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ORIGINAL PAPER

QTL for yield and associated traits in the Seri/Babax population grown across several environments in Mexico, in the West Asia, North Africa, and South Asia regions

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Abstract Heat and drought adaptive quantitative trait loci (QTL) in a spring bread wheat population resulting from the Seri/Babax cross designed to minimize confounding agronomic traits have been identified previously in trials conducted in Mexico. The same population was grown across a wide range of environments where heat and drought stress are naturally experienced including environments in Mexico, West Asia, North Africa (WANA), and South Asia regions. A molecular genetic linkage map including 475 marker loci associated to 29 linkage groups was used for QTL analysis of yield, days to heading (DH) and to maturity (DM), grain number (GM2), thousand kernel weight (TKW), plant height (PH), canopy temperature at the vegetative and grain filling stages (CTvg and CTgf), and early ground cover. A QTL for yield on chromosome 4A was confirmed across several environments, in subsets of lines with uniform allelic expression of a major phenology QTL, but not independently from PH. With terminal stress, TKW QTL was linked or pleiotropic to DH and DM. The link between phenology and TKW suggested that early maturity would favor the post—anthesis grain growth periods resulting in increased grain size and yields under terminal stress. GM2 and TKW were partially associated with markers at different positions suggesting different genetic regulation and room for improvement of both traits. Prediction accuracy of yield was improved by 5 % when using marker scores of component traits (GM2 and DH) together with yield in multiple regression. This procedure may provide accumulation of more favorable alleles during selection.

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Introduction

Physiological characterization is used to dissect the components of stress adaptation in crops and can, therefore, increase rates of genetic gains through: (1) strategic trait-crossing to combine complementary traits in the progeny, (2) high-throughput phenotyping to enrich for desirable alleles in intermediate generations, and (3) exploration of genetic resources to broaden the genetic base for hybridization (Reynolds et al. 2009). In addition, precision phenotyping plays a crucial role in gene discovery and in understanding the complex interactions between genes,

genetic background, and environment (Reynolds et al. 2009). The advent of ever cheaper and more abundant molecular markers are enabling the accumulation of knowledge on the genetics of yield, yield components, and physiological traits, for application in molecular breeding.

Physiological traits such as canopy temperature at the vegetative and grain filling stages (CTvg, CTgf), and early ground cover (EGC) have been identified as useful in breeding for both drought and heat environments (Pinto et al. 2010). Expression of low CT epitomizes a mechanism of dehydration avoidance expressed throughout the cycle and across latitudes, which can be utilized as a selection criteria for performance under drought, as well as indicating adaptation to hot, irrigated conditions for wheat (Olivares-Villegas et al. 2007; Pinto et al. 2010). Moreover, CT has been shown to be associated with better root functionality under drought (Lopes and Reynolds 2010a, 2010b). Another valuable trait is the rapid development of leaf area and/or above ground biomass to improve water harvest of rain fed wheat in Mediterranean-type environments through reduced soil evaporation (Mullan and Reynolds 2010).

Phenotyping physiological traits for gene discovery, especially under stress conditions, are difficult due to the interactions of physiological traits with major genes like height and flowering. By avoiding segregation for genes of major phenology effects in mapping populations, the probability of identifying genes of minor effect (i.e., those affecting complex traits) is expected to increase (Reynolds and Tuberosa 2008; Reynolds et al. 2009; Pinto et al. 2010). The Seri/Babax population has proved to be useful for mapping quantitative trait loci (QTL) associated with yield and several agronomic traits in experimental environments with minimal phenological variation (McIntyre et al. 2010; Pinto et al. 2010). This study aimed to confirm the value of previously identified, stress-adaptive QTL at a number of sites representing important breeding target regions where climate change is already manifesting increases in heat and drought stress, namely South Asia, West Asia, and North Africa. Specific objectives included: (1) comparing QTL associated with yield, yield components, and physiological traits in each environment with other published works; (2) to confirm and validate these QTL across a broad range of environments in the WANA region; (3) to detect interactions between traits; and (4) to predict yield using different QTL models.

Materials and methods

Plant material and environments

A wheat recombinant inbred line (RIL) population, comprising 165 sister lines derived from the Seri M82/Babax cross (Olivares-Villegas et al. 2007), was studied in managed environments in Mexico and in several countries in West Asia, North Africa (WANA), and South Asia.

The population was characterized by its narrow range of plant height (PH) and flowering time (in the present study, averaged across all environments, ca. 17 cm and 9 days, respectively), and was developed primarily for genetic mapping and screening for the physiological basis of stress tolerance in drought and heat environments (Olivares-Villegas et al. 2007).

In Mexico, experiments were conducted at the Norman E. Borlaug experimental station (CENEB), near Ciudad Obregon, northwest Mexico, during the 2009–2010 spring season (late November sowing and April harvest) for irrigated and drought experiments, and with delayed sowing (late February sowing and June harvest) for heat stress (with or without drought) experiments. The environment is a high radiation, irrigated environment for which meteorological data are summarized in Table 1. Appropriate fertilization and weed, disease, and pest controls were implemented to avoid yield limitations. The experimental design was a randomized lattice with two replications in 2 m long and 0.8 m wide plots, consisting of one raised bed with two rows per bed (0.25 m between rows) with seed rates of 120 kg/ha.

The same population was grown during the 2008–2009 season in two environments in Sudan (Dongola and Wad Medani), one environment in Iran (Darab), two environments in India (Ludhiana and Karnal), and one environment in Egypt (Sohag). Environmental data are summarized in Table 1.

Yield, yield components, and physiological traits

Grain yield, grain number (GM2), thousand kernel weight (TKW) were determined using standard protocols (Sayre et al. 1997). Days to heading (DH) was taken as the point when more than 50 % of plants were displaying heads (Zadoks stage 59, Zadoks et al. 1974), and day to maturity (DM) was recorded when 50 % of the spikes in a plot showed a total loss of green color (Zadoks stage 89, Zadoks et al. 1974). Early ground cover (EGC) was measured during the first 2 weeks after emergence, using a visual scale of 0–10 (0 corresponds to 0 % cover and 10 to 100 % cover) by looking at the crop through a circle formed by the thumb and index finger held 10 cm from the eye. Calibrations were made by first observing several circles drawn on a piece of paper, containing different percentages of known cover. CTvg and CTgf were measured at the vegetative and grain filling stages, respectively, using a portable infrared thermometer (Mikron M90 Series, Mikron Infrared Instrument Co., Inc., Oakland, NJ, USA) at midday (between 11am and 1pm). When all plots reached physiological maturity, PH was determined by measuring

Table 1 Meteorological data including peak temperature of the hottest day (month of occurrence is shown in brackets), average maximum and average minimum temperature (avgMax/avgMin), precipitation (rain), total amount of water applied by irrigation (Irr), relative humidity (RH), evapotranspiration (ETo) and yield in environments where the Seri/Babax population was grown in the 2008–2009 cycle

