

Genomic characterization of drought tolerance-related traits in spring wheat

Sundeep Kumar · Sunish Kumar Sehgal ·
Uttam Kumar · P. V. Vara Prasad ·
Arun Kumar Joshi · Bikram Singh Gill

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Abstract Drought tolerance was investigated in ‘C306’, one of the most drought tolerant wheat cultivars bred in India in the 1960’s. An intervarietal mapping population of recombinant inbred lines of the cross ‘C306’ × ‘HUW206’ was evaluated for drought tolerance components, namely potential quantum efficiency of photosystem (PS) II (F_v/F_m), chlorophyll content (Chl), flag leaf temperature (Lt), and grain yield per plant (Gyp) under stress. Three independent experiments were conducted under well-watered and water-stressed conditions in greenhouses and growth chambers at Kansas State University (USA). Five hundred and sixty microsatellite markers covering the entire genome were screened for polymorphism between the parents. A QTL (*QLt.ksu-1D*) for Lt (low flag leaf temperature

under stress) on the short arm of chromosome 1D between markers *Xbarc271* and *Xgwm337* at LOD 3.5 explained 37% of the phenotypic variation. A QTL for F_v/F_m (*QF_v/F_m.ksu-3B*) and Chl (*QChl.ksu-3B*) controlling quantum efficiency of PS II and chlorophyll content under stress were co-localized on chromosome 3B in the marker interval *Xbarc68–Xbarc101* and explained 35–40% of the phenotypic variation for each trait. A QTL (*QGyp.ksu-4A*) for Gyp on chromosome 4A at a LOD value of 3.2 explained 16.3% of the phenotypic variation. Inconsistent QTLs were observed for F_v/F_m on chromosomes 3A, 6A, 2B, 4B, and 4D; for Chl on 3A, 6A, 2B and 4B; and for Lt on 1A, 3A 6A, 3B and 5B. The identified QTLs give a first glimpse of the genetics of drought tolerance in C306 and need to be

S. Kumar · S. K. Sehgal · B. S. Gill
Wheat Genetic and Genomic Resources Center,
Department of Plant Pathology, Kansas State University,
Manhattan, KS 66506, USA

S. Kumar
NRC on DNA Fingerprinting, National Bureau of Plant
Genetic Resources, Pusa Campus, New Delhi 110012,
India

U. Kumar
The Energy and Resources Institute (TERI), IHC
Complex, Lodhi Road, New Delhi 110003, India

P. V. V. Prasad
Department of Agronomy, Kansas State University,
Manhattan, KS 66506, USA

A. K. Joshi (✉)
CIMMYT South Asia, Singha Durbar Road, Kathmandu,
Nepal
e-mail: a.k.joshi@cgiar.org; joshi_vns@yahoo.co.in

A. K. Joshi
Genetics and Plant Breeding, Institute of Agricultural
Sciences, Banaras Hindu University, Varanasi, India

B. S. Gill
Department of Biological Sciences, Faculty of Science,
Genomics and Biotechnology Section, King Abdulaziz
University, Jeddah 21589, Saudi Arabia

validated in field experiments using the marker-phenotype linkages reported here.

Keywords Chlorophyll content · Chlorophyll fluorescence · Leaf temperature · *Triticum aestivum* · QTL

Introduction

Soil water deficit is a chronic limitation to wheat productivity and drought has become one of the main environmental threats to grain production in many regions, especially the arid and semiarid regions of the world (Chaves et al. 2003). Changing weather patterns indicate that even irrigated wheat will have to be produced with less applied water. Irrigated wheat in most parts of India is already cultivated under partial irrigation, receiving 1–2 irrigations over a cropping season (Joshi et al. 2007a). Water will become a limiting factor for sustained production of wheat and other crops, and this includes the water-rich Indo-Gangetic Plains (Joshi et al. 2007b). Therefore, breeding for enhanced drought tolerance is essential for achieving improved crop productivity and greater food security for the hundreds of millions of rural poor (Ortiz et al. 2008).

Abiotic stress reactions, especially to water deficits, are complex morphological and physiological phenomena in plants (Nachit et al. 1998). Numerous traits identified as being responsible for drought tolerance are heritable, additive in nature, and display continuous variation; this is an indication that there is considerable room for improvement in drought tolerance (Tuberosa and Salvi 2006). However, progress in breeding drought-tolerant wheat varieties has been slow. For instance, C306, a variety released in 1969, is still considered the most dependable wheat variety under drought and is used as the standard check in trials throughout India (Joshi et al. 2007a). Molecular approaches may need to be investigated in order to breed superior drought tolerant varieties. The first molecular approaches to assist breeders in their efforts to increase drought tolerance were with molecular markers, genomics and “post-genomics strategies” (Nguyen et al. 2004; Lanceras et al. 2004).

Molecular markers for traits that contribute to drought tolerance can be used in molecular assisted

breeding. Traits that could be selected more efficiently include photosystem (PS) II (F_v/F_m) efficiency (Jiang et al. 2004; Blum and Ebercon 1981), chlorophyll content (Chl) (Li et al. 2006), and leaf temperature (Lt) (Inagaki and Nachit 2008). However, much more information is required for a better understanding of the genomic regions in wheat that contribute to variability in such traits. Dissection of quantitative traits into their genetic components, or QTLs, provides direct access to genetic diversity for important physiological processes that regulate adaptive response to drought. This allows scientists/breeders to use molecular markers in marker-assisted selection (MAS) for enhancing plant performance under drought stress conditions. Despite significant progress in molecular techniques and identification of QTLs, the overall impact of MAS on the production and release of drought-tolerant cultivars has not been significant (Tuberosa and Salvi 2006; Ortiz et al. 2008). However, few studies have been conducted to identify QTLs, particularly for F_v/F_m , Chl, and Lt under drought stress. The present study focuses on identifying QTLs for these traits under drought stress conditions.

