6	1.27**	1.37			
<i>Stress environment</i>											
(Fv/Fm)	<i>QFv/Fm-1A</i>	wPt-3904-wPt-9592	11.4	<i>QFv/Fm-1D</i>	wPt-5503-wPt-9380	41.0	0.009**	1.59	0.006*	– 0.006*	1.53
	<i>QFv/Fm-2B</i>	act/cte-1-wPt-0047	61.5	<i>QFv/Fm-7B</i>	wPt-3723-gwm132c	12.7	– 0.01*	1.45			
GY (gm ⁻²)	<i>QGY-1A</i>	agg/cac-6-agg/cac-6	45.3	<i>QGY-2D</i>	wPt-6657-gdm035	4.0	– 0.01**	1.99			
	<i>QGY-1A</i>	agg/cac-6-agg/cac-6	45.3	<i>QGY-3A</i>	wPt-2478-gwm369	25.5	– 0.01**	2.25			
WSC (mg g ⁻¹)	<i>QWSC-3B</i>	wPt-2757-barc147	86.8	<i>QWSC-5A</i>	wPt-3563-gwm617a	74.0	0.003*	0.84			

ns non-significant. *a* additive by additive epistasis effect of the pairs QTLs. *b* percentage of phenotypic variation explained by epistasis effect. *c* epistasis effect and environment interaction in E1 (2015 year). *d* epistasis effect and environment interaction in E2 (2016 year). *e* percentage of phenotypic variation explained by epistasis and environments interaction

* and ** significant at 0.05 and 0.01 probability level, respectively

which one and one had significant epistasis $\times$ environment (AAE) interaction effects in the non-stress and heat stress conditions, respectively. Two to six main-effect QTLs were detected for each of the studied traits in this study, which confirms these traits are genetically complex and also inherited quantitatively as previously reported (Blum 1988; Pinto et al. 2010; Lopes et al. 2013). The results of this study are in agreement with those of reported in previous studies (McIntyre et al. 2010; Talukder et al. 2014; Azam et al. 2015). For example, Pinto et al. (2010) reported eight QTLs for GY. Talukder et al. (2014) identified three QTLs for CMS and Azam et al. (2015) detected eight QTLs for Fv/Fm. It has been found that epistasis and QTL $\times$ environment interaction effects are a limiting factor for plant breeders and reduce the effectiveness of marker-assisted selection (MAS). Epistatic effects have been recognized as important part for quantitative traits (Rebetzke et al. 2007; Zhang et al. 2009; Xu et al. 2012). In the present study, epistatic effects were mostly observed between additive QTLs that showed significant main effects. The phenotypic variances explained by these epistatic QTLs varied from 0.55% for WSC in non-stress to 2.25% for GY in heat stress conditions. However, the results showed that the QTLs for yield and yield-related traits were mostly additive and the role of epistasis in the genetic control of these traits was important. Genotype $\times$ environment interaction has been shown to make significant contributions to the variability of a quantitatively inherited trait (Rebetzke et al. 2007; Tahmasebi et al. 2016). Therefore, for the unbiased estimation of QTL parameters, QTL $\times$ environment interactions should be considered in QTL mapping studies. In the present study, most of detected QTLs in the two conditions did not have interactions with the environment. From these QTLs, *QGY-2B*, *QPro-5B*, *QWSC-4A*, *QFv/Fm-6A* and *QCMS-4B* that did not show significant G $\times$ E interaction effects and were also common between these two conditions, could be useful for MAS in order to develop heat tolerant and high-performance wheat varieties.

QTL comparison between two conditions

Investigation of a recombinant inbred lines (RIL) population in the two environmental conditions allowed us to compare detected QTLs for different traits and also to identify common QTLs between two conditions. In this study, six common QTLs were detected between the two conditions. These common QTLs were located on chromosomes 2B, 2D, 4A, 4B, 5B and 6A and affected GY, GY, WSC, CMS, PRO and Fv/Fm in both conditions, respectively. However, several QTLs with major effects (for example *QChl-4A*) were also detected for different traits only in one of the two environmental conditions.

Table 5 Co-localized QTLs for the evaluated traits in the N and S environments, identified by mixed linear model using QTLNetwork

Traits (environments)	Chromosome	Marker interval
GY (N), WSC (N)	1B	wPt-0944-aac/caa-4
GY (N), PRO (N)	2B	aac/cta-1-wPt-0615
Fv/Fm (S), CMS (S)	2B	act/ctc-1-wPt-0047
GY (S), CHL (S)	2B	gwm301a-acc/ctg-3
GY (N), PRO (N), CMS (N), CHL (N)	2D	gdm035-acg/cta-6
PRO (N), Fv/Fm (N), CHL (N)	6A	aca/caa-5-wmc0163
CMS (N), CHL (N)	6B	wPt-3774-acg/cta-5
GY (S), PRO (S)	7A	wPt-2260-aca/cac-8
CMS (S), Fv/Fm (S)	7B	wPt-3723-gwm132c
WSC (N), CHL (N)	7D	gwm130-acc/cat-10

These environment-specific QTLs can also be used in the marker-assisted selection (MAS) approach to increase breeding efficiency. A similar result was reported in another study; Mathews et al. (2008) detected different QTLs for grain yield and its components in this population (Seri M82/Babax) under normal and drought stress conditions, suggesting the different set of genes (QTLs) being expressed under drought stress condition. Pinto et al. (2010) also reported several environment-sensitive QTLs for grain yield and yield-related traits in this population under heat and drought stress conditions.