Country	Environment	Peak Temp (month) (°C)	Emerg Date	Harv Date	avgMax/avgMin (°C)	Rain (mm)	Irr (mm)	RH (%)	ETo (mm)	Yield (g m^{-2})
Iran	Darab(1)	40 (June)	19 Dec	13 Jun	25.3/10.2	175	~450	49.3	4.4	453
Egypt	Sohag(3)	45 (May)	1 Dec	15 May	26.5/10.2	0	~694	62.6	3.5	752
Sudan	Dongola(4)	NA	8 Dec	10 Apr	NA	0	~650	NA	NA	666
	Medani(5)	45 (March)	20 Nov	30 Mar	36.9/17.8	0	~600	30.1	10.7	274
Syria	Tel Hadya(6)	NA	NA	NA	NA	NA	NA	NA	NA	355
Mexico	Irr-Mex(7)	39 (May)	16 Nov	28 May	27.4/9.4	16	~600	62.6	4.1	658
	Dr-Mex(8)	39 (May)	16 Nov	28 May	27.4/9.4	16	~250	62.6	4.1	386
	He-Mex(9)	39 (May)	1 Mar	15 June	30.7/11.9	0	~800	57.7	5.4	350
	HD-Mex(10)	39 (May)	1 Mar	15 June	30.7/11.9	0	~400	57.7	5.4	268
India	Ludhiana(11)	38 (Apr)	1 Oct	29 Apr	26.9/12.3	120	NA	66.8	21.4	408
	Karnal(12)	31 (Apr)	8 Nov	9 Apr	27.8/11.6	0	NA	66.0	NA	660

Numbers within brackets after each environment are an identification number specific to each environment

NA not available

the distance between the base of the stem and the top of the spike, excluding awns.

Phenotypic analysis

The first step of the analyses consisted of computing the adjusted means for each environment and genotype. This was done by adjusting the experimental design (i.e., alpha lattice) with environments, replicates within environments, and incomplete blocks within environments, replications and genotype all considered as random effects using the MIXED procedure from SAS (2004). To obtain information on the genotype by environment interactions, cluster and site regression analysis was used. Cluster of environments based on genetic correlations for yield performance of the Seri/Babax progeny and parents grown across several environments, was generated using the CLUSTER procedure from SAS (2004) with the Ward method and plotted using the TREE procedures from SAS (2004). Site regression analysis was performed according to Samonte et al. (2005) using normalized yield values in SAS (2004) to characterize the genotype by environment interactions.

To determine the reproducibility of each trait across environments, broad sense heritability was estimated for each trait individually across all environments as:

$$H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma^2/re)$$

where, r is the number of replications, e is the number of environments, σ^2 is the error variance, σ_g^2 is the genotypic variance, and σ_{ge}^2 is the genotype by environment interaction variance.

To evaluate the associations between traits measured in Mexico, genetic correlations (r_g) were estimated as the ratio of the genetic covariance between two traits and the square root of the product between genetic variances of the two traits. For broad sense heritability and genetic correlations, all the variance components were estimated using the MIXED procedure from SAS (2004) considering all the terms in the model as random effects. Phenotypic correlations between yield and physiological variables were calculated using the CORR procedure (SAS 2004) in each location.

Map construction

A map with 425 markers (68 simple sequence repeat, SSR), 212 amplified fragment length polymorphism (AFLP), and 145 diversity array technology (DArT) markers distributed over 39 linkage groups (LGs) had been previously constructed for this population (McIntyre et al. 2010). An additional 63 SSR markers, with known chromosomal locations, were scored in the population. A new map was constructed for each chromosome using JoinMap 4.0 (van Ooijen 2006), which combined the segregation data from the 425 marker map and the new SSR markers. As described by McIntyre et al. (2010), segregation ratios were examined for each marker within an LG or chromosome; single markers exhibiting segregation distortion at $P < 0.01$ were removed, if their presence affected marker order and/or marker distances in the LG. LGs were named according to the chromosome to which they were assigned on the basis of the known map location of the SSR and

DArT markers. The chromosome name was followed by a suffix (a, b, c, or d) if more than one LG was assigned to a chromosome. The suffix indicated the relative chromosomal position of LGs assigned to the same chromosome (LG-a above LG-b, consistent with short arm above long arm), if known. If the position of a LG, relative to other LGs assigned to the same chromosome, could not be determined, it was assigned the next suffix in the sequence. The final map comprises 475 markers (120 SSR, 211 AFLP, and 144 DArT) distributed over 29 LGs. There are multiple small LGs for seven chromosomes: 1D, 2A, 3A, 5D, 6A, 6D, and 7A.

QTL mapping

Multi-environment single trait (QTLxE) and multi-trait QTL (single environment) effects were estimated using the approach of Malosetti et al. (2004, 2006) which is implemented in GenStat Version 14.0 (VSN International 2011). The QTLxE effects were determined after modeling the GxE variance covariance matrix, and then performing a whole genome scan where genetic predictors were calculated at 10 cM intervals. Each QTL in the final multi-QTL model was tested for an overall main effect. The size of each QTL effects, the contributing allele and variance explained (R^2) are presented. A Bonferroni-based multiple test control threshold was applied, using the estimation of the effective number of tests along the genome proposed by Li and Ji (2005) with a point-wise alpha level of 0.05 divided by the effective number of tests along the genome (a corresponding LOD of 3.9 in this study, but LOD values higher than 3.5 were not discarded).

To calculate marker prediction values, first average additive effects of each marker and trait were computed across environments. Prediction values were then calculated as the sum of additive effects of all markers associated with a specific trait, according to the allele present in each genotype. Finally, predicted values (from additive effects) were correlated with observed values of each trait (adjusted means of each genotype across all environments). Simple and multiple regression were used to detect how well do marker additive effects of several traits predict observed traits and grain yield, using the add-in POPTOOLS for Microsoft® Excel (Hood 2010).

Results

Environmental conditions and yield

The performance of the Seri/Babax population was evaluated under a diverse set of environmental conditions, though most had temperatures above 35 °C and some had drought (Table 1). Cluster analysis based on genetic correlations of grain yield across all environments and using the entire progeny and parental lines (Fig. 1) showed two main groups: one encompassed all environments in Mexico, Egypt, Iran, and India, whereas the second cluster