Materials and methods

Plant materials

A total of 104 single-seed descent (SSD) derived recombinant inbred lines (RILs; F_8 generation) from the cross ‘C306’ × ‘HUW206’ were evaluated in greenhouses and growth chambers for F_v/F_m , Chl, Lt, and grain yield per plant (Gyp). Cultivar ‘C306’ (RGN/CSK3//2*C591/3/C217/N14//C281) of Indian origin is still one of the best sources of drought tolerance, whereas ‘HUW206’ (KAVKAZ/BUHO//KALYANSONA/BLUE BIRD) is a Veery (originally developed at CIMMYT, Mexico) selection released for irrigated conditions by Banaras Hindu University, Varanasi.

Planting of RILs in greenhouses and growth chambers

The RILs were evaluated in three independent experiments under controlled conditions at Kansas State University during 2008–2009. Experiments 1 and 2 were conducted in a greenhouse and Experiment 3 in a

growth chamber. Each experiment comprised two environmental sets, with each set containing 3 pots of 20 cm², which were used as replications. There were five plants of each line in each pot. One set in each experiment was evaluated under well-watered conditions, and the other set was evaluated under water-stressed conditions.

Water-stressed conditions were created at early anthesis (GS63, Zadoks et al. 1974). The same level of moisture was maintained in all pots of both sets, until GS63, by supplying water at 100 ml per day to each pot. The level of moisture in each pot was confirmed using a soil moisture probe (Type ML2X attached to a HH2 Moisture Meter, Delta-T Devices, Burwell, UK). Readings were converted from microvolts to volumetric soil moisture content (h), based on a two-point calibration (field capacity and oven-dried soil) with the same potting soil. Normal agronomic practices, including fertilization and the use of insecticides and fungicides, were applied to both sets. After GS63, the water supply to the ‘water-stressed’ set was stopped whereas it was maintained for the plants grown under well-watered conditions. Other factors, such as 25 °C day/18 °C night temperature and 16 h light/8 h dark regimes, remained constant for both sets of plants following Yang et al. (2002).

Physiological characterization

The physiological traits (F_v/F_m , Chl, and Lt) were measured on fully expanded flag leaves of three tagged plants in each pot in the well-watered and water-stressed sets. The first observations were recorded on the final day of irrigation (i.e. early anthesis; GS63). The same observations were recorded on both sets of plants on alternate days for the next 10 days. QTL analysis was conducted using the data recorded following 6 days of water stress, earlier determined to be the critical stage for distinguishing between drought-tolerant and susceptible cultivars (Tambussi et al. 2000). Chlorophyll fluorescence values were recorded using clips, which created dark conditions for at least 30 min before measurements were taken using a pulse modular fluorometer (Model OS5-FL, Opto-Sciences, Hudson, NH, USA). A SPAD-502 chlorophyll meter (Spectrum Technologies, Plainfield, IL, USA) was used to measure chlorophyll content by inserting the flag leaf into the sample slot of the measuring head and automatically storing the values

in the internal memory. Leaf temperature was recorded using a thermal imaging camera (FLIR Systems Inc., Wilsonville, OR, USA), which renders infrared radiation as visible light (Sirault et al. 2009). Drought tolerance index (DTI) was estimated by:

$$DTI = [(1 - GY_{\text{drought stress}}/GY_{\text{control}})/D]$$

where, $GY_{\text{drought stress}}$ is the grain yield in droughted conditions, GY_{control} is the grain yield in normal conditions,

$$D(\text{stress intensity}) = (1 - X_{\text{drought stress}}/X_{\text{control}})$$

where, $X_{\text{drought stress}}$ is the mean of $GY_{\text{drought stress}}$ of all RILs, X_{control} is the mean of GY_{control} of all RILs.

Microsatellite analysis

DNA was extracted from all RILs and the two parents by the CTAB method (Saghai-Marooif et al. 1984). A total of 560 microsatellite primers from the Beltsville Agriculture Research Centre (BARC), Gatersleben Wheat Microsatellite (GWM), Wheat Microsatellite Consortium (WMC), CFA, and GDM sets were used to screen the parents for polymorphism. The RIL population was genotyped with 141 polymorphic microsatellite markers. DNA amplification was carried out in a 96-well thermocycler (Applied Biosystems, Foster City, CA, USA) as described by Röder et al. (1998). The following PCR profile was used: initial denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 94 °C for 1 min, annealing at 50–61 °C (depending on information provided in Röder et al. 1998; Somers et al. 2004) for 1 min, polymerization at 72 °C for 2 min with a final extension step of 10 min at 72 °C. Microsatellite fragments were detected on high-resolution, low-melting agarose gels (4.5%). Fragments were compared using a 100-bp ladder.

Map construction and QTL detection

The reference International Triticeae Mapping Initiative (ITMI) map was used to select approximately 20 microsatellite markers from each chromosome to cover the entire genome (Röder et al. 1998; Ganai and Röder 2007). Microsatellite primers, polymorphic between ‘C306’ and ‘HUW206’, were used to screen

72 (36 most tolerant and 36 most susceptible) RILs of the population for initial analysis.