Co-location of QTLs

In the present study, the co-location of two or more QTLs confirmed the strong correlations among the studied traits. For example, in the non-stress condition, *QChl-7D* and *QWSC-7D* QTLs coincided in their genomic regions with each other and suggested that the strong positive correlation between CHL with WSC would come from these co-localized QTLs. Another example of co-location was the QTL near the marker of *aca/cac-8* on chromosome 7A, which affected GY (*QGY-7A*) and PRO (*QPro-7A*) and confirmed their significant positive correlations in the heat stress condition. Similar co-locations were also reported in previous studies; McIntyre et al. (2010) reported several co-located QTLs for different traits in this population, for example, QTLs for WSC and GY were detected in the same location on 6D. Tahmasebi et al. (2016) also detected common QTLs for GY and SPAD on chromosome 7D. Common genomic regions for different traits can be the result of a linkage between two genes or the pleiotropic effect of one gene (Pinto et al. 2010). However, the results showed that co-located QTLs with appropriate effects for grain yield and yield components in non-stress and heat stress conditions could be useful for marker assisted selection or gene cloning to produce high-performance genotypes.

Comparison with previous studies

The results demonstrated that QTLs were mapped for GY trait on chromosomes 1B, 2B and 2D in both conditions. Previous studies also reported that QTLs for GY were located on chromosomes 1B, 2B and 2D (Pinto et al. 2010; Bennett et al. 2012). The *QFv/Fm-6A*, which was common between the two conditions, was detected for Fv/Fm on chromosome 6A and could be useful for MAS. This result is in agreement with that reported by Azam et al. (2015). For CMS, QTLs were found on chromosomes 2B, 3B and 7A in the heat stress condition. Previous studies also showed that QTLs for CMS were located on chromosomes 1D, 2B, 3B and 7A (Talukder et al. 2014; Gupta et al. 2015). However, although most of the genomic regions identified in this study have been reported previously, new QTLs were also identified for different traits. For example, QTLs for PRO were mapped to chromosomes 5A, 5B and 7A. For CHL, QTLs were located on chromosomes 4A, 7A and 7D and QTLs associated with WSC were detected on chromosomes 4B, 5A, and 7D. Tolerance to heat in field crops, including bread wheat, is associated with a variety of physiological, biochemical and morphological traits (Pinto et al. 2010; Tahmasebi et al. 2016). In this study, CHL was positively associated with GY in non-stress condition but no significant association was found between CHL and GY in the heat stress condition. Tahmasebi et al. (2016) also reported that there was no consistent association between CHL and GY under different environments. The association of WSC and GY under heat stress condition was positive, which was in agreement with the reports of Pinto et al. (2010) and McIntyre et al. (2010). Fv/Fm had a positive association with GY under both environmental conditions. Vijayalakshmi et al. (2010) and Azam et al. (2015) also reported that Fv/Fm could be used as an indirect selection criterion for improving grain yield under heat stress condition. CMS was negatively correlated with GY under both environmental conditions. CMS as an indicator of heat tolerance was also associated with yield under heat

stress condition (Reynolds et al. 1994; Blum et al. 2001). PRO showed positive correlation with GY under heat stress condition but no significant relationship was observed between PRO and GY in non-stress condition. These findings confirmed that WSC, PRO, CMS and Fv/Fm could be used as heat-adaptive traits for better understanding of the mechanisms involved in heat tolerance in wheat.

Conclusion

The results showed that most of the detected QTLs in the two conditions did not have interactions with the environment. Six major QTLs, *QGY-2B*, *QGY-2D*, *QPro-5B*, *QWSC-4A*, *QFv/Fm-6A* and *QCMS-4B* were common between the two conditions. Moreover, heat-specific QTLs with major effects were also identified for all traits. Co-localized QTLs associated with yield and physiological traits were identified on chromosomes 1B, 2B, 2D and 7A. In this study, several pairs of QTLs with epistatic effects (AA) were identified for all studied traits except CMS. Thus, for the unbiased estimation of QTL parameters, epistatic and QTL $\times$ environment interactions should be considered in QTL mapping studies. However, the major QTLs detected during this study especially *QGY-2B*, which was not influenced by the environment and also was common between these two conditions could be used in marker-assisted selection (MAS) and gene pyramiding to develop heat tolerant and high-performance wheat varieties.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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