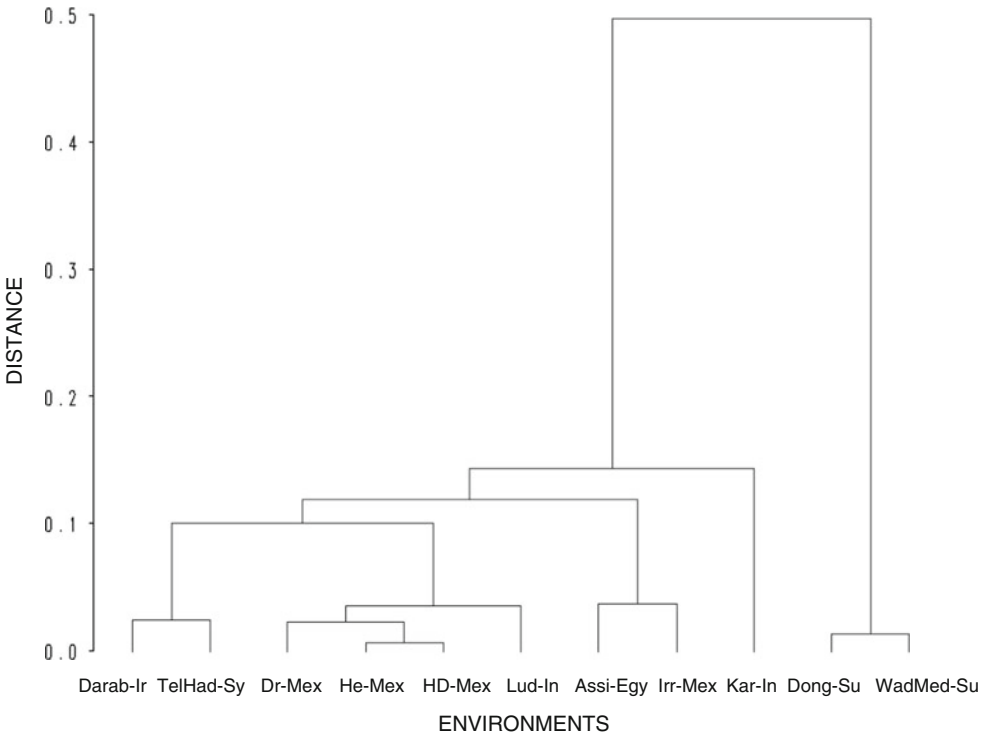

Fig. 1 Cluster of environments based on genetic correlations for yield performance of the Seri/Babax progeny and parents grown across several environments in Mexico (*irrigated* irr-mex, *drought* dr-Mex, *heat* he-mex, and *heat plus drought* hd-mex), Sudan (Dongola Dong-Su and Wad Medani, WadMed-Su), Iran (Darab, Darab-Ir), Egypt (Sohag, Soh-Egy), and India (Karnal Kar-In and Ludhiana Lud-In)

comprised the two Sudanese environments (Fig. 1). Trial yields are shown in Table 1. Site regression analysis was performed for yield to find the most stable and the highest yielding genotype across all environments, determined here as line SBS 21 (Fig. 2; Table 2). Site regression analysis confirmed the groups of environments clustered in Fig. 1, with the Sudanese sites negatively correlated with all other sites (Fig. 2).

Physiological traits and associations with yield

The across trial trait averages for the total population, the parents, and the most stable line are shown in Table 2. The parents mainly contrasted (i.e., showed significant differences Table 2) for yield, TKW, and PH. However, the progeny showed a range of contrasting values for all traits measured. Traits showing high heritability include DH, DM, TKW, and PH. Grain yield, CTvg, and CTgf showed intermediate heritability, whereas EGC showed low heritability (Table 2). Compared to the parents, the most stable line, SBS 21, had higher yield and GM2, lower DH and PH and TKW was between the two parents (Table 2). Average values (between the two parents) of other traits (EGC and CTvg) were observed in SBS 21. Using all genotypes in the population (data obtained from the four trials conducted in the experimental station CENEB in Mexico), the traits

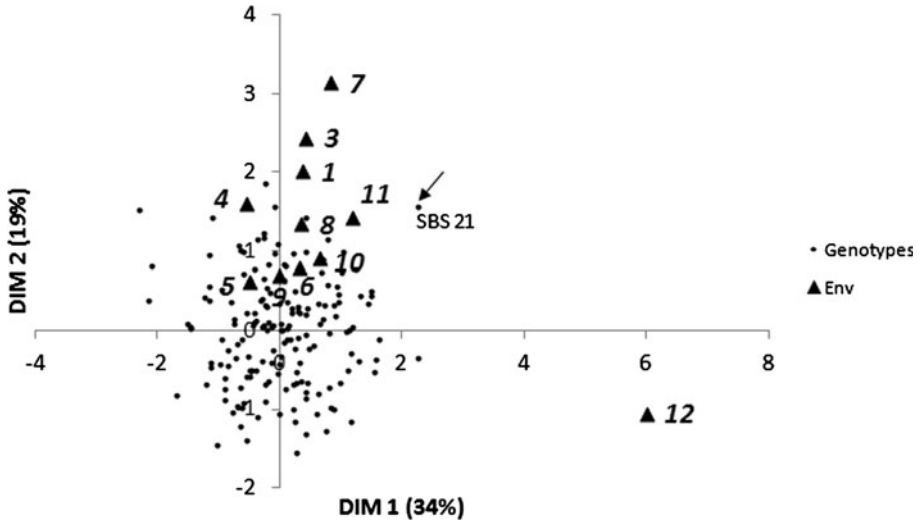

Fig. 2 Site regression for yield in the Seri/Babax progeny and parents (*points*) grown in several environments (*triangles*) including: environments in Mexico (irrigated 7, drought 8, heat 9 and heat plus drought 10), Sudan (Dongola 4 and Wad Medani 5), Iran (Darab 1), Egypt (Sohag 3) and India (Karnal 11and Ludhiana 12). The highest yielding and more stable genotype, SBS 21, is *highlighted* with an *arrow*. Percentage of variation explained in the first and second dimensions (DIM) are shown (total of 53 %)

Table 2 Seri/Babax population average (AVG), Seri/Babax line 21 (SBS 21), minima, maxima, LSD test at 5 % confidence, heritability (H^2) and probability of significance of genetic effects (Gen), environment effects (Env) and genotype by environment (GenxEnv), for yield (YLD), days to heading and maturity (DH and DM), early ground cover (EGC), canopy temperature measured at the vegetative (CTvg) and grain filling stage (CTgf), and plant height (PH) of the Seri/Babax population grown across different environments (the same described in Table 1)

	GY (gm^{-2})	DH (days)	DM (days)	TKW (g)	GM2 ($\#m^{-2}$)	EGC (%)	CTvg (^oC)	CTgf (^oC)	PH (cm)
AVG	435.7	74.5	114.5	36.5	12178	51.9	24.8	26.0	92.1
Seri	438.4	77.8	116.0	35.7	12059	45.6	24.9	26.9	90.1
Babax	489.5	77.3	116.1	38.5	12885	50.7	24.9	26.7	95.3
SBS 21	571.2	73.1	111.6	36.3	14280	42.9	24.5	27.3	87.1
MIN	404.8	71.9	109.9	32.2	9410	38.4	23.9	24.8	82.6
MAX	571.2	80.5	118.6	46.3	14698	48.6	25.4	29.5	99.8
LSD	46.9	1.9	2.0	2.4	1680	5.4	0.61	0.89	2.8
H^2	0.59	0.87	0.92	0.75	0.68	0.25	0.24	0.29	0.89
Nb Envs	11	11	10	9	4	6	4	10	11
Gen	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Env	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0425	<0.0001	<0.0001	<0.0001
GenxEnv	<0.0001	<0.0001	<0.0001	0.0326	<0.0001	0.24	<0.0001	<0.0001	<0.0001

showing the most significant correlations with yield were: GM2, DH, CTgf, CTvg, DM, TKW, and EGC (in order of significance level, see Table 3). Moreover, the above traits were inter-related as shown in Table 3, specifically: DH and DM were negatively correlated with TKW and positively correlated with EGC, TKW was negatively correlated with GM2, and CTgf was negatively correlated with GM2 and with EGC. However, the former correlations were not particularly strong as shown by the relatively low r_g values (Table 3).