Mapmaker v2.0 (Lander et al. 1987) was used to develop a framework map for initial QTL detection. Later, more polymorphic markers were added to enrich the neighboring regions of markers found significant in the initial analysis. Finally, Mapmaker v2.0 was again used to create a linkage map of QTL regions on chromosomes 1A, 1D, 2B, 3A, 3B, 3D, 4A, 4B, 4D, 5B, and 6D. Chi-squared tests were performed to test the segregation of markers for 1:1 segregation ratios. To assign markers to chromosomes, the ‘default linkage criteria’ was set with a LOD of 3 and a maximum recombination distance of 50 cM (Kosambi units) between markers. The initial anchored markers were determined on the basis of published consensus maps (Somers et al. 2004; Ganal and Röder 2007) and subsequently, other markers were assigned to linkage groups. These groups were then ordered and validated with the ‘order’ and ‘ripple’ commands. The best order of markers was designated as the framework for each linkage group. QTL analysis was performed using QTL Cartographer version 2.0 (Wang et al. 2007) following Kumar et al. (2009, 2010). Initially, single marker regression analysis was performed to test each marker for significance of drought stress tolerance based on the 36 most tolerant and 36 most susceptible lines. When a marker was significant ($P < 0.001$), all RILs were genotyped and the QTL was confirmed by interval mapping (IM) and composite interval mapping (CIM). QTLs were considered to have a significant effect when the LOD threshold was 2.5 or higher. Adjusted mean values for F_v/F_m , Chl, Lt, and Gyp were calculated before pooling the data from all three independent experiments. The names of the QTLs were assigned according to the International Rules for

Genetic Nomenclature (IRGN) (<http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm>).

Statistical analysis

All statistical analyses were conducted using SAS version 8.2 (SAS Inst. Inc., Cary, NC 1988). Analyses of variance (ANOVA) for all three experiments were performed using PROC GLM of SAS. ANOVA and least square means of traits were estimated using the statistical procedure PROC MIXED. The average from the three replications of each set of experiments was used and % decline was calculated by comparing the average of the water-stressed and well-watered sets. Correlations were performed using Pearson’s correlation in the statistical procedure PROC CORR. Narrow sense heritabilities (h^2) were estimated from the analyses of variance following Nyquist (1991):

$$h^2 = 1 - [\text{MS}(\text{Genotype} \times \text{Environment})] / \text{MS}(\text{Genotype}).$$

Results

Analyses of variance of morpho-physiological traits

Significant differences in F_v/F_m , Chl, Lt, and Gyp were observed between the drought tolerant ‘C306’, the susceptible cultivar ‘HUW206’ (Table 1). The ANOVA carried out on 104 RILs revealed significant differences in F_v/F_m , Chl, Lt and Gyp evaluated under the water-stressed conditions indicating transgressive segregation for all traits (Table 2). The traits associated with drought tolerance assessed in this study appeared to be quantitatively inherited as shown by the nearly continuous distribution of 104 RILs derived from ‘C306’ \times ‘HUW206’ (Fig. 1).

Table 1 Mean values of chlorophyll fluorescence (F_v/F_m), chlorophyll content (Chl), leaf temperature (Lt) and grain yield per plant (Gyp) of parents and 104 RILs derived from ‘C306’ \times ‘HUW206’ under water-stressed conditions

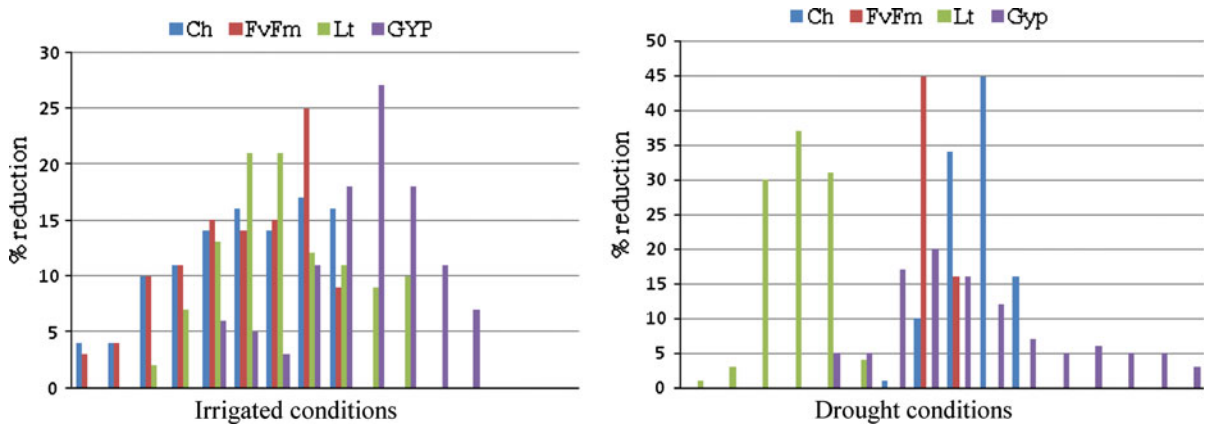
Parent/RIL	Trait			
	F_v/F_m	Chl	Lt	Gyp
C306	0.81 (0.79–0.82)	45.70 (44.1–47.6)	22.37 (22.2–22.4)	1.40 (1.36–1.44)
HUW 206	0.25 (0.24–0.26)	15.97 (15.83–16.14)	28.67 (28.41–28.94)	0.54 (0.46–0.65)
RIL	0.59 (0.16–0.84)	37.16 (13.72–62.33)	26.84 (20.93–34.27)	1.08 (0.38–1.84)
LSD $P = 0.05$	0.008	1.33	0.55	0.08