In more detail, correlations between physiological traits and yield according to the environment are shown in Table 4. DH and DM were mainly related negatively with yield except in Sudanese environments where positive associations were observed (Dongola and Wad Medani). TKW was positively associated with yield except in Sudanese sites where negative correlations were observed (Table 3). CTvg and CTgf were negatively associated with yield in several environments, but in others the correlations were not significant (Table 4).

QTL associated with phenology and plant height

Several QTL were identified by multi-environment single trait analysis for DH on chromosomes 2B, 3A-a, 4A, 5D-b, 6A-a, 6B, and 7D-b (Table 5), and all showed significant QTLxE interactions. The marker explaining the highest percentage of total variation accounting for DH in most environments was located on chromosome 7D-b, positioned at 12.5 cM (30 % of variation in some environments) (Supplementary Table 1).

Analysis of QTLxE for DM showed significant QTL on several chromosomes: 1D-a, 2B, 4A, 4D, 5D-b, 6A-a, 6B, 7D-a, and 7D-b (Table 5). The marker explaining the highest percentage of total variation accounting for DM in most environments was located on chromosome 7D-b, positioned at 2.73 cM (Supplementary Table 1).

Ten QTL were identified for PH on chromosomes 1D-a, 2B, 2D, 4A, 4B, 4D, 5A, 5D-a, and 7D-b (Table 5). Three of these QTL were identified for PH without significant QTLxE interactions on chromosomes 2B, 4B, and 5D-a,

Table 3 Genetic correlations (r_g) of yield (YLD), days to heading and maturity (DH and DM), thousand kernel weight (TKW), grain number (GM2), early ground cover (EGC) and canopy temperature measured at the vegetative and grain filling stages (CTvg and CTgf) and plant height (PH) of the Seri/Babax population grown in Mexico

r_g	YLD	DH	DM	TKW	GM2	EGC	CTvg	CTgf	PH
YLD		**−0.63**	**−0.43**	**+0.33**	**+0.77**	**+0.19**	**−0.46**	**−0.58**	−0.01
DH			**+0.99**	**−0.24**	−0.07	**+0.47**	−0.15	−0.11	+0.11
DM				**−0.48**	−0.00	**+0.30**	−0.14	−0.11	−0.03
TKW					**−0.38**	−0.11	−0.05	+0.21	−0.05
GM2						+0.09	**−0.27**	**−0.43**	−0.01
EGC							+0.03	**−0.32**	0.06
CTvg								**+0.56**	−0.01
CTgf									**+0.16**

Figures in bold indicate significant genetic correlations at $p < 0.05$

Table 4 Associations of yield in each environment (Darab, Sohag, Dongola, Wad Medani, Tel Hadya, Mexico Irrigated (Irr-Mex), Mexico drought (Dr-Mex), Mexico-Heat (He-Mex), Mexico-Heat plus Drought (HD-Mex), Karnal and Ludhiana) with days to heading and to maturity (DH and DM), thousand kernel weight (TKW), grains per square meter (GM2), canopy temperature at the vegetative and grain filling stages (CTvg and CTgf), early ground cover (EGC) and plant height (PH)

YIELD	Darab	Sohag	Dongola	Wad Medani	TelHadya	Irr-MexIrri	Dr-Mex	He-Mex	HD-Mex	Karnal	Ludhiana
DH	**−0.37**	**−0.14**	+0.08	**+0.25**	**−0.20**	+0.07	**−0.25**	**−0.38**	**−0.48**	**−0.44**	**−0.25**
DM	**−0.23**	−0.12	**+0.21**	**+0.30**	**+0.21**	+0.11	**−0.30**	**−0.41**	**−0.45**	**−0.42**	NA
TKW	**+0.32**	+0.04	**−0.14**	**−0.14**	NA	**+0.20**	+0.13	**+0.29**	**+0.51**	**+0.27**	+0.01
GM2	NA	NA	NA	NA	NA	**+0.86**	**+0.87**	**+0.73**	**+0.78**	NA	NA
CTvg	NA	NA	NA	NA	NA	**−0.23**	−0.11	**−0.20**	**−0.25**	NA	NA
CTgf	**−0.17**	−0.05	−0.08	**−0.26**	NA	**−0.36**	−0.08	**−0.20**	**−0.54**	+0.08	+0.07
EGC	**+0.45**	+0.06	NA	+0.02	NA	**+0.16**	+0.00	**−0.38**	+0.06	**+0.39**	NA
PH	+0.03	**+0.21**	+0.01	+0.05	**−0.20**	+0.06	−0.03	+0.01	−0.04	+0.00	+0.03

Figures in bold indicate significant Pearson correlation coefficients at $p < 0.05$

Table 5 Multi-environment QTL for yield (YLD), thousand kernel weight (TKW), days to heading and maturity (DH and DM), plant height (PH), canopy temperature at the vegetative (CTvg) and grain filling (CTgf) stages and early ground cover (EGC) analyzed for each trait at the time across all environments

Trait	Marker	Chr	Pos	QTLxE	Dar	Soh	Don	WM	THa	MIR	MD	MH	MHD	Kar	Lud
YLD	4A-act/cag-3	4A	13.15	Yes	4.2	2.2	**17.3**	**9.1**	3.4	**21.7**	**15.1**	**9.9**	**13.7**	6.6	**−27.8**
	4A-barc070	4A	99.46	Yes	6.5	**−13.1**	−8.7	−4.5	**5.9**	−9.8	−4.1	**−5.8**	−2.9	−7.2	**−30.1**
	4B-gwm375	4B	14.09	Yes	3.9	−4.3	−2.1	−2.7	**7.9**	**16.4**	**7.3**	**5.7**	**6.2**	**31.3**	**21.9**
	5A-barc040	5A	48.36	Yes	2.2	−1.5	−2.8	**8.5**	**−6.0**	**16.3**	−1.3	1.6	0.4	−7.7	**−34.3**
	6B-agg/ctg-8	6B	77.72	Yes	**−19.6**	0.3	−2.6	2.0	−9.4	4.1	**−11.1**	**−6.4**	**−5.6**	−5.4	−6.7
	7D-acc/cat-10	7D-b	2.73	Yes	**−23.7**	**−17.5**	4.9	3.3	**−11.8**	−11.9	−3.6	**−6.7**	**−12.7**	**−15.2**	−14.3
TKW	5B-gwm133	5B	7.47	Yes	**0.59**	0.03	0.29	0.27	NA	0.37	**0.49**	0.24	**0.49**	0.29	**0.67**
	6A-wmc0163	6A-a	62.18	Yes	**1.1**	0.0	**0.6**	**0.5**	NA	**0.7**	0.1	−0.1	0.0	**0.6**	0.1
	6D-gwm325	6D-a	36.98	Yes	**0.7**	**0.9**	0.4	**1.0**	NA	**1.1**	0.3	**0.8**	0.3	**0.9**	**0.4**
	C29P13	7D-b	12.5	No	**−1.4**	**−1.4**	**−1.4**	**−1.4**	NA	**−1.4**	**−1.4**	**−1.4**	**−1.4**	**−1.4**	**−1.4**
GM2	C1P48	1A	48.08	No	NA	NA	NA	NA	NA	**−364.1**	**−364.1**	**−364.1**	**−364.1**	NA	NA
	C14P6	4A	5.55	No	NA	NA	NA	NA	NA	**415.3**	**415.3**	**415.3**	**415.3**	NA	NA
	4B-aag/cta-5	4B	11.57	No	NA	NA	NA	NA	NA	**359.6**	**359.6**	**359.6**	**359.6**	NA	NA
	6D-cfd0188	6D-a	41.41	No	NA	NA	NA	NA	NA	**−289.3**	**−289.3**	**−289.3**	**−289.3**	NA	NA
DH	2B-act/ctc-11	2B	38.9	Yes	0.0	−0.1	−0.3	**−0.9**	0.1	−0.4	−0.4	−0.3	−0.4	−0.1	−0.1
	3A-wPt-2478	3A-a	23.5	Yes	−0.1	**−0.5**	−0.3	**−0.5**	−0.1	−0.2	−0.2	−0.2	−0.1	**−0.4**	**−1.2**
	4A-wmc048d	4A	12.9	Yes	−0.2	0.1	0.3	**0.6**	−0.2	0.3	0.3	0.3	0.3	−0.1	**−0.5**
	5D-wPt-5505	5D-b	12.6	Yes	0.2	**0.8**	**0.9**	**0.8**	0.2	**0.5**	**0.5**	0.4	**0.5**	**0.7**	**0.5**
	6A-wPt-7599	6A-a	50.8	Yes	−0.2	**−0.4**	**−0.5**	−0.2	−0.2	0.0	0.0	0.0	0.0	**−0.7**	**−1.0**
	6B-agg/ctg-8	6B	77.7	Yes	−0.1	0.4	**0.8**	**1.2**	0.0	0.2	0.2	0.2	0.2	0.3	**1.0**
	C29P13	7D-b	12.5	Yes	**0.6**	**1.9**	**1.3**	**0.6**	**1.0**	**1.1**	**1.1**	**0.9**	**1.0**	**1.6**	**2.2**
DM	1D-gdm0111	1D-a	132.2	Yes	**0.2**	**1.0**	**0.7**	**0.6**	**0.3**	**0.4**	**0.6**	**0.5**	**0.6**	**0.3**	NA
	2B-act/ctc-11	2B	38.9	Yes	−0.1	0.0	**−0.7**	**−0.9**	0.2	−0.2	**−0.3**	**−0.3**	**−0.5**	−0.3	NA
	4A-gwm397	4A	23.7	Yes	−0.1	0.4	0.5	**0.5**	−0.2	**0.4**	0.0	**0.5**	**0.5**	−0.1	NA
	C16P7	4D	7.3	Yes	0.0	**−0.7**	**−0.7**	**−0.5**	0.2	**−0.4**	**−0.6**	**−0.5**	−0.3	**−0.4**	NA
	C20P6	5D-b	6.3	Yes	0.2	**0.6**	**1.0**	**0.8**	0.1	**0.7**	**0.6**	**0.6**	**0.9**	0.1	NA
	6A-barc0113	6A-a	68.2	Yes	0.0	−0.1	−0.1	0.1	**0.4**	0.3	−0.1	0.2	**0.3**	**−0.4**	NA
	6B-aac/ctc-3	6B	83	Yes	−0.1	0.2	**0.7**	**0.8**	−0.3	0.2	**0.3**	**0.3**	**0.3**	0.1	NA
	7D-aca/cag-11	7D-a	11.1	Yes	−0.2	**−0.8**	**−1.0**	**−0.9**	**−0.1**	**−0.6**	**−0.7**	**−0.5**	**−0.7**	**−0.4**	NA
	7D-acc/cat-10	7D-b	2.7	Yes	**0.4**	**1.8**	**1.7**	**0.7**	**0.4**	**0.8**	**1.2**	**0.7**	**0.8**	**0.8**	NA
PH	1D-gdm0111	1D-a	132.2	Yes	−0.3	−0.7	−0.3	0.4	0.4	−0.6	0.3	−0.4	**−0.6**	**−0.7**	−0.6
	2B-aag/ctg-5	2B	26.8	No	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**
	C9P62	2B	62.3	Yes	**1.8**	**1.4**	**1.1**	−0.4	−0.4	**1.3**	−0.1	0.3	0.4	0.7	0.7
	2D-aac/ctg-6	2D	12	Yes	0.3	**0.9**	0.2	−0.4	−0.4	0.2	0.1	0.2	**−0.5**	0.3	**0.9**
	4A-agg/cta-12	4A	13.6	Yes	**−1.5**	**−1.9**	**−1.0**	0.0	0.0	−0.6	−0.4	0.2	−0.1	**−1.4**	**−1.7**

Table 5 continued

Trait	Marker	Chr	Pos	QTLxE	Dar	Soh	Don	WM	THa	MIR	MD	MH	MHD	Kar	Lud
4B-aag/cta-5	4B	11.6	No	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	**−0.6**	
4D-wmc048b	4D	0	Yes	0.5	**1.0**	0.2	−0.6	−0.6	0.0	0.0	0.2	**−0.6**	0.3	0.3	
5A-gwm617a	5A	76	Yes	**1.7**	**1.4**	**1.2**	**1.1**	**1.1**	0.6	0.3	**0.5**	0.0	**0.9**	**1.1**	
C19P17	5D-a	17	No	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**	
7D-gwm130	7D-b	0	Yes	0.3	−0.2	0.2	0.3	0.3	**1.1**	0.0	**0.7**	**1.3**	−0.2	−0.4	
CTvg	4A-wmc048d	4A	12.92	Yes	NA	NA	NA	NA	NA	**−0.1**	**−0.1**	**−0.1**	**−0.1**	NA	NA
CTgf	6A-gwm617b	6A-b	28.39	Yes	0.0	**−0.1**	−0.1	−0.2	NA	−0.1	0.0	0.0	0.0	0.0	**0.3**
	C29P13	7D-b	12.5	Yes	−0.1	−0.2	**−0.2**	**0.1**	NA	0.0	−0.1	0.1	**0.1**	−0.1	−0.1
EGC	7D-acc/ctc-7	7D-a	11.7	Yes	0.2	NA	NA	0.1	NA	**−1.1**	0.1	**−0.4**	**−0.8**	**0.6**	NA
	7D-acc/cat-10	7D-b	2.7	Yes	**−0.7**	NA	NA	−0.2	NA	0.2	0.2	**0.8**	−0.3	**−0.6**	NA