Values in parentheses indicate range for each trait

Table 2 Mean squares for chlorophyll fluorescence (F_v/F_m), chlorophyll content (Chl), leaf temperature (Lt) and grain yield per plant (Gyp) in the parents and 104 recombinant inbred lines(RILs; F_8 generation) derived from ‘C306’ × ‘HUW206’ under controlled conditions

Source of variation	d.f.	F_v/F_m	Chl	Lt	Gyp
Treatment (optimum vs. stress)	1	7.407***	21414.76***	1692.789***	86.565***
Replicates	4	0.00009 NS	2.44 NS	0.425 NS	0.0036 NS
RILs	105	0.07018***	285.99***	9.272***	1.2922***
Irrigation*RIL	105	0.06827***	281.11***	8.187***	0.4029**
Error	420	0.00006	1.39	0.240	0.0052
Heritability		0.41	0.40	0.42	0.68

d.f. degrees of freedom, NS not significant

, * Significant at $\alpha = 0.01$ and 0.001, respectively**Fig. 1** Distributions of percentage reductions in chlorophyll fluorescence (F_v/F_m), chlorophyll content (Chl), leaf temperature (Lt), and grain yield per plant (Gyp) averaged separately

under well-watered and water-stressed conditions over three experiments for 104 RILs derived from ‘C306’ (drought tolerant) × ‘HUW206’ (drought susceptible)

Correlation among traits

Significant and positive correlations ($\alpha = 0.001$ and 0.01) were observed between F_v/F_m and Chl (0.967, $\alpha = 0.01$ with DTI and 0.955, $\alpha = 0.01$ without DTI), F_v/F_m and Gyp (0.538, $\alpha = 0.01$ with DTI and 0.551, $\alpha = 0.01$ without DTI) and between Chl and Gyp (0.534, $\alpha = 0.01$ with DTI and 0.527, $\alpha = 0.01$ without DTI), indicating that increased F_v/F_m and Chl increases Gyp (Table 3). Moderate but negative correlation coefficients were observed between Lt and F_v/F_m (-0.630 , $\alpha = 0.01$ with DTI and -0.648 , $\alpha = 0.01$ without DTI), Lt and Chl (-0.620 , $\alpha = 0.01$ with DTI and -0.640 , $\alpha = 0.01$ without DTI) and Lt and Gyp (-0.284 , $\alpha = 0.01$ with DTI and -0.400 , $\alpha = 0.01$ without DTI). Heritabilities were low to moderate for F_v/F_m (0.4063), Chl (0.4025) and Lt (0.4214), and moderate to high for Gyp (0.6795).

QTL detection and mapping

Molecular marker map and genetic characterization of yield traits

To identify polymorphism between ‘C306’ and ‘HUW206’ 560 microsatellite markers were tested. Of these, 141 (25.2%) SSR markers were polymorphic and were scored on the RIL population. About 2.1% of loci were heterozygous, presumably representing unfixated loci in the RILs. All except 14 marker loci segregated in the expected 1:1 ratios ($P < 0.05$).

Three major QTLs (*Q_{Lt.ksu-1D}*, *Q_{F_v/F_m.ksu-3B}* and *Q_{Chl.ksu-3B}*) were detected for the traits associated with drought tolerance (i.e. Lt, F_v/F_m , and Chl) and were consistent over several experiments. Another QTL (*Q_{Gyp.ksu-4A}*) from grain yield under water stress was identified from data averaged over all

Table 3 Pearson's correlation coefficients between chlorophyll fluorescence (F_v/F_m), chlorophyll content (Chl), leaf temperature (Lt), and grain yield per plant (Gyp) under water-stressed and well-watered conditions and using a drought tolerance index (DTI)

Traits	F_v/F_m	Chl	Lt
Water-stressed conditions			
Chl	0.955***		
Lt	-0.648**	-0.640**	
Gyp	0.551**	0.527**	-0.400**
Well-watered conditions			
Chl	-0.091		
Lt	0.001	0.059	
Gyp	0.083	-0.308**	-0.032
DTI			
Chl	0.967***		
Lt	-0.630**	-0.620**	
Gyp	0.538**	0.534**	-0.284**

, * Significant at $\alpha = 0.01$ and 0.001 , respectively

experiments. In addition to these 4 major QTLs another 14 QTLs were detected in only one experiment or the mean of all experiments. The 18 QTL detected by CIM were distributed on 10 chromosomes and explained phenotypic variances (R^2) ranging from 13.3 to 56.1% with LOD values ranging from 2.5 to 8.0 (Table 4).

A robust QTL ($QF_v/F_m.ksu-3B$) for F_v/F_m on chromosome 3B, detected in the first and second experiments as well as the overall mean, was flanked by markers *Xbarc68* and *Xbarc101* and explained 35.1% of the mean phenotypic variation with a LOD value of 6.3 (Table 4; Fig. 2). Other QTLs for F_v/F_m were inconsistent as they were observed only in single experiments. Four QTLs were identified for F_v/F_m in Experiment 1 on chromosomes 2B, 3A, 3B and 4D, one on chromosome 4B in Experiment 2, and one in the interval *Xgwm427–Xgwm169* (15 cM) on 6A in the overall mean (Table 4). No QTL for F_v/F_m was detected in Experiment 3.