Marker, chromosome (Chr), position (Pos), QTL by environment interactions (QTLxE) and additive effects in each environment are shown. Environments include Darab (Dar), Sohag (Soh), Dongola (Don), Wad Medani (WD), Tel Hadya (THa), Mexico irrigated (MIR), Mexico drought (MD), Mexico heat (MH), Mexico heat plus drought (MHD), Karnal (Kar) and Ludhiana (Lud)

Figures in bold indicate significant QTL effects in one particular environment

NA not available

with additive effects coming either from the Seri or Babax parent. All QTL identified for PH had small effects (accounting each for not more than 10 % of PH variation) (Table 5 and Supplementary Table 1).

QTL mapping for agronomic traits

Several QTL were identified for yield across all environments using QTL by environment analysis (Table 5): on chromosomes 4A (two), 4B, 5A, 6B, and 7D. All these QTL have shown significant QTL by environment (QTLxE) interactions, with effects coming either from the Babax or Seri parent. The QTL accounting for the highest percentage of total yield variation in most environments was located on chromosome 4A, positioned at 13.15 cM (Supplementary Table 1).

Multi-environment single trait QTL analysis for TKW detected significant QTL on chromosomes 5B, 6A, 6D, and 7D-b, all showing interaction with the environment (Table 5). The marker explaining the highest percentage of total variation accounting for TKW (in most environments) was located on chromosome 7D-b, positioned at 12.5 cM (up to 39.7 %) (Supplementary Table 1). GM2 showed four consistent QTL on chromosomes 1A, 4A, 4B, and 6D-a (Table 5 and Supplementary Table 1), each marker accounting for <12 % of GM2 variation.

QTL associated with canopy temperature at the vegetative and grain filling stages and early ground cover

For CTvg, only one QTL, on chromosome 4A at 12.92 cM, was considered significant in the multi-environment analysis (Table 5). Two QTL were detected for CTgf on chromosomes 6A and 7D, positioned at 28.39 and 12.5 cM, respectively (Table 5). However, these markers accounted for a small proportion of CTvg and CTgf variation (Supplementary Table 1).

Two QTL were identified on chromosomes 7D-a and 7D-b (at 11.72 and 2.73 cM, respectively) for EGC (in multi-environment analysis), with positive effects coming from both parents, depending on the environment, but accounting for a small percentage of trait variation (Table 5 and supplementary Table 1).

Multi-trait QTL analysis

Using all sister lines in the population, several clusters of QTL were identified for two or more traits (Supplementary Table 2). Under irrigated and drought conditions, co-locations of yield and GM2 QTL were the most frequent (Supplementary Table 2) on chromosomes 1A, 1B, 4A, 5A, and 6A. However, under heat and heat plus drought,

co-locations of yield with many other traits were observed (Supplementary Table 2). Particularly, co-locations of yield and GM2 were reported on chromosomes 4A, 4B, and 7D-b, but yield also clustered with TKW on chromosomes 2B, 4B, and 7D-b (Supplementary Table 2); co-locations of yield and DH/DM were depicted on chromosomes 4A, 4B, and 7D-b; co-locations of yield and PH were observed on chromosomes 2B, 4B, and 7D-b; and finally, co-locations of yield with CTvg and CTgf were reported on chromosome 4A (Supplementary Table 2). In this multi-trait approach (as in the multi-environment analysis), marker 7D-acc/cat-10 was identified in all environments, explaining QTL effects for yield, physiological, and phenological traits (Supplementary Table 2). This marker accounted for 30 % of trait variation in some cases, particularly for DH (Supplementary Table 2). To remove the effect of this major QTL for DH, a subsequent analysis was performed using two distinct groups of sister lines, with and without marker 7D-acc/cat-10. Supplementary Tables 3 and 4 show the results, whereby removing the effects of marker 7D-acc/cat-10, it was possible to separate (for heat and heat plus drought environments) the effects of DH from CTgf and CTvg on chromosome 4A (Supplementary Table 3, 4). Moreover, the percentage of variation explained by these QTL was slightly improved, particularly for CTvg, compared with the first analysis (containing all genotypes with the interactive effect of markers associated with DH), as shown in supplementary Tables 3 and 4. The only exception to this was observed on chromosome 6A-b and 6D-a where CTvg and phenology QTL were co-located (Supplementary Tables 3, 4), but further division of the population would greatly reduce the number of genotypes used in the analysis and any QTL discoveries would be insignificant. Yield components were regulated by different QTL, but co-location of TKW, GM2, and yield QTL was also observed under the heat and heat plus drought environments on chromosomes 4B and 7D-b, and not under the irrigated and drought environments (Supplementary Table 2). When the effect of marker 7D-acc/cat-10 was removed, a common region for TKW, GM2, yield, DH and DM was still identified on chromosome 4B (Supplementary Table 3); but as explained above, further division of the population would make any QTL discovery insignificant.

Prediction accuracy of marker scores associated with yield, yield components and physiological traits

Traits showing the best prediction accuracy according to marker scores, across all environments included TKW, DH, PH, yield, and GM2 showing R^2 between 0.26 and 0.40 (Table 6). Prediction accuracies of marker scores associated with CTvg, CTgf, and EGC were not significant. To find the best QTL model to predict yield, multiple regression was used (Table 6). Prediction accuracy of marker scores associated with yield alone had R^2 of 0.33 (Table 6). However, a better model was identified using marker additive effects and scores associated with yield, GM2, and DH together to predict yield with R^2 of 0.37 (Table 6). Furthermore, scores for the highest yielding genotype (SBS 21) as compared to the lowest yielding genotype (SBS 105) were used to confirm how well the scores in SBS 21 could predict its better performance (Supplementary Table 5), and around 49 % of the markers showed different alleles in the two genotypes.

Discussion

Phenotyping of the Seri/Babax population

In this study, useful phenotyping data have been generated for several heat- and drought-prone environments in Mexico, WANA, and South Asia. Based on genetic correlations in cluster analysis and site regression analysis for yield, the Sudanese environments were the most distinct from all other environments. These are particularly warm environments with short wheat cycles. Despite this segregation, it was still possible to find at least one genotype showing stable and high yields across most environments. This genotype showed a balanced combination of traits, but particularly, it showed decreased DH and PH and increased GM2 as compared to the average in the population. This confirms the main mechanisms and strategies underlying grain yield, associated with increased grain number (Peltonen-Sainio et al. 2007) and stress escape (Ludlow and Muchow 1990; Blum 2009).