Four genomic regions had significant effects on Chl in individual experiments and an additional two genomic regions were detected when using means. Notably, *QChl.ksu-3B* was detected in the same interval as $QF_v/F_m.ksu-3B$ (Fig. 2). *QChl.ksu-3B* explained 40% of the mean phenotypic variation with a LOD score of 7.0 (Table 4; Fig. 2). Inconsistent QTLs for Chl were detected on chromosomes 3A, 6A,

2B and 4B. Although detected only in Experiment 3, *QChl.ksu-2B* flanked by markers *Xgwm356* and *Xwmc154* (26 cM) (Table 4) explained 50% of the phenotypic variation.

The most consistent QTL (*QLt.ksu-1D*) for Lt on chromosome 1DS between *Xbarc271* and *Xgwm337* was detected in all experiments and the overall mean (Table 4; Fig. 2). Inconsistent QTLs for Lt were identified on chromosomes 1A, 3A, 6A, 3B and 5B. *QLt.ksu-3A* was flanked by the same markers, *Xbarc12* and *Xgwm369*, as F_v/F_m and Chl. *QLt.ksu-6A* was detected in Experiment 1 and the overall mean (Fig. 2). It is notable that QTLs $QF_v/F_m.ksu-3B$ *QChl.ksu-3B* and *QLt.ksu-3B* were flanked by the same markers (*Xbarc68–Xbarc101*). The QTL LOD curves of significant chromosome regions are shown in Fig. 2.

The only QTL (*QGyp.ksu-4A*) identified for Gyp was on chromosome 4A detected in mean data with a LOD value of 3.2 and explaining 16.3% of the phenotypic variation (Fig. 2). *QGyp.ksu-4A* was flanked by the markers *Xwmc161* and *Xwmc313* in an interval of 7.0 cM. The contributions of each allele to the physiological traits associated with drought tolerance, and standard errors are presented in Table 5.

The QTL \times QTL and QTL \times environment interactions were calculated using QTL Mapper. Of the 21 maximum possible QTL \times QTL interactions for F_v/F_m , four had significant epistatic interactions effects with other QTLs (Table 4). Similarly, of the maximum 15 possible combinations of QTL \times QTL interactions, three combinations for Chl, and three combinations for Lt displayed significant and positive epistatic effects. The QTL \times QTL \times environment interactions were not significant.

Discussion

QTL mapping was employed in the present study to dissect the relationship between physiological traits and drought stress using a set of 104 intervarietal RILs. Four different parameters, viz. chlorophyll fluorescence (F_v/F_m) (Krause and Weis 1991), chlorophyll content (Chl) (Nikolaeva et al. 2010; Pradhan et al. 2012a), leaf temperature (Lt) (Blum et al. 1989), and grain yield (Gyp) reduction under drought (Kumar et al. 2007) were used as measures of drought

Table 4 Effect of QTLs, QTL × QTL and QTL × Environment interactions for chlorophyll fluorescence (F_v/F_m), chlorophyll content (Chl), and leaf temperature (L_t) in the ‘C306’ × ‘HUW206’ RIL population detected by composite interval mapping

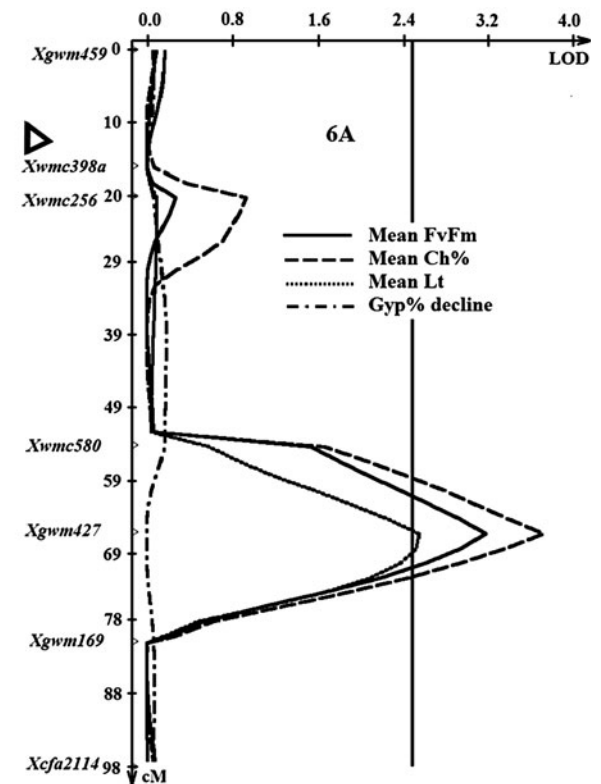
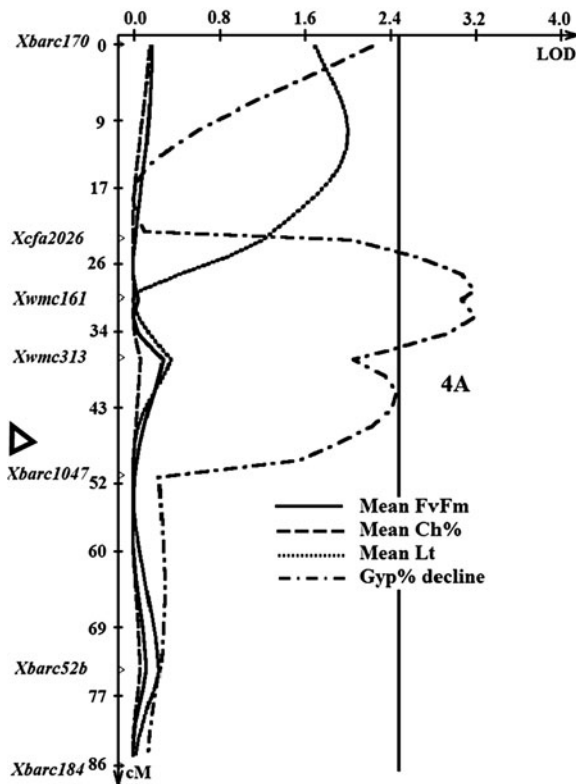
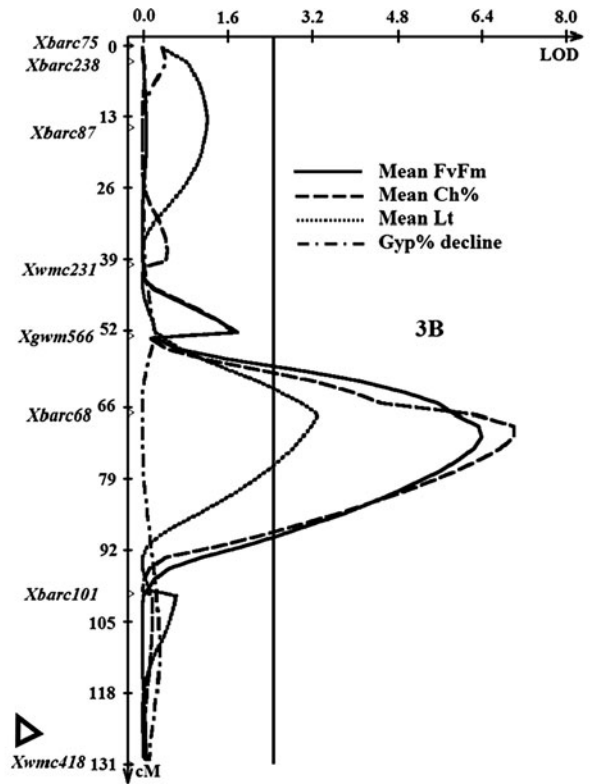
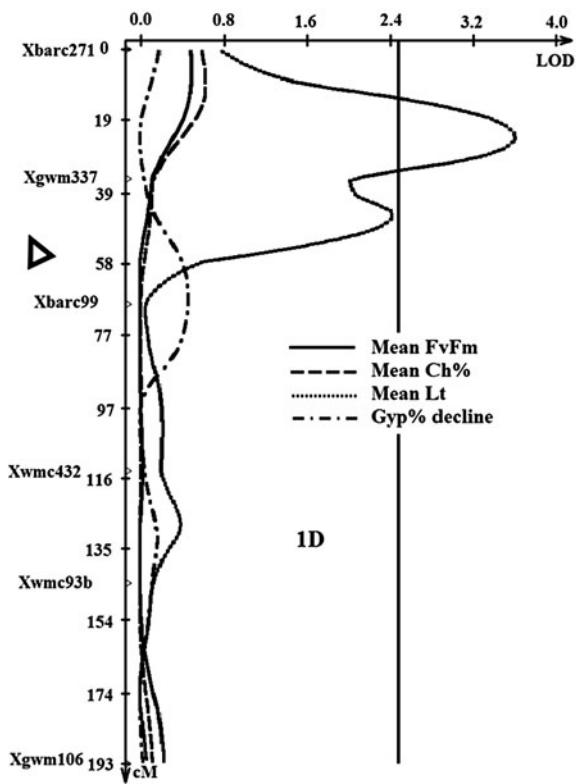
QTL	Marker interval	Interval (cM)	F _v /F _m						Chl						L _t											
			Exp1		Exp2		Exp3		Mean		Exp1		Exp2		Exp3		Mean		Exp1		Exp2		Exp3		Mean	
			LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²	LOD	R ²
QF _v /F _m -ksu-2B	Xcfa175-Xgwm388	33	4.3	46.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QChl-ksu-2B	Xgwm356-Xcfa154	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QL-ksu-1A	Xbarc176-Xcfa2153	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QL-ksu-1D	Xbarc271-Xgwm337	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QF _v /F _m -ksu-3A	Xbarc12-Xgwm369	17	2.8	20.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QChl-ksu-3A	Xbarc12-Xgwm369	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QL-ksu-3A	Xbarc12-Xgwm369	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QF _v /F _m -ksu-3B	Xbarc68-Xbarc101	33	3.5	16.2	8	56.1	-	-	6.3	35.1 ^a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QChl-ksu-3B	Xbarc68-Xbarc101	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QL-ksu-3B	Xbarc68-Xbarc101	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QF _v /F _m -ksu-4B	Xcfa349-Xcfa2149	44	-	-	3.3	39.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QChl-ksu-4B	Xcfa349-Xcfa2149	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QF _v /F _m -ksu-4D	Xcfa285-Xgwm265	48	3.5	46.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QL-ksu-5B	Xgwm540-Xgwm159	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QF _v /F _m -ksu-6A	Xgwm427-Xgwm169	15	-	-	2.9	16.1	-	-	3.2	13.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QChl-ksu-6A	Xgwm427-Xgwm169	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QL-ksu-6A	Xgwm427-Xgwm169	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total R ² by joint analysis			4.6	57.2	5.4	18.5	-	-	7.5	47.6	3.4	14.7	6	19.5	5.3	58.4	7.3	46.6	6.1	57.2	5.9	56.6	3.3	31.2	6.1	57.1
QxQ interactions																										
2B × 4D			7.4**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1A × 3A			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1A × 1D			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3A × 4A			4.4**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1D × 6A			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3B × 3D			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3B × 4B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3B × 4D			-	-	6.1**	-	-	-	-	-	-	-	-	5.8**	-	-	-	-	-	-	-	-	-	-	-	-
4D × 5B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The marker intervals cited are those flanking the peak of the LOD scan. R² represents the percentage of phenotypic variance explained for each QTL; significant QTL × QTL are also presented