Localization of QTL associated with phenological, agronomic and physiological traits

Phenology

Flowering time is an important trait for the adaptation of wheat to its target environments (Jung and Müller 2009). This trait is controlled by three major groups of genes, vernalization response genes (*Vrn*) on chromosome 5, photoperiod response genes (*Ppd*) on chromosome 2, and development rate genes ('earliness per se', *Eps*) (Snape et al. 2001; Mayfield et al. 2011). Besides vernalization, photoperiod sensitive varieties will not initiate floral primordial until the requirement for an extended period of long days is met and QTL for photoperiod sensitivity are located on the short arm of chromosome 2 (Snape 1998). Flowering per se genes act independently of environmental signals, and are usually responsible for fine-tuning flowering time (Flood and Halloran 1984). In the present study,

Table 6 Individual prediction accuracies of marker scores associated with each trait: yield (YLD), thousand kernel weight (TKW), grain number (GM2), days to maturity (DH), plant height (PH), canopy temperature at the vegetative and grain filling stages (CTvg and CTgf) and early ground cover (EGC)

Trait/Component	Equation	R^2
YLD	0.87 x + 451.3	**0.33**
TKW	0.67 x + 36.2	**0.26**
GM2	0.99 x + 12254	**0.40**
DH	0.76 x + 79.6	**0.28**
PH	0.94 x + 91.1	**0.31**
CTvg	0.68 x + 24.8	0.07
CTgf	0.54 x + 27.3	0.01
EGC	2.77 x + 41.3	0.03
MR	452.0 − 4.0DH + 0.007GM2 + 0.575YLD	**0.37**

Prediction accuracy is also shown for multiple regression (MR) using the best model (including GM2, DH and YLD). Regression equations (Equation) and R^2 are shown. Figures in bold were statistically significant at $p < 0.05$

we mapped QTL associated with DH using a spring wheat population, so vernalization is not expected to be the major factor causing the variation in phenology. Moreover, the parental lines Seri and Babax have been screened for known phenology alleles, and both parents have the photoperiod-insensitive allele at Ppd-D1, as well as spring-type alleles for at least two vernalization loci (Pinto et al. 2010). Hence, the population is characterized by its narrow range of flowering time, and most QTL are likely to be associated with earliness per se genes. In the study presented here while parents did not contrast for heading and maturity time, the progeny still showed on average across all environments a range of 9 days. This variation caused significant and consistent QTL for DH and DM on chromosomes 2B, 5D-b, and 7D-b. Moreover, QTL on chromosome 7D-b, accounted for more than 30 % of DH variation. These results confirm the complex nature of phenology and how difficult it can be to control these effects, when targeting the study of QTL for drought and heat areas without this confounding effect.

Plant height

Despite the fact that diagnostic markers have been identified for the dwarfing *Rht* genes in wheat (Ellis et al. 2002), it is generally accepted that PH in wheat is also a complex trait and its components include spike length and internode lengths (Cui et al. 2011; Wang et al. 2010; Mao et al. 2010). In the study presented here, the difference between the two parents Seri and Babax was of about 5 cm on average with Babax showing the highest values for PH across all environments. However, the whole population had a 17 cm range of variation for PH averaged across all environments. The most interesting QTL associated with PH (not showing QTLxE interactions) were identified on chromosomes 2B, 4B, and 5D, and these are promising markers that deserve further investigation. Other QTL were identified for PH on chromosomes 1D, 2B, 2D, 4A, 4D, 5A, and 7D-b. Seri and Babax do not segregate for any known *Rht* genes, and given their common pedigree, these two parental lines are likely to share the same *Rht* allele regardless (Pinto et al. 2010). Therefore, other genes for PH are yet to be found and studied having minor but significant effects, showing the complex genetic nature of PH (as for DH).

Yield

In recent decades, the widespread use of molecular markers has made it possible to study complex, quantitative traits in different crop species (Bernardo 2008). Recently, Pinto et al. (2010) identified QTL of several physiological and agronomic traits including common QTL for adaptation to both hot-irrigated and water stressed conditions. In this study, yield QTL were identified across all environments on chromosomes 4A, 4B, 5A, 6B, and 7D. Important QTL for yield and yield components have previously been associated with chromosome 4A in drought environments and other stressed environments (Kirigwi et al. 2007; Pinto et al. 2010; Liu et al. 2010). In this study, the QTL for yield on chromosome 4A was linked or pleiotropic with DH, and in some cases with DM and CTvg/CTgf in multi-trait (single environment) analysis. However, when the effect of marker 7D-acc/cat-10 (with major DH QTL effects) was removed, the same QTL on chromosome 4A was still identified for yield, but co-location with DH or DM disappeared. Co-location on chromosome 4A was only observed for CTgf and yield traits after removing the effects of marker 7D-acc/cat-10. These results indicate that, even if DH varied (range of 9 days) and significant negative genetic correlations between yield and DH were observed, some QTL could still be uniquely identified for yield and CTvg/CTgf in the Seri/Babax population. Moreover, the amount of variation accounting for CTvg/CTgf and yield QTL was slightly increased after removing the effects of marker 7D-acc/cat-10, which is in agreement with Reynolds and Turberosa (2008; see Introduction). While the influence of DH was removed from this yield QTL on chromosome 4A, the influence of PH could not be ruled out as shown by the identification of a QTL for PH across a wide range of environments in this same chromosome (significant in Darab, Sohag, Dongola, Karnal, and Ludhiana, but not in Mexico).

Thousand kernel weight and grain number

TKW has been a yield component for which breeders have made significant efforts to improve at CIMMYT (Lopes et al. 2012). Larger grains not only directly relate to grain yield, but also have favorable effects on seedling vigour and early growth and quality as well (Botwright et al. 2002; Wiersma et al. 2001). Large grain size has been an important trait selected during domestication and modern wheat breeding (Peng et al. 2003). In the study presented here, across most of the environments associations between TKW and yield were positive (both genetic and phenotypic). However, this association was not present in all environments, e.g., in Sudan, the association even trended to be negative; Sudanese environments are, however, an isolated example (not representative of most other environments where wheat is grown) with very short wheat cycles due to warm temperatures and where irrigation is applied very frequently to avoid periods of drought. Overall, contributions of TKW to yield became more important at higher levels of stress (e.g., heat plus drought environment in Mexico where the highest significant associations and TKW and yield QTL co-locations were observed).

Multi-environment (single trait) QTL analysis showed that the marker explaining the highest percentage of total variation accounted for TKW in most of the environments was located on chromosome 7D-b positioned at 12.5 cM (39 % of variation accounted for this QTL in at least one environment), but other QTL for TKW were also identified on chromosomes 5B, 6A-a, and 6D-a. Almost all QTL identified for TKW were linked or pleiotropic with DH and DM: (1) in the irrigated and drought environments, TKW was consistently linked or pleiotropic with DH on chromosome 7D-b and (2) in the heat and heat plus drought environments, co-location with phenology was consistently observed on chromosome 4B and 7D-b. The important link between phenology (DH and DM) and TKW, further confirms that in environments with terminal stress (during and after anthesis) early maturity would favor the post—anthesis grain growth periods resulting in increased grain size and yields.