LOD likelihood of odds ratio

^a QTL × environment interaction (Q × E)

** Significant at P = 0.05



◀ **Fig. 2** LOD curves obtained by composite interval mapping for quantitative trait loci (QTL) mapped on chromosomes 1D, 3B, 4A, and 6A for stress-tolerance parameters in the ‘C306’ × ‘HUW206’ recombinant inbred population. The vertical line indicates the threshold LOD 2.5 value indicating QTLs. Short arms of chromosomes are toward the top and open triangles indicate the approximate positions of centromeres

tolerance. The quantum efficiency of PS II, F_v/F_m , plays a vital role in photosynthesis, and can be used as screening tool for drought tolerance (Blum and Ebercon 1981; Prasad et al. 2007). A low F_v/F_m ratio indicates low photosynthetic efficiency, thus, genotypes with higher F_v/F_m values under stress conditions may be more tolerant to drought stress. High temperature stress or higher leaf temperatures decrease leaf chlorophyll content (Ristic et al. 2007; Pradhan et al. 2012b) leading to reduced photosynthesis. Drought susceptible genotypes, which are those with higher yield losses under drought stress, also tended to have higher canopy temperatures (Blum et al. 1989). Havaux and Tardy (1999) observed that chlorophyll loss was also associated with decreased numbers of photosystems in the thylakoid membranes. Overall, drought tolerant genotypes tend to have higher photosynthetic efficiency (greater F_v/F_m), higher

chlorophyll content (higher SPAD readings) and lower canopy temperatures under drought stress conditions. The role of F_v/F_m , Chl, and Lt in relation to Gyp under water-stressed conditions is well recognized in the selection of drought and heat tolerant wheat plants (Blum 1988; Blum et al. 1989; Krause and Weis 1991).

The RILs were evaluated in three independent experiments under controlled greenhouse or growth chamber conditions. Planting of seed in three independent experiments enabled the study of genotype × environment interactions as each experiment served as an environment. The data for physiological traits at different time points, viz. 0, 2, 4, 6, 8, and 10 days under water stress, gave an accurate evaluation of the population for drought tolerance. Although genotype × environment interactions for drought stress were significant, the presence of significant correlations between F_v/F_m , Chl, and Gyp and Chl and Gyp based on a DTI (Table 3) reflects the accuracy and reproducibility of these experimental conditions and the scoring method used for drought stress evaluation.

Five hundred and sixty microsatellite markers providing whole-genome coverage were tested and 25.1% were polymorphic. The order and orientation of

Table 5 Relative contribution of alleles to the phenotypic values of chlorophyll fluorescence (F_v/F_m), chlorophyll content (Chl), and leaf temperature (Lt)

Chromosome	Marker	Trait	Allele A ^a	SE of allele A	Allele B	SE of allele B
1A	<i>Xbarc176</i>	Lt	22.3	±1.3	17.5	±1.4
1D	<i>Xgwm337</i>	Lt	14.4	±1.7	23.2	±1.8
2B	<i>Xgwm388</i>	F_v/F_m	36.0	±5.1	23.4	±4.1
		Chl	30.7	±5.9	23.2	±3.5
3A	<i>Xgwm369</i>	F_v/F_m	38.5	±5.9	22.8	±5.5
		Chl	32.3	±4.9	20.7	±3.5
		Lt	21.4	±1.9	18.3	±2.2
3B	<i>Xbarc68</i>	F_v/F_m	41.5	±5.7	22.0	±3.2
		Chl	35.1	±4.6	19.7	±2.7
		Lt	23.3	±1.6	17.6	±1.2
3D	<i>Xgwm3</i>	F_v/F_m	35.5	±4.5	26.3	±4.7
		Chl	30.9	±3.8	22.6	±3.7
4B	<i>Xcfa2149</i>	F_v/F_m	38.1	±8.2	26.2	±3.2
		Chl	32.9	±6.6	22.9	±2.6
4D	<i>Xgwm265</i>	Gyp	26.7	±3.9	30.5	±4.7
5B	<i>Xgwm159</i>	Lt	20.0	±1.1	18.1	±2.0
6A	<i>Xgwm427</i>	F_v/F_m	31.2	±3.7	28.8	±6.1
		Chl	26.9	±3.1	18.3	±4.2
		Lt	20.1	±1.2	17.4	±2.1

^a Allele A and B are the mean phenotypic values for alleles derived from the tolerant (‘C306’) and susceptible (‘HUW206’) parents, respectively

the mapped microsatellite markers were in agreement with those in the ITMI map (Ganal and Röder 2007). Some loci (14) showed segregation distortion; these were randomly distributed throughout the genome, and as the linkage map was not affected we included them in the linkage map.