TKW, GM2, and yield QTL were linked or pleiotropic on chromosome 4B only, but also dependent on phenology (co-location observed with DH and DM). Interestingly, we observed some QTL linked or pleiotropic to TKW and DH or DM, but far fewer co-locations were found between GM2 and phenology. A more frequent co-location of TKW and DH or DM as compared to GM2 would support more important contributions of phenology to define grain size than number with terminal drought or heat stress. Probably the importance of TKW under terminal stress and contributions to yield are mainly related with stress escape, whereas GM2 is defining yield with or without stress. Finally, the results presented here showed that GM2 and TKW were partially regulated by different genes (several QTL identified at different positions) suggesting room for improvement of both traits.

Pinto et al. (2010) identified QTL for TKW on chromosome 4B, but also strong effects were detected for this trait on chromosomes 3B-a and 4A-b, however, these authors used an early map version with no markers mapped on chromosome 7D (where important QTL were identified in the present study). Several studies have identified QTL at different positions and in different chromosomes for grain size (Nezhad et al. 2011; Sun et al. 2009, 2011). However, a lot of work is still necessary to confirm the role of important markers associated with TKW across different genetic backgrounds and environments. Association mapping has been applied previously to detect kernel size/milling quality genomic regions and these were identified on chromosomes 2D, 5A, and 5B in bread wheat (Breseghello and Sorrels 2006). In CIMMYT, a diverse panel of advanced spring wheat lines was developed for association genetics and evaluated in several environments for which analysis and results will soon be shared. In this association mapping, initiative GM2, TKW and mechanisms associated with phenological adjustment will be also addressed.

Canopy temperature

CTvg and CTgf shows a strong and reliable association with yield under drought and heat stress and is used in wheat breeding to select for yield (Olivares-Villegas et al. 2007; Reynolds et al. 2009; Saint-Pierre et al. 2010; Lopes and Reynolds 2010a, 2010b). This study confirmed this association under several environments, but heritability was moderate and this was probably associated with the fact that different cultivars have different mechanisms to adapt to particular environmental conditions (Liu et al. 2005; Mathews et al. 2008).

The work of Pinto et al. (2010) was the first in determining and locating QTL for CTvg/CTgf in wheat on chromosome 4A. In this study, QTL for CTvg and CTgf (across all environments and by multi-trait single environment) were found on chromosomes 4A, 6A-b, and 7D-b, with favorable effects from both parents. After removing the effects of a major QTL for phenology, interactive with CTvg and CTgf, on chromosome 7D-b, consistent QTL were identified on chromosome 4A particularly for the heat and heat plus drought environments in Mexico. An increased percentage of variation accounted for CTgf (the highest being 16 %), compared to the analysis that used all genotypes and alleles (up to 9 % variation). CTvg and CTgf are complex and integrative traits reflecting stress adaptation through several possible phenotypic and genetic

mechanisms: (1) a root system that can match evaporative demand at high vapour pressure deficit, (2) high intrinsic radiation use efficiency, and (3) photo-protective mechanisms that maintain radiation use efficiency and green area throughout the growth cycle (Reynolds et al. 2012). This complexity explains why QTL discovery for this trait is so difficult.

Early ground cover

Rapid development of leaf area and/or aboveground biomass has the potential to improve water harvest of rainfed wheat in Mediterranean-type environments, via reduced soil evaporation (Mullan and Reynolds 2010). Overall, differences in ground cover between Seri and Babax (Babax generally displays earlier ground cover than Seri) were nearly significant, but in some environments (particularly in Mexico late sowing, Iran-Darab, and India-Karnal) these differences were clear. Babax shows a more prostrate architecture with more ground cover than Seri. Heritability for this trait was, however, the lowest of all traits measured in all environments, but correlations with yield were positive and significant (but weak). In this study, very few QTL were detected to be associated with EGC, but the most robust QTL was identified on chromosome 7D-a and 7D-b; though for most environments the amount of variation accounting for EGC was very small, except for the Mex-Heat environment with 17.4 %. Moreover, multi-trait analysis showed a QTL on chromosome 1B co-located with yield and GM2, but only in the drought environment. Very few QTL have been identified for EGC in the literature, and this is probably related to the very low heritability observed for this trait.

Yield dissection into component traits

Ideally, each genotype is represented by a set of response parameters or traits valid in a large range of conditions, and can be deduced from the alleles present at specific QTL. Each trait included in the model to explain yield could be computed as the sum of QTL effects detected for that specific trait. QTL for some of these yield component traits were identified in the present study, and their prediction accuracies were calculated. QTL additive effects were particular accurate to detect variation in GM2, TKW, DH, PH, and yield. Using the additive effects of component traits including yield, DH, and GM2 in multiple regression, the prediction accuracy of the grain yield was improved by 5 % as compared to the simple model with yield alone. These results suggested that the probability of finding a high yielding genotype is improved if component traits are also included in the model. By selecting the positive alleles associated with yield and component traits, the chance of accumulating more favorable alleles increases.

Conclusions

This study identified several QTL associated with yield, yield components, and physiological traits in spring bread wheat, across a diverse set of wheat-growing environments. The study showed that in one bi-parental population, for traits showing high heritability, putative QTL could be identified across a diverse set of environments (including well irrigated, drought, heat, and heat plus drought). Moreover, some of the highly heritable traits such as DH, DM, TKW, and PH were still showing a complex nature, with several markers having small effects.

A stable genotype across several environments, based on the expression of yield per se, was identified within the progeny. The increases in yield across all environments for the highly stable genotype were mainly explained by earliness, increased GM2, and decreased PH, as compared to the average observed for the whole population (despite the narrow variation of these traits in this particular population). TKW, EGC, CTvg, and CTgf in the stable genotype were similar to the parents and progeny average. If the former traits were to be combined in the stable, early, and short genotype, yields would probably increase even further.

A major QTL for DH was identified on chromosome 7D-b, which was linked or pleiotropic with almost all other traits. This marker was used to carry out QTL detection in subsets of lines contrasting for allelic expression of this major DH QTL. In these subsets, chromosome 4A was associated with grain yield, GM2, CTvg, and CTgf, and not linked or pleiotropic with phenology. However, the PH effects on the 4A QTL could not be ruled out.

Contributions of TKW to yield became more important in heat and heat plus drought environments. TKW QTL were linked or pleiotropic to DH, even when the effect of major QTL for DH was removed (on chromosome 7D-b). The important link between phenology (DH and DM) and TKW further suggested that in environments with terminal stress (during and after anthesis), early maturity would favor the post—anthesis grain growth periods resulting in increased grain size and yields. GM2 and TKW were partially regulated by different genes suggesting room for improvement of both traits.

Prediction accuracy of markers for grain yield was improved if marker scores and additive effects of yield components were used in multiple regression; markers associated with GM2, DH, and yield, were the traits generating the best prediction accuracy of yield.

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