Separate QTL analyses were performed for each experiment and for mean values from all three experiments. Tambussi et al. (2000) suggested that measurements of drought tolerance were most reliable on the sixth day after cessation of irrigation and we adopted the same time point. QTLs for drought tolerance-related traits F_v/F_m (6 QTL), Chl (6), Lt (5) and Gyp (1) were identified and mapped. The six QTLs for F_v/F_m , which is related to drought tolerance of photosynthesis, identified on chromosomes 3A, 6A, 2B, 3B, 4B and 4D showed significant positive correlations to DTI; $QF_v/F_m.ksu-3B$ was the most stable explaining up to 56.1% of the mean phenotypic variation. QTLs for F_v/F_m were earlier reported on chromosomes 1B, 2A and 4A (Czyczyło-Mysza et al. 2011). No QTL for F_v/F_m was detected in Experiment 3. This indicated that compared to other traits, F_v/F_m could be more sensitive to environmental conditions. Previous findings also suggest that F_v/F_m is influenced by environmental conditions (Pruvot et al. 1996).

Leaf chlorophyll content determines capacity to absorb sunlight and is thus a measure of photosynthetic potential. A major QTL ($QChl.ksu-3B$) for chlorophyll content was identified on chromosome 3B. In addition there were four other QTLs ($QChl.ksu-2B$, $QChl.ksu-3A$, $QChl.ksu-4B$, and $QChl.ksu-6A$). QTLs for Chl were identified in durum and wild emmer on chromosomes 1A, 4A, 5A, 6A, 7A, 1B, 2B and 5B (Peleg et al. 2009). The ability of a genotype to stay green (high chlorophyll content) under drought stress could help in efficient assimilation leading to increased grain filling and enhanced productivity.

$QLt.ksu-1D$ detected on chromosome 1D was the most stable of six QTLs for Lt. At all loci cooler leaf temperatures were associated with lower DTI. The benefits of cooler canopy are well documented (Blum et al. 1989). $QLt.ksu-1D$ could be introgressed into different genetic backgrounds through MAS to develop genotypes with enhanced drought tolerance. A major QTL on chromosome 3B for canopy temperature suppression was identified in a Kukri/RCA875 mapping population (Fleury et al. 2010). The relationship of this QTL to $QLt.ksu-3B$ identified

in the present study remains to be determined. The co-localization of QTLs for different parameters on chromosomes 3A, 3B, and 6A suggests the importance of these genomic regions in drought tolerance and indicates the reliability of experiments for QTL mapping. These QTLs could also be termed as multi-trait QTLs although further work with saturated maps and precise mapping on further segregating populations is needed to determine the extent to which co-localization is due to pleiotropy or tightly linked loci. However co-localization would be simpler for MAS for drought tolerance (Zaharieva et al. 2001). Co-location of QTLs for these traits was also reported by Peleg et al. (2009), McIntyre et al. (2010) and Czyczyło-Mysza et al. (2011).

Although there were gaps in some chromosome regions it is unlikely that major QTLs escaped detection because the identified QTLs explained large parts of the phenotypic variances, ranging from 31.2% (Experiment 3) to 57.2% (Experiment 1) for Lt in the joint analysis. Similarly, up to 57.2% of the phenotypic variation was explained jointly by QTLs identified for F_v/F_m (Experiment 1) and 58.4% of variation was explained by the QTL for Chl (Experiment 3). Some minor QTLs for these traits were possibly not detected due to their relatively minor effects. Additionally, the presence of small but undetectable epistatic interactions cannot be ruled out. Epistatic interactions are relatively difficult to identify compared to single-marker effects as they involve linkage of markers and QTLs at more than one locus. Associations of QTLs with markers could remain undetected if the marker linkages are not tight enough. The D genome chromosomes in the present population were less saturated with molecular markers than the A and B genomes, and they may contain gaps of more than 30 cM. Therefore, it is possible that minor QTLs and interactions on D-genome chromosomes remain undetected. Analysis of a much larger population with highly saturated maps could confirm further minor QTLs and detect epistatic interactions associated with drought tolerance.

QTLs detected only under drought stress are presumed to contain genomic regions contributing to drought tolerance and could be used to enhance yield under drought stress. The QTL \times QTL and QTL \times QTL \times Environment interactions were also analysed to study the gene action and interaction with environment. The detected QTLs for physiological

traits were influenced by the environmental conditions. However, the effects were moderate when compared to the main effects across the environments. We estimated the effects of individual alleles by dividing the RILs into two groups. Grouping was determined by the presence of alternative alleles. The closest marker linked to each QTL was used in the analysis. For example, by screening the population with *Xgwm369* and using chlorophyll content as a parameter it is predicted that tolerant lines with the 189 bp allele will have a $38.5 \pm 5.9\%$ gain relative to the mean chlorophyll content value. It is worth noting that lower reductions in chlorophyll content or stable chlorophyll contents under water stress in drought tolerant lines demonstrates that this parameter is associated with drought tolerance. The QTL mapping conducted in this study provides preliminary information of genomic regions that may be important for drought tolerance, but further mapping and validation of the QTLs in field trials should occur before it is applied in MAS.